FACTORS AFFECTING THE GROWTH OF *CLADOPHORA* IN RELATION TO RIVER POLLUTION.

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Peter Keith ROBINSON Ph.D. 1983

Summary

Nuisance growths of *Cladophora* have been associated with eutrophication. A review of the literature, however, reveals a scarcity of relevant experimental growth studies.

Sampling experimental streams reveals that the addition of sewage effluent to good quality water alters the flora from that dominated by *Potamogeton crispus* to one dominated by *Cladophora*. Spatial and temporal differences in biomass of taxa present are discussed in the context of accompanying physico-chemical data.

In laboratory batch culture, growth of unialgal *C. glomerata* was accompanied by elevation of medium pH - considered largely responsible for the poor growth in such culture. However, appropriate experimental conditions and indices of growth were selected and the effects of various herbicides assessed. Diquat and terbutryne were shown to possess algicidal activity towards *Cladophora*.

A closed continuous culture apparatus was developed : growth proceeded through lag, logarithmic and linear phases. Inoculum size and medium flow rate had significant effects on growth, and were standardized.

In continuous culture, specific growth rate increased linearly with increased duration of light per day, up to 24 hours, and increased light intensity, up to 6000 lux - the highest intensity tested.

Comparison of field and laboratory results suggests that ammonia toxicity is attributable to the undissociated form. In the laboratory, 185 µg/l undissociated ammoniacal nitrogen reduced specific growth rate to 50% of that at 10 µg/l undissociated ammoniacal nitrogen.

0.077-1.057 mg/l NO_2-N had no significant effect on growth.

7.2-15.2 mg/l NO_3-N had no significant effect on specific growth rate. Neither was any nitrate/phosphate interaction significant.

At 4.9 mg/l PO_4-P specific growth rate was only 48% of that at 1.9 g/l PO_4-P. The critical medium PO_4-P concentration was <0.1 mg/l.

Specific growth rate was reduced to 50% of that in natural water by 0.036 mgCu/l, 0.070 mgZn/l and 1.03 mgPb/l. Metal uptake was evaluated.

KEY WORDS : *Cladophora*/growth/laboratory culture/pollution.

DEDICATED to my wife

Valerie

who, in the first 8 months of marriage, has tolerated this rival in her husband's affections with absolute equanimity.

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1. INTRODUCTION

Aquatic pollution is an important issue in today's world. By their nature pollutional problems almost inevitably lead to emotive response from a proportion of the population. The controversy surrounding such problems, however, is beyond the scope of this work, suffice to say that the author does not accept that a reduction in the quality of our environment is necessarily the consequence of the economic growth of our society (as measured by gross national product), but that economic growth can be reconciled with pollution control so long as public opionion and political will are in favour. Before further consideration of aquatic pollution it would seem wise to define some basic terminology.

Aquatic pollution may be legally defined as the addition of something to water which changes its natural qualities so that the riparian proprieter does not get the natural water transmitted to him (Coulson and Forbes, 1952). Hawkes (1969) defined the term as the discharge of something to the river which so changes its nature that the general amenities of the river are adversely affected, its suitability for man's legitimate use being impaired. The numerous additional definitions available in the literature do little to develop the concept further.

Pollution (and pollutants) may be categorised in a variety of ways. Owens (1970) categorised pollution according to source i.e. point (industrial and domestic effluents) or diffuse (agricultural run-off). Hawkes (1968) found it convenient to classify according to the ecological factor most affected (physical, chemical or nutritional), whilst classification by nature (physical state and chemical composition) may also be adopted. Thorpe (1981), however, points out that the problem with such systems is that the distinctions between different factors may be difficult to define or essentially arbitrary, and that in reality pollution involves the interaction of several factors. Holdgate (1979) in defence of classification systems accepts that no one system is 'ideal' but that classification is usually carried out for a purpose, and selection of a classification system must be use-related. In this introductory section it seems

adequate to divide aquatic pollution into three broad divisions, viz. organic, inorganic and toxic pollution. The first two categories should thus be considered as 'nutritional' according to the classification of Hawkes (1968).

Inorganic pollution may occur through the addition to a waterway of a well-treated domestic sewage or agricultural run-off. The increased level of inorganic nutrients available to the producer population, and the subsequent rise in productivity have been termed eutrophication. A strict definition of eutrophication is difficult : Landner (1976) points out that it was Naumann (1919) who first developed the concept and later (Naumann, 1931) defined it as an increase of the nutritional standards, with special reference to nitrogen and phosphorus. Hasler (1947) defined the term as intentional or unintentional enrichment of water; whilst Vollenweider (1968) describes eutrophication as the enrichment in nutrients and the ensuing progressive deterioration of water quality, due to the luxuriant growth of plants, with its repercussions on the overall metabolism of the affected waters. The subject of eutrophication has been thoroughly reviewed by Stewart and Rohlich (1967), Vollenweider (1968) and Landner (1976).

Gross organic pollution generally arises as the consequence of an input of poorly treated domestic sewage, or industrial effluent with a high organic content. Elevated levels of organic matter stimulate the development of an increased decomposer population whose respiratory demand results in a lowering in dissolved oxygen concentration in the water. Suspended organic matter may reduce light penetration or may settle out and alter substrate type. A 'replacement community' comprising animals and plants able to tolerate these environmental conditions subsequently develops, frequently dominated by organisms of the 'sewage-fungus' complex. Moving away from the source of pollution levels of organic matter drop, as a result of the activity of the decomposer organisms, and levels of inorganic salts rise. The size of the decomposer population subsequently drops, and as a result of surface diffusion processes the dissolved oxygen concentration rises. At this point the physico-chemical conditions of the waterway are similar to one undergoing eutrophication, except that in this case elevated levels of simple organic materials (such as organic acids and vitamins) accompany the elevated levels of inorganic mineral salts.

The enrichment of waterways with inorganic mineral salts (added directly or through the action of decomposers on an organic input) therefore acts so as to stimulate growth of the producer (= plant) community. Some species, however, seem particularly responsive to such stimulation : in lentic waters planktonic algal species such as *Anabaena, Microcystis* and *Asterionella* may reach 'bloom' proportions, whilst in lotic waters it is the benthic algal species and the attached vascular hydrophytes (commonly termed macrophytes) which show the greatest growth response. Of particular notoriety in this context are species of the genus *Cladophora* (usually fitting the description of *C. glomerata* (L.) Kutz.) a filamentous alga known commonly as 'blanket weed'. *Cladophora* species are common in unpolluted waters where they may grow as a tufted mat on the rocky substrate of riffle zones. Under conditions of nutrient enrichment, however, streamers may grow upto 12m in length (Hawkes, 1977) seriously affecting the amenity value of the waterway. At night the respiratory demand of such growths may lead to deoxygenation of the water, amd subsequent fish deaths (Tweed RPB, 1957). Upon detachment streamers may damage water-flow measuring structures (DOE, 1977) or if washed up on the shores of lakes give cause for public complaint from their unsightly appearance, smell, and associated fly nuisance. Filaments remaining within the waterway may, upon decay, give rise to secondary organic pollution.

Toxic pollution usually arises from the addition of an industrial effluent which may, or may not, have an associated high organic content. Any toxic component may act on members of the producer, consumer or decomposer community alike, and through action on the decomposer community may prevent normal self-purification processes from proceeding if the waterway also receives an organic input. Conditions of high dissolved oxygen and high organic load may then occur in the same stretch of water. *Cladophora* is characteristically absent from the benthic flora of such waters. The alga has been found to be rather sensitive to some of the toxic pollutants which commonly occur, especially heavy metals (Whitton 1967, 1970a), and in such conditions it is frequently the alga *Stigeoclonium* which dominates the flora.

Cladophora thus characteristically dominates the benthic flora of waterways undergoing nutrient enrichment but is rarely present in conditions of gross organic or toxic pollution. Whitton (1970b) has thoroughly reviewed the literature concerning the biology of *Cladophora* in freshwaters revealing that the genus as a whole seems to be absent from completely quiet waters and is almost always associated with conditions of relatively high pH (>7.0), favouring hard or very hard waters. Growth is apparently favoured markedly by high light intensities, whilst it has been suggested that high summer temperatures may suppress growth. The alga avoids waters which are very low in phosphate and nitrate and it is assumed that the obvious increase in crops over the past thirty years is a response to the increased levels of nutrients resulting from increased use of detergents and fertilizers and from increased population. However, considering the nuisance caused by some species of *Cladophora* it is perhaps suprising that the physico-chemical factors which affect growth have not been more fully established. A cursory glance at the literature reveals that much of our understanding of the growth response of this alga stems from field observations - experimental investigations being rather scarce. Indeed Whitton's review article (1970b) devotes 315 lines of text to the findings of environmental (= field) studies but could only fill 24 lines with findings from experimental studies. At first sight such a situation might seem paradoxical in that experimental studies, in which one environmental factor may be varied at a time, are more able to provide the findings necessary to understand the growth response of *Cladophora* than field studies in which a multiplicity of environmental factors fluctuate. Whitton (1967), however, felt that the lack of experimental studies was simply a result of the difficulty of such studies.

The experimental growth studies which have been reported in the literature have invariably involved the use of one of three types of experimental system, viz. stimulated stream ecosystems, laboratory experimental streams and laboratory batch culture techniques. Experimental studies from stimulated stream ecosystems are, however, scarce; notable exceptions being those of Zimmerman (1961) and Eichenberger (1967a, 1967b) from Zurich. This is undoubtedly a result of the scarcity of such expensive facilities themselves.

Smaller scale laboratory streams have been little used owing to the difficulty in obtaining adequate replication of treatments (Whitton 1967); whilst laboratory batch culture techniques suffer from difficulties of standardization of inocula, measurement of growth, and interpretation of the results in relation to the field situation (Whitton, 1967).

The work presented in this report was thus carried out as a direct response to the need for further experimental investigation of the physico-chemical factors affecting the growth of *Cladophora* and necessitated the trial and evaluation of a variety of experimental techniques.

2. OBJECTIVES

The broad objectives of the current research program were to investigate aspects of the biology of *Cladophora* in relation to its growth in eutrophic conditions and its absence under conditions of gross organic and toxic pollution; and to develop techniques which would allow such investigations to proceed.

Work was carried out during the period October 1979 to September 1982.

Experimental investigation into the growth response of *Cladophora* was planned along three lines :

(a) A study of the effects of a well-treated sewage effluent on the benthic flora, making use of the stimulated stream ecosystem facility situated at Checkley, Staffordshire and run by the Applied Hydrobiology Section of the University of Aston in Birmingham.

(b) An investigation into the growth of *Cladophora* in batch culture, with a view to using the technique to evaluate the growth response of the alga to various nutrients and toxicants.

(c) Development of alternative techniques which would allow the study of the growth response of *Cladophora* under controlled conditions.

With these aims in mind it was felt necessary to initially review the literature relevant to such a study.

3. LITERATURE REVIEW

At the outset the author feels it necessary to mention the general review of the biology of *Cladophora* in freshwater produced by Whitton (1970b). This comprehensive work, together with the works of van den Hoek (1963) and Fjerdinstad (1965), provides an excellent coverage of the literature up to 1970 This review, therefore, updates that of Whitton (1970b) and expands areas of specific relevance to this particular study.

3.1 THE BIOLOGY OF *CLADOPHORA*

3.1.1 Taxonomy

The genus *Cladophora* is by most authors placed in the family Cladophoraceae, which until 1935 was included in the well established order Siphonocladales. Though Smith (1933) included the Cladophoraceae in the sub-order Cladophorineae of the Ulotrichales. Fritsch (1935), however, placed most of the genera of the Cladophoraceae into the order Siphonales but retained the family Cladophoraceae, making it into a separate order - the Cladophorales. Later Feldman (1938) re-established the order Siphonocladales, Egerod (1952) accepted the order but, unlike Feldman, excluded the Cladophoraceae from it. Chapman (1962) felt this to be unacceptable and his classification includes the Cladophoracae as a family in the order Siphonocladales. The orders Siphonocladales (Chapman 1962) or Cladophorales (Fritsch 1935, Prescott, 1962) are placed in the class Chlorophyceae. Chapman (1962) arranges this class under the division Euphycophyta whilst Smith (1933) and Prescott (1962) recognise the division Chlorophyta.

Van den Hoek (1963) gives perhaps the best description of the genus. "Thallus filamentous, monosiphonous profusely to very sparsely branched, composed of multinucleate cells. Numerous disciform chloroplasts which are either densely packed in the parietal protoplasmic layer, extending also into the central part of the cell, or united into a reticulate structure in the partial meshes of the protoplasmic reticulum. Some of these chloroplasts contain a bilenticular pyrenoid around which two bowl-shaped starch bodies are formed. Growth by apical and/or intercalary cell divisions. Cell division independent of nuclear division, but simultaneous or almost simultaneous mitoses may precede formation of a cross-wall, which grows in from longitudinal walls in a diaphragm-like fashion. Insertion of a branch either lateral

below the apical pole of a cell (the branch being separated from its parental cell by a vertical cross-wall that does not change its position or hardly so) or apical on a cell (the branch being separated from its parental cell by an oblique wall which can grow into an almost horizontal position, thus giving rise to pseudodichotomy). Attachment either by branching rhizoids growing out from the basal cell or from other cells mostly in the basal region, or by a simple disciform holdfast formed by the lower cell wall of the basal cell. In some species, attachment organs are lacking and in other species their is a primary basal stratum of branching rhizoidal branches from which upright shoots arise. Reproduction either entirely asexual, by biflagellate or quadriflagellate zoospores, or by biflagellate isogametes and quadriflagellate zoospores in a regular alternation of isomorphic generations, with meiosis preceding spore formation. In several species, however, only multiplication by thallus fragmentation has been observed. In most species swollen, thick-walled, starch filled cells (akinetes) are formed under unfavourable conditions. In general, thick-walled rhizoids and basal parts of main axes are also able to survive unfavourable conditions". The genus *Cladophora* is thus a rather heterogeneous assemblage of species, the extremes of which *C. prolifera* and *C. basiramosa* exhibit a strikingly different morphology. In general the only feature which distinguishes *Cladophora* from other members of the family Cladophoraceae is its ramification (van den Hoek, 1963). A full discussion of the delimitation of the genus is given by van den Hoek (1963); for present purposes, however, it suffices to say that as regards freshwater members of the genus few problems are encountered making identification to the generic level, but identification of *Cladophora* species remains a problem.

The taxonomy of the genus is, to say the least, rather confused. The genus was created when Kützing (1843) united species of *Conferva* with uniform branches and named them *Cladophora* (Gk. *klados*, a branch and *phoreo*, I bear). His list contained 69 supposed species, a number which was expanded to more than one hundred by contributions from Areschoug (1850) and Harvey (1851). The confusion thus generated persists to the present day. Whitton (1970b) comments that the best attempt to sort out the chaos was that of van den Hoek (1963) who recognises 38 European species, or varieties, 27 being marine and 11 occurring in freshwater habitats. Starmach (1972) adds to the description of the species recognised by van den Hoek commenting that of the freshwater species *C. cornuta*, *C. fracta* var. *fracta* and *C. glomerata* var. *crassior* are typical of still waters whilst above all others *C. glomerata* is quoted

from flowing waters. Soderstrom (1963) studying primarily the marine species of *Cladophora* found in Europe proposed *C. fracta* and *C. glomerata* to be fresh or brackish water species. Whitton *et al.* (1978) list only *C. aegagropila*, *C. fracta* and *C. glomerata* in their coded list of freshwater algae.

The morphological and cytological criteria used in speciating members of the genus *Cladophora* are varied and include apical and filament cell diameter, length : diameter ratio, basal cell length, degree and pattern of branching, cell colour, cell shape, type and method of release of spores, position of fruiting bodies, chromosome count, and the overall length of the plant. The plasticity of several of these characters was,however, demonstrated by Dean (1966) in her experimental investigation into the taxonomic problem. Bliding (1936) used swarmer (zoospore) morphology and infertility between populations as criteria to aid speciation. Schussnig (1954), however, considered that only rarely, if ever, did *C. glomerata* produce functional gametes, whilst van den Hoek (1963) and Bellis and McLarty (1967) are contradictory as to the number of flagella possessed by *C. glomerata* zoospores (reporting 2 and 4 flagellae respectively) Dean (1966) thus felt that separation of species using such criteria appeared to have little value for direct indentification. Chromosome enumeration seems, at first sight, to be a promising technique to aid speciation ; the situation is, however, rather more complex than may be envisaged. Schussnig (1954) considered *Cladophora* to be a polyploid series with n = 12. Patel (1961), in agreement, found 2n = 24 in *C. flexuosa*, Sinha (1958) found 2n = 24 in *C. crispata*, *C. limaei* and *C. brandii* and 4n = 48, 6n = 72 and 8n = 96 in *C. glomerata*, and Faridi (1961) found 2n = 24 in both *C. crispa* and *C. sauteri* and 4n = 48 in *C. glomerata*. Shyam (1980) found chromosome numbers of 12, 24 and 48 recognizable in different isolates of *C. rivularis*. However, Chapman (1962) felt chromosomes to be present in multiples of 4, the diploid chromosome numbers being 8 + 1 for *C. repens*, 12 + 1 for *C. suhriana*, 24 for *C. flavesens* and *C. flaccida*, 32 for *C. pellucida* and 92 + 4 for *C. glomerata* (octaploid). Preparation and staining of samples is relatively easy but the intraspecific variability caused by polyploidy and the fact that many species have

the same chromosome number anyway makes speciation more difficult than at first envisaged.

The use of differential tolerance to various environmental factors (e.g. salinity) may be used as a method of achieving speciation. It is likely, however, that intraspecific variation arising from the adaptation of communities of the same species, and the likely large overlap in the tolerances of different species will make information concerning such tolerances rather difficult to interpret. Further, such information is as yet unavailable for many species of the genus. The use of such information together with morphological and cytological studies does, however, seem the best approach to this difficult problem of speciation within the genus *Cladophora*.

3.1.2 Life cycle

Many species of *Cladophora* are perennial; *C. glomerata*, for example, has been found throughout the winter months by many authors including List (1930), Raabe (1951), Jürgensen (1928) and Flint (1950). Under unfavourable conditions much of the algal thallus dies back and certain cells (akinetes) develop a dense cytoplasm and become swollen in appearance. Such cells are able to overwinter and give rise to new vegetative growth when conditions become more favourable.

The existence of an alternation of a diploid, spore producing, generation with a morphologically identical haploid, gamete producing, generation has been well documented for many species of *Cladophora*. Czempyrek(1930) describes the events leading up to the formation of asexual zoospores, which in most species are pear-shaped quadriflagellate structures. Schussnig (1928) found that in some *Cladophora* species meiosis occurred during the nuclear divisions leading to the formation of zoospores. Zoospores are liberated through a lateral pore in the cell wall and upon release they settle and develop into germlings (= plantlets) possessing two axes, one of which will form the erect portion of the thallus and the other which will form the rhizoidal holdfast (Brand, 1909). Biflagellate isogamous gametes produced by the haploid sexual generation are liberated in the same manner as zoospores, gametes from some species being heterothallic

(Foyn, 1929). Zygotes germinate immediately.

The life cycle of *C. glomerata* is, however, very different from that of most other members of the genus. In this species both zoospore and gamete producing plants are diploid. No meiotic division occurs prior to zoospore formation; zoospores are therefore diploid and produce a diploid gamete producing generation. Meiosis occurs during gamete production, gametes are thus haploid and fuse to form diploid zygotes which give rise to a new generation of spore producing diploid plants (List, 1930). List (1930) reported fusion of gametes of *C. glomerata*, and Whitton (1967) noted 'zoospore' fusion. However, no case of viable zygotes being formed has ever been reported in the literature, and Schussnig (1954) considered *C. glomerata* to seldom, if ever, produce functional gametes.

The importance of vegetative propagation in the life cycle of *Cladophora* awaits investigation. Fritsch (1935), however, felt such propagation, by fragmentation, was of common occurrence in species of the genus.

3.1.3 Distribution

The distribution of *Cladophora* seems cosmopolitan although Fritsch (1907) suggested the alga to be more abundant in temperate regions than in tropical climes. Luxuriant growths of *Cladophora* have been reported in the waterways of Britain (e.g. Pitcairn and Hawkes, 1973; Thorpe, 1981; Tweed River Purification Board, 1957; Dean, 1966) and Europe (e.g. Chudyba, 1965 and 1968; Sladeckova, 1968) In Iceland Schwabe (1928) reported growths of *Cladophora* in cooled thermal springs. Whitton (1970b) discussed observations in Saudi Arabia and (1971) in the tropical pools of Aldabra; whilst Betzer and Kott (1969) obtained *Cladophora* from a channel west of Lake Kinnereth, Israel. Whitton (1970b), after personal communication felt this species to be *C. glomerata*. In North America most reports have dealt with the Great Lakes region. As far back as 1872 Wood (1872) found *Cladophora* to be present in all of the Great Lakes except Lake Superior. Most of the current interest, however, has arisen following the accumulations occurring from the late 1950's onwards. Detached masses of *Cladophora* washed ashore formed foul smelling black heaps and attracted flies, providing a major source of public complaint. Several authors have

recorded such luxuriant algal growths including Kishler (1967), McNaught (1964), Neil and Owen (1964) and Bellis and McLarty (1967). Herbst (1969) succinctly reviews their work and describes the distribution of *Cladophora* in the Great Lakes as do Shear and Konasewich (1975). Most authors regard the species of *Cladophora* responsible for the problem in the Great Lakes as being *C. glomerata* (Prescott 1962, Bellis and McLarty 1967, Herbst 1969). Neil and Owen (1964), however, felt the predominant species was *C. fracta*. *Cladophora* has also been reported in North America by Mason (1965) who found *C. glomerata* growing profusely in streams and farm ponds around Ithaca, New York. Thurman and Kuehne (1952) reported growths of *C. glomerata* near Dallas, Texas; whilst after his survey of the Saline River Blum (1957) remarked that "*C. glomerata* was the most abundant and conspicuous filamentous alga in unpolluted parts of the Saline River as it appears to be in most streams throughout the world".

3.2 *CLADOPHORA* GROWTH/BIOMASS STUDIES

3.2.1 Methods

3.2.1.1 Field Surveys

The filamentous growth-form of *Cladophora* does not lend itself to easy enumeration, consequently many field surveys have simply recorded the presence or absence of the alga at a particular site with some quantitative description of abundance (e.g. Thurman and Kuehne, 1952; Tweed River Purification Board (RPB), 1957; and Neil and Owen, 1964). Neil and Owen (1964) collected information from the Great Lakes area from direct observation from boats, photographic evidence from aircraft and from scuba divers, as well as indirectly estimating the location and extent of growths by recording accumulations washed up on the shores. This indirect approach was felt to be the most practical on a lake-wide basis. Various semi-quantitative estimates of abundance have also been used in an attempt to reduce the subjectivity of estimation. Bellis and McLarty (1967), for example, used a relative abundance scale of 'dominant', 'common', 'present', and 'rare'. McNaught (1964) adopted the scale V = dominant, IV = abundant, III = equal with other species, II = scarce, I = 1-2 individuals; whilst Thorpe and Williams (1980) suggested the scale abundant (5), common (4), frequent (3), occasional (2), or rare (1). The use of such semi-quantitative scales appears to be a useful compromise between true quantitative work which is often difficult and always time consuming, and simply recording the presence or absence of taxa.

True quantitative procedures involve the removal of all plant material from a known area of substratum. Quantitative samplers include various forms of cylinder samplers, Surber samplers and grab samplers, as well as various artificial substrates of known surface area. Thorpe and Williams (1980) thoroughly review the literature concerning sampling methodology in relation to the use of algae in biological surveillance. In performing field surveys both the size of each sample and the frequency of sampling are important.

Kershaw (1964) commented that it is impossible to make a general rule as to the number of sample quadrats or measurements to be taken from an area and also since it is usually impossible to predetermine the scale of pattern (i.e. distribution of individuals), and since such patterns are often repeated on larger scales, the size of quadrat has to be chosen quite arbitrarily. There are, however, some general rules which can be applied. If the dispersion of a population is truly random then quadrat size is immaterial except from the standpoint of convenience. In many cases however, population dispersion is contagious and in such cases, the advantages of a small quadrat (over a large one) are threefold. First, more small quadrats can be taken with the same amount of effort in sample processing. Second, as a sample of many small quadrats has more degrees of freedom than a samples with a few large quadrats, the statistical error is reduced. Finally, since many small quadrats cover a wider range of habitats then a few large quadrats, the catch of the small units is more representative. Thus Elliott (1977) - considering the contagious distribution of benthic invertebrates - concludes that the smaller the sampling units employed the more accurate and representative will be the results. Kershaw (1964) adding that the most suitable quadrat on theoretical grounds is the smallest possible, relative to the type of vegetation and to the practicability of the enumeration of such a quadrat size. Pitcairn and Hawkes (1973) adopted a $0.1m^2$ cylinder sampler is their study of *Cladophora* growth in rivers in the West Midlands area. Sampling at fortnightly intervals the 'crop' was returned to the laboratory, washed and dried to constant weight, allowing standing crop to be expressed as dry weight of alga per square metre. Similarly, Thorpe (1981) used a $0.05 m^2$ sampler in his survey of *Cladophora* in the River Tean, Staffordshire; and Kishler (1967) used a $1 ft^2$ quadrat constructed from bicycle chain.

In relatively few cases a variety of sampling methods have been used together to provide a very full account of algal distribution. Noticable in this context is the study of Blum (1957) who made quantitative observations of sites on the Saline River, Michigan, at 10 day intervals noting the algal species present in 'significant' amounts, as well as using two quantitative sampling techniques. First, he took

transects of riffles by passing a rope, marked in metres and decimetres across the river ; presence was recorded of all algal species, an individual or colony of which was crossed by the rope, in alternate decimetres. Two or more transects were made until 50-100 decimetres were recorded at each station. Secondly, he took 20 rocks from sites and layed them out on the river bank in random order. Alternate rocks were selected (10 in all) and weighed; if they weighed 12 ± 0.5lb they were used as a sample, if not one or more rocks were chosen from the 10 left until 10 rocks were found which together did weigh 12 ± 0.5lb. The flora was then removed from these rocks and placed in a collecting bottle. On return to the laboratory the algal mass was washed and, after manual removal of stones, was dried to constant weight. Similarly, Wong *et al.* (1978) used a variety of techniques in their study of rivers in south-western Ontario. Visual estimates of the percentage abundance of each species were obtained by harvested material, quadrat photographs and drifting plant material collected on wire screens.

3.2.1.2 Field experimentation

As well as field surveys various experimental studies have also been carried out in the field situation. Butcher (1937), Neil and Owen (1964), Dean (1966) and Whitton and Buckmaster (1970) have all studied the effect of transplanting *Cladophora* colonized rocks from one site to another, to investigate the effects on growth and morphology. Blum (1957) alternatively investigated colonization using large, glacially-polished, rocks (3-5 kg) which were dry sterilised at 200°c for 5 hours and placed in the Saline River at successive dates. These were carefully removed, at intervals, and observed so as to provide information as to the times and conditions of reproduction of the colonizing algae. Thorpe (1981), however found *Cladophora* to be particularly reluctant to colonize artificial substrates including red quarry tiles, polythene strips and perspex plates.

3.2.1.3 Experimental streams

Studies involving the growth of *Cladophora* in 'field-scale' environmental streams include those of Eichenberger (1967a, 1967b) and Zimmerman (1961) from Zurich. Balloch (1977) briefly describes seasonal

growth of *Cladophora*, *Vaucheria*, *Stigeoclonium* and *Amblystegium* in the experimental streams at Checkley, Staffordshire; whilst the studies of Bolas and Lund (1974) involved growth of *Cladophora* in small bankside 'streams' on the banks of the Kentish Stour.

'Laboratory-scale' experimental streams, all involving recirculation of nutrients, have been used by Whitton (1967) and Turano (1963) with some success. Similar streams at the University of Aston (described by Peters, 1977) have been used in *Cladophora* studies with little success (Hawkes, personal communication).

3.2.1.4 Culture studies

Growth of *Cladophora* in the laboratory has almost exclusively involved batch culture techniques. It is unfortunate, however, that there has been little standardization in experimental procedures, making direct quantitative comparison of results difficult. Techniques reported in the literature differ in four main areas, which seem best considered in turn.

(a) Inoculum size and condition

An ideal inoculum for *Cladophora* growth studies would likely be an axenic spore suspension. Unfortunately, however, the sporulation of *Cladophora* although observed by several workers (e.g. Zuraw, 1969; Bellis, 1968a 1968b; and Whitton 1967) has rarely been controlled or induced, although Cook and Price (1928) were able to alter the time taken for *C. crispa* and *C. glomerata* to sporulate in the laboratory by altering the wavelength of light illuminating the cultures. Axenic cultures of *Cladophora* have rarely been maintained : Thomas (1963) succeeded in isolating an axenic culture of *C. glomerata* but could only stimulate growth on a mineral salt agar if an unidentified bacterial substance or lake water was used as a supplement. Sikes (1976), however, was successful in maintaining an axenic culture isolated from a breakwall in Milwaukee harbour; whilst Gerloff and Fitzgerald (1976) used axenic isolates from Lake Michigan , Superior and Erie in their studies. Unialgal (but not necessarily axenic) cultures have been frequently used in batch culture studies. Zuraw (1969) isolated a unialgal culture of *C. glomerata* from Lake Michigan and used unialgal

filaments and plantlets derived from this isolate for inocula in culture work. Bellis (1968a, 1968b) obtained a unialgal stock culture derived from Medway Creek, Near London, Ontario and used sparsely branched, 1 cm long, segments of akinetes as inocula. Whitton (1967) did not use a unialgal inoculum, but removed *Cladophora* covered stones from the rivers Tees and Wear and placed them in experimental streams at 20°C, illuminated continually at an intensity of 6500 lux. After four days healthy filaments of *Cladophora* 1 cm long, having 2-4 main branches, and appearing free from epiphytes were removed and three such pieces were used per flask as inoculum for growth and toxicity tests. Attempts to refine the inoculum, including homogenization of filaments and dissecting 4-cell apical pieces, failed as a result of the apparent sensitivity of the alga to cutting and other mechanical damage (Whitton, 1967). Pitcairn and Hawkes (1973) and Thorpe (1981) used perhaps the simplest method of obtaining an inoculum ; *Cladophora* was collected from the nearby River Blythe and kept in an open bottle in natural water at 14°C, in indirect sunlight. 2 cm long, well-branched filaments were then washed free of epiphytes and used as inocula.

(b) Experimental duration

It seems likely that experimental duration is directly related to inoculum type. Bellis (1968a, 1968b) and Zuraw (1969), for example, were able to use experimental durations of 22 and 42 days respectively, since cultures were unialgal. The use of non-unialgal cultures, however, necessitates shorter experimental periods in order to avoid major contamination problems. Pitcairn and Hawkes (1973) and Thorpe (1981) thus used only a 10 day experimental period; whilst Whitton (1970a) used only a 6 day period and in an earlier study (Whitton, 1967) a period as short as 4 days.

It is unfortunate that the choice of experimental duration has tended to be arbitrary, only Whitton (1967) and Zuraw (1969) giving any indication of the accompanying kinetics of growth. Whitton (1967) commented that the replication between flasks (i.e. within treatment variability) was not adequate to justify a detailed study of growth rates but that in most tests with periods up to four days the fresh-weight doubling time at 20°C and 6500 lux was 21-26 hours with shaken

cultures and 30-45 hours with static cultures. Zuraw (1969) found that growth of filaments under stagnant conditions and 200 ft-candles proceeded exponentally for one week and that the cellular doubling time of plants originating from 12-24 cell fragments was 29-58 hours.

(c) Experimental conditions

The great diversity of media, etc. reported in the literature suggests that no one set of growth conditions has been found to be entirely satisfactory in stimulating optimum growth of *Cladophora*.

Bellis (1968a, 1968b) grew *Cladophora* at various temperatures (1968a) and Ca/Mg ratios and pH's (1968b). Cultures were grown in 250 ml Erlenmeyer flasks or 1 x 6 inch culture tubes containing a medium consisting of 50% TRIS (tris (hydroxymethyl) aminomethane) buffered inorganic medium (TBlM) (Smith and Wiedman, 1964), 35% distilled water and 15% soil water extract (SWE). The final medium was adjusted to the appropriate pH with 0.1N HCl prior to autoclaving. At temperatures of 15-30°C (1968a) culture tubes were illuminated on a 12:12, light/dark, cycle at 400-600 ft-candles. At temperatures below 15°C cultures were grown in a greenhouse illuminated by sunlight at 2600 ft-candles on a 16:8, light/dark, photoperiod. Moore and McLarty (1975), in their study of the active component in soil water extract used a medium rather similar to that of Bellis (1968a, 1968b) - comprising 90% TBlM and 10% soil water extract. The pH was adjusted to 8.4 with 0.1N HCl. *Cladophora* was maintained in 2.8 litre Fernback flasks containing 1 litre of medium. Cool white fluorescent tubes and incandescent tubes provided an intensity of 500 ft-candles for 14 hours per day. Flasks were shaken on a reciprocating shake table at 14 ± 1°C, Pitcairn and Hawkes (1973) grew the alga in 100 ml medium in 250 ml conical flasks at 15°C, illuminated from beneath on 18:6, light/dark, photoperiod. Their inorganic growth medium was supplemented with a trace element solution of Kevern and Ball (1965) and to obtain good growth 10% membrane filtered, nutrient-poor, pond water was added. The pH was adjusted to 7.7-8.0. Experiments were also carried out with natural river waters. Thorpe (1981) similarly carried out bioassay experiments, using natural waters. 250 ml flasks were placed on an orbital shaker and rotated at 100 cycles/minute. Illumination was provided, 18 hr/day, by two 30W white fluorescent tubes and two 60W tungsten filament bulbs, maintaining a mean illuminance at the level

of the cultures of 4500 lux. Whitton (1967) based his growth medium on Chu's No. 10 medium (Chu, 1942), modifying it by addition of 10% membrane filtered river water and the trace element solution of Allen and Arnon (1955); sodium silicate was omitted to reduce growth of epiphytic diatoms and iron was added as FeEDTA (ethylendiaminetetra-acetic acid). Conditions of temperature amd light intensity were 20°C and 6500 lux respectively. In a later study (Whitton, 1970a) alga was placed in 5 ml medium in a boiling tube, which was incubated at an angle and shaken gently. Illumination was provided continually at an intensity of 6000 lux and temperature was maintained at 20°C. Medium was based on Chu's No. 10 medium (Chu, 1942) modified by the addition of the trace element 'stock C' solution of Kratz and Myers (1955). Zuraw (1969) obtained good growth of *Cladophora* in a specific defined medium free from river water or soil water extract supplements. Cultures were grown either in petri-plates or stoppered 700 ml test tubes. Petri-plates containing 30 ml of medium were incubated at 25°C under cool-white fluorescent tubes, providing an intensity of 200 ft-candles; whilst test tubes contained 500 ml of medium were incubated at 25°C under 500 ft-candle light intensity. The culture medium comprised 50% of a medium based upon a formulation developed by Trama and Benoit (1960) and 50% synthetic seawater (Rila products, P.O. Box 114, Teaneck, New Jersey). Gerloff and Fitzgerald (1976), however, were unable to grow *Cladophora* isolated from Lake Michigan in the medium developed by Zuraw (1969), but found good growth of *Cladophora* in Gorhams medium (Zehnder and Gorham, 1960) fortified with 10-25% final effluent from the Madison, Wisconsin sewage disposal plant amd 2 $mg \cdot l^{-1}$ vitamin B_1. Sikes (1976) used the same medium as Gerloff and Fitzgerald but omitted the B_7 and C_{13} trace elements - considering that evidence of their growth enhancement potential was inconclusive. Hoffman, *et al.* (1974) grew *Cladophora* in sterilised gallon plastic containers. Light was provided by a 20W soft-white fluorescent light in conjunction with two 15 watt (blue-tinted) bulbs, providing 185 ft-candles incident at the surface for 16 hours per day. A temperature of 25°C was maintained and culture media were magnetically stirred during the photoperiod. After experimentation a medium comprising 99% river water and 1% soil extract was adopted. Betzer and Kott (1969) similarly used natural water, continuously aerated, in 1 litre beakers. A light intensity of 1500 lux and temperature of 30°C were maintained. Water

was replaced every 3 days. Cook and Price (1928) maintained small quantities of *Cladophora* in a beaker of tap water, subjected to constant aeration, although Knops solution (McLean and Cook, 1941) was also used with success. Storr and Sweeney (1971) cultured *Cladophora* in autoclaved Lake Erie water, using 14 gallon aquaria as test chambers. Lighting was provided by cool white fluorescent tubes, on 12 and 14 hours photoperiods, maintaining an intensity of 800 ft-candles at aquarium level. Dean (1966) used an organic medium (referred to as a 'Bristol-Knop medium') with membrane filtered stream water as the solvent, and a marine algal culture solution - both were found suitable for growth. Van den Hoek (1963) in the course of his taxonomic work maintained *Cladophora* strains in a medium comprising 95% pond water and 5% Erdschreiber medium (Foyn, 1934).

(d) Evaluation of growth

A variety of destructive and non-destructive techniques are available to evaluate biomass and thereby growth. The majority of culture studies involving growth of *Cladophora*, including those of Pitcairn and Hawkes (1973), Thorpe (1981) and Zuraw (1969), have used dry weight determinations. Bellis (1968a, 1968b), however used various morphological features to compare growth of the alga in culture including relative degree of branching, number of cells/branch, cell shape, dimensions, colour, and possession of specialized structures (e.g. zoosporangia). Whitton (1967) attempted a cell counting technique to assess growth, but found that difficulties owing to the time consuming nature of such determinations and complications arising from alterations in cell size and shape made such a procedure impracticable. Further work, was successfully performed, however, using a wet weighing technique - filaments were blotted free of surplus moisture with filter paper and weighed in a small glass container which contained a small reservoir of water at the bottom. Zuraw (1969) did obtain some results using a cell counting technique but using smaller inocula. Whitton (1970a) assessed the acute effects of toxicants on a semi-quantitative scale of 'survival' (ranging from maximum amount of toxicant to cause no lag in algal growth to the minimum concentration able to kill all of the population of cells). Similarly Betzer and Kott (1969) visually assessed the effects of halogens.

3.2.2 Results

Whitton (1970b) comments that it is generally assumed that the obvious increases in crops of *Cladophora* over the last couple of decades are a response to increased levels of nutrients resulting from increased population, detergents and fertilizers. In an effort to find the substance responsible for this stimulation Pitcairn and Hawkes (1973) carried out both river surveys and laboratory, flask culture, experiments. River survey data revealed a significant correlation between *Cladophora* crop and total organic phosphate concentration ($r = +0.54$, $P<0.05$) but failed to find significant correlation between crop and oxidised nitrogen concentration ($r = +0.22$, $P>0.05$). Culture experiments confirmed the importance of phosphorus in that growth of *Cladophora* taken upstream of a discharge could be increased to downstream levels by the addition of K_2HPO_4. Growth experiments in synthetic media indicated no significant growth increase above 1 mg/l phosphorus but a significant reduction below that value. The maximum level of phosphorus for growth varied, however, with alteration in the nitrate-nitrogen concentration - the highest level of NO_3 reduced growth at 3 mg l^{-1} PO_4 but elevated growth at 0.5 mg l^{-1} PO_4. Wong and Clark (1976) similarly investigated *Cladophora* to determine the critical nutrient levels below which growth would cease. Field results revealed a significant correlation between total phosphorus concentration in the water and the phosphorus content in *Cladophora* tissue in six rivers in Ontario ($r^2=0.80$), but failed to show any significant correlation between total Kjeldahl nitrogen content of the tissue and the total nitrogen concentration in the water. Critical levels, below which maximum growth would not take place, were 12-15 mg N g^{-1} dry weight and 1.6 mg P g^{-1} dry weight. Since water and tissue phosphate were correlated, the critical water level was calculated as 0.06 mg P l^{-1}. Lin (1977) similarly demonstrated a significant correlation between total dissolved phosphorus in Lake Michigan water and the concentration of hot water extractable phosphorus. Hoffman *et al.* (1974) similarly considered phosphorus to be the most probable limiting element in controlling algal growth in the Eau Gallie River, Florida; whilst Bolas and Lund (1974) after extensive long-term field work concluded

that there was no doubt that phosphorus was a major controlling element, in *Cladophora* growth, while nitrogen was not.

Rarely has *Cladophora* been found to grow well in synthetic, inorganic media without the addition of approximately 10% soil water extract or membrane filtered river water. Hoffman *et al.* (1974) for example concluded that predominantly inorganic nutrient solutions were unable to sustain growth and that the 'catalytic' effect of soil extract on growth was readily apparent. Moore and McLarty (1975)- investigating the factor responsible for the activity of soil water supplements revealed the importance of an organic heat-labile component, and when thiamine was substituted it provided 80% of the stimulation of the soil water extract. The presence of thiamine in soil water extract was subsequently shown and further experiments demonstrated that 1 $\mu g l^{-1}$ thiamine stimulated 'satisfactory' algal growth, whilst 10 $\mu g l^{-1}$ was the apparent optimum concentration.

Storr and Sweeney (1971) investigated the growth response of *Cladophora* to temperature and photoperiod, extrapolating results to develop a predictive model which fit the seasonal biomass data of Kishler (1967) with good agreement. Wong *et al.* (1978) similarly investigated the seasonal periodicity of *Cladophora* and found temperature to be an important factor controlling such periodicity. The tolerance limits of both *Cladophora glomerata* and *Potamogeton pectinatus* at fixed values of the daily mean or daily maximum temperature were found to change with daily range. *C. glomerata* was found to be dominant at lower temperatures, and *P. pectinatus* at higher temperatures; at intermediate temperatures (mean = 18-22^{o}C, <4^{o}C daily range) a mixed community was found.

3.3 PHOTOSYNTHESIS STUDIES

Several studies have been made of the photosynthetic rate of *Cladophora* under a variety of environmental conditions. Most involve the, so called, light-and-dark bottle technique reviewed by Vollenweider (1974). Adams and Stone (1973) used a modification of this technique involving carbon-14. Light-and-dark bottles were incubated for two hours, suspended 10 cm below the surface of Lake Michigan, after which time ^{14}C uptake was analysed by scintillation. Results indicated that lower rates of photosynthesis occurred when temperatures were between 9 and 16°C than when between 19 and 24°C. Relatively efficient use of low illumination was claimed. Wood (1975a), however, criticised the light-and-dark bottle technique on the grounds that errors may arise owing to $CaCO_3$ precipitation, self-shading, pH/CO_2 limitation, or variations in the condition of the alga. Consideration of such errors led Wood to refute the conclusions of Adams and Stone (1973) that *Cladophora* is capable of utilizing relatively low levels of light and of light saturation at low irradiance levels. Mantai (1974) similarly criticised the light-and-dark bottle technique and in his study, using an oxygen electrode, found photosynthetic rates for *Cladophora* 3-4 fold higher than reported by either Wood (1968) or Adams and Stone (1973). Mantai considered this discrepancy attributable to the light limiting conditions resulting from self-shading of the alga in the 'light' bottles. Mantai also found no evidence to support the hypothesis that photosynthesis, in *Cladophora*, saturates at low light intensities.

Turano (1963) carried out an interesting study using both artificial streams and light-and-dark bottle studies for investigation into photosynthetic/respiratory rates linked with nutrient status. Results showed optimal temperature for photosynthesis to be within the range 17-21°C. Photosynthetic rate was maximal at pH 7.5-8.0 whilst respiratory rate was minimal at pH 8.0 : pH 9.0 was found to be limiting in photosynthetic studies. Medium containing 0-0.3 mg l^{-1} phosphate elicited no change in photosynthetic rate; whilst between 0.7 and 5.0 µg l^{-1} a linear relationship between phosphate concentration and photosynthetic rate was found so that the rate at 5.0 mg l^{-1} phosphate was approximately double that at 0.7 mg l^{-1}. Little relationship was found between photosynthetic rate and nitrate concentration.

3.4 BIOACCUMULATION STUDIES

In view of the common occurrence of *Cladophora*, knowledge of its bioaccumulative ability is of some practical importance. Keeney *et al.* (1976) assessed the use of the alga as a biological monitor for metals, concluding that *C. glomerata* can act as a biological monitor, concentrating trace metals present in the aqueous environment with a reasonably constant concentration factor for each element. Thorpe (1981) studied metal bioaccumulation by *Cladophora* in the River Tean, Staffordshire. Results demonstrated both temporal and spatial differences in concentration factors, Thorpe concluding that *Cladophora* appeared to exhibit many of the characteristics mentioned by Phillips (1977) as being desirable of a biological monitor. Wood (1975b), unlike the above authors did not crop algal material growing in the watercourse for study, but placed quantities of *Cladophora* (approximately 1 g fresh weight) in Dacron bags and exposed them for either 2 hours or overnight in experimental and control streams in mining areas of north-western USA. Results were, unfortunately, widely variable but indicated that even in the short periods of immersion used bioaccumulation may proceed upto the levels found in the indigenous biota. Williams (1970) used the radioisotopes ^{85}Sr and ^{137}Cs to show that uptake of these metals proceeded with greatest speed in rapidly growing cells. Burkett (1975) investigating the bioaccumulation and release of methylmercury by *C. glomerata* under laboratory conditions revealed living cells to accumulate more metal than those which had been killed. Accumulation reached a peak after only two days. Desorption was nominal during the 16 day release period when the alga was held in uncontaminated water. Sikes (1976, 1978) studied ^{45}Ca uptake in batch and closed continuous culture, Results indicated that total calcium increased as cells grew to maturity. Calcium uptake was suppressed by treatments that inhibited photosynthesis but allowed diffusion - cold and darkness - and since uptake demonstrated saturation kinetics and could occur against a 50 fold concentration gradient an active uptake mechanism was suggested.

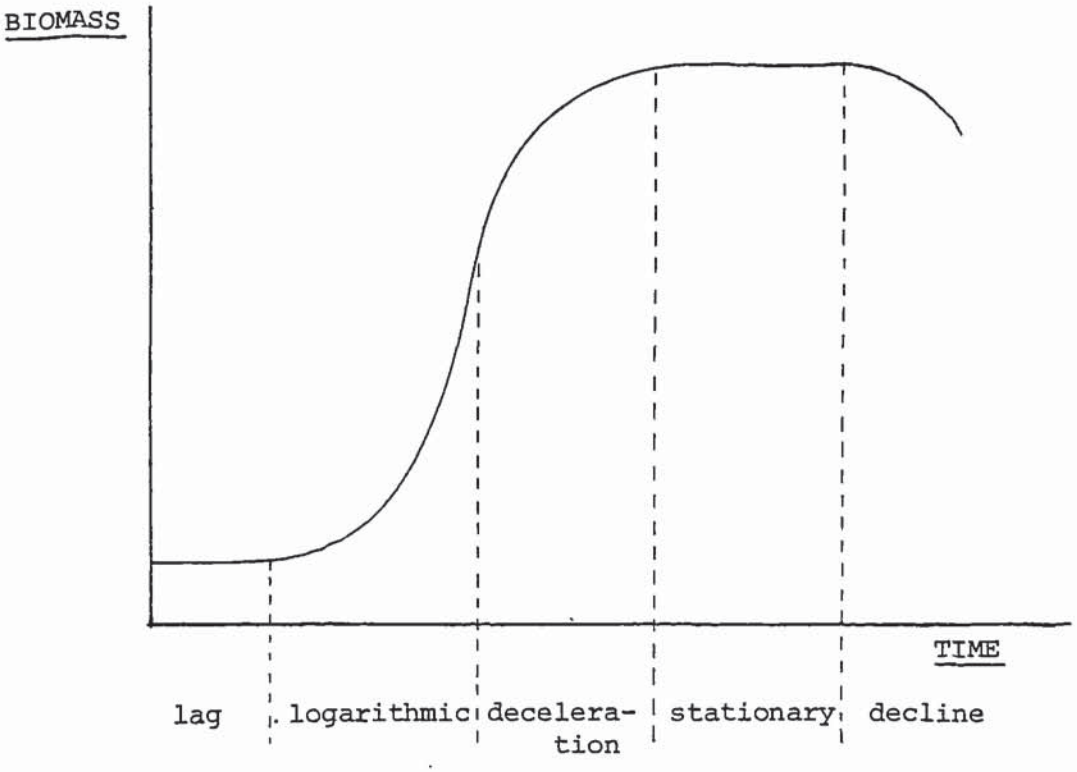
3.5 THEORETICAL ASPECTS OF MICROBIAL GROWTH IN CULTURE

3.5.1 Growth in batch culture

A substantial literature exists, dealing with the growth kinetics of unicellular organisms in batch culture (e.g. Monod, 1942; Dean and Hinshelwood, 1966; Fencl, 1966). This can, however, be applied to studies involving filamentous organisms with appropriate modifications.

Batch culture may be defined as the growth of an organism in a medium containing a limited supply of nutrients, with no removal of waste products (other than gaseous products to the atmosphere). Growth proceeds through a series of definable phases producing the characteristic growth curve shown in fig. 3.1.

Fig. 3.1. Typical microbial growth curve in batch culture.



The LAG phase

The lag phase, also described as the phase of adjustment (Winslow and Walker, 1939) and the acceleration phase (Monod, 1942), is generally considered to be a period during which the organism adapts to the ambient conditions. This likely involves the induction of vital enzymes and an increase in the activity of the cellular machinery responsible for the synthesis of protein (Kjeldgaard *et al.*, 1958). Alternatively, the phase may occur as a result of the requirement of the organism to modify the environment before growth may proceed at the maximal rate (Hughes and Wimpenny, 1969). The phase may also occur as a result of the time taken for a stimulatory metabolic product to accumulate ; carbon dioxide, for example, has been reported as stimulating the growth of several fungi (Hartman *et al.*, 1972).

The LOG phase

Once adapted to the environment many organisms typically grow 'autocatalytically' - growth results in the production of new biomass which is itself capable of growth so that the growth curve is exponential (Monod, 1942) or logarithmic (Buchanan, 1918). In this phase of growth the increase in biomass at any time is proportional to the initial biomass (at time t = o) and the specific growth rate (μ).

$$\text{i.e. } W_t = W_o e^{\mu t}$$

$$\ln W_t = \ln W_o + \mu t$$

$$\mu = \frac{\ln W_t - \ln W_o}{t}$$

Experimental growth therefore requires all, or a constant proportion, of the biomass to contribute to new growth. In unicellular forms this is achieved simply by parent cells undergoing binary fission to produce two daughter cells, each with the same capacity for growth and replication as the parent. It may be envisaged that the situation is identical

in many unbranched, filamentous forms where the filament may simply be comsidered as a linearly arranged aggregate of unicells - each capable of binary fission. In some branched filamentous forms, however, growth is totally or largely restricted to the apical cells, and if all growth takes place at the apex of a filament exponential growth will only occur if new branches (and therefore new growing points) are produced at a rate proportional to the increase in cell mass. Katz *et al.* (1972) indeed found frequency of branching to be proportional to the specific growth rate in the fungus *Aspergillus nidulans;* whilst Smith (1924) revealed that an exponential increase in the total length of the hyphal system occurred in *Botrytis cinerea* even though individual filaments were only found to be growing at a linear rate. Righelato (1975) therefore concluded that the overall picture of an exponential increase in biomass and total hyphal length as a result of branching was well established for homogeneous cultures.

The DECELERATION phase

In practice it is rare, in batch culture, for exponential growth to persist for more than a few days, after which time growth decelerates, usually as a result of a decrease in nutrient concentrations or an increase in the concentration of inhibitory metabolic products. Ignoring the latter, growth rate during the phase may be considered to be a function of the limiting substrate concentration (Monod, 1942; Novick and Szilard, 1950). The mathematical expression describing this situation is usually given in the form :-

$$\frac{\delta W}{\delta t} = \mu \max \left(\frac{s}{S_k + s} \right)$$

where $\frac{\delta W}{\delta t}$ is the growth rate

μ max is the maximum growth rate

S_k is the half saturation constant for the uptake of the growth limiting substrate

s is the substrate concentration

The STATIONARY and DECLINE phases

As nutrient depletion progresses growth ceases and as the limiting substrate concentration falls below the maintenance level (i.e. that required to maintain zero growth rate) lysis occurs and biomass decreases (Trinci and Righelato, 1970). Additionally, waste products may accumulate to concentrations which lead to cell necrosis.

3.5.2 Growth in continuous culture

The term 'continuous culture' may be applied to any process involving passage of fluids through a reactor in which they are exposed to microbial action. Herbert (1960), however, comments that it is unfortunate that there is a tendency to think that all continuous culture processes have a great deal in common simply because they are continuous, as opposed to batch, processes. There are indeed some underlying principles common to all continuous culture systems but these have to do with the fundamental mechanisms of growth and apply equally to batch culture. In most other respects different types of continuous culture system may be as unalike to each other as any of them is unalike a batch process (Herbert, 1960).

Excluding large-scale industrial/commercial reactors (e.g. percolating sewage filters and activated sludge plants) the two best known continuous culture systems are probably the chemostat and the turbidostat. Both are open, homogeneous, single-stage systems (according to the classiffication of Herbert, 1960) and have been used extensively for the culture of unicellular organisms. Prance and Benson-Evans (1973), however, developed a closed continuous culture system for the culture of filamentous green algae; and succeeded in growing *Ulothrix zonata* in such a system. However, no study of *Cladophora* growth has been made in continuous culture, although Sikes (1976, 1978) used the apparatus of Prance and Benson-Evans (1973) in his study of calcification in *Cladophora* to circumvent problems of nutrient depletion and metabolite accumulation.

4. INVESTIGATION INTO THE GROWTH RESPONSE OF *CLADOPHORA*, AND ASSOCIATED FLORA, TO THE ADDITION OF WELL-OXIDIZED SEWAGE EFFLUENT IN SIMULATED STREAMS.

4.1. Introduction

Balloch (1977) points out that environmental (=field) surveys alone do not demonstrate true cause and effect relationships between the responses of aquatic organisms and the observed water quality criteria, and that the usefulness of field data may be limited by the fact that unmeasured environmental variables may have interposed between the aquatic organisms under consideration and the water quality criteria monitored. The validity of such criticism may be illustrated in respect to the study of the growth of *Cladophora* by considering the following example. The addition of a poorely treated sewage effluent to a waterway results in the stimulation of a *Cladophora* dominated community some distance downstream. Comparison of biomass upstream and downstream of the discharge will indicate the extent of the differences in the two sites. Since, however, the two sites likely differ in pertinent physical variables such as river flow, velocity and topography the differences in the two communities may not be described by the differences in chemical nature of the two sites alone. Balloch (1977) considers that a solution to this fundamental problem is afforded by the use of simulated streams in which physical variables may be experimentally controlled, allowing replicate streams to have similar attributes, such that the responses of biological communities to water quality treatments may be directly investigated.

The objectives of this study were to quantitatively investigate the growth response of *Cladophora* and associated floral taxa (as indicated by standing crop) to variation in levels of chemical variables associated with the addition of a well- oxidised sewage effluent by carrying out a general survey of the flora of the simulated stream ecosystem at Checkley, Staffordshire. A sampling and data processing methodology was designed to show variation in the spatial and temporal distribution of taxa present. It was felt that systematic observations on the growth response of the floral taxa (as indicated by biomass) to known water qualities may reveal effects not demonstrable in laboratory experiments, especially those effects expressed at the population (synecological) level and those expressed as a response to the intera-

ction of two or more chemical variables. Results would thus complement those of laboratory experiments (chapters 5 onwards) and aid in the extrapolation of such data to the environmental situation.

Further study of the flora of the simulated stream ecosystem at Checkley was unfortunately not possible owing to the closure of the facility in September 1980.

4.2 Description of the experimental facility

Checkley applied hydrobiology field station was situated on the banks of the River Tean near Checkley, Staffordshire (Nat. grid. ref. SK 035373). The field station was established by the Applied Hydrobiology Section of the University of Aston in Birmingham and was supported by the Water Research Centre. Permission was obtained to make use of the facility.

The facility basically comprised three parallel channels, each 300m long, made up of two riffles 90m long and 1m wide alternate to two pool sections 60m long and 1.25m wide (fig. 4.1). Riffle sections were of 5‰ gradient producing a water velocity of 43cm s^{-1} for a flow of 52.7 l s^{-1} (1.0 x 10^6 gal day^{-1}) and had a substratum of gravel and pebbles ranging from 1.0-4.0 cm in diameter. Pool sections were of 0‰ gradient, having been allowed to accumulate silt naturally, and had a water velocity of 10 cm s^{-1} for a flow of 52.7 ls^{-1}. At the upper end of each stream a system of pumps and mixing chambers allowed good quality from the River Tean (chemical class 1A : STWA, 1979) to be mixed with a well-oxidised sewage effluent from the nearby Blithe Valley Water Reclamation Works. This was then fed into each channel to provide experimental stream A(0) with no effluent addition, stream B(25) with 25% effluent and 75% river water and C(50) with 50% effluent and 50% river water. At their lower ends channels discharged to the River Tean, downstream of main sewage works discharge.

4.3 Materials and methods

4.3.1 Physico-chemical sampling

Determinations were routinely carried out by the technical staff of the research station in their research program. Parameters measured and methods of measurement are included as table 4.1.

Fig. 4.1 Plan of the Checkley simulated stream ecosytem. (diagrammatic).

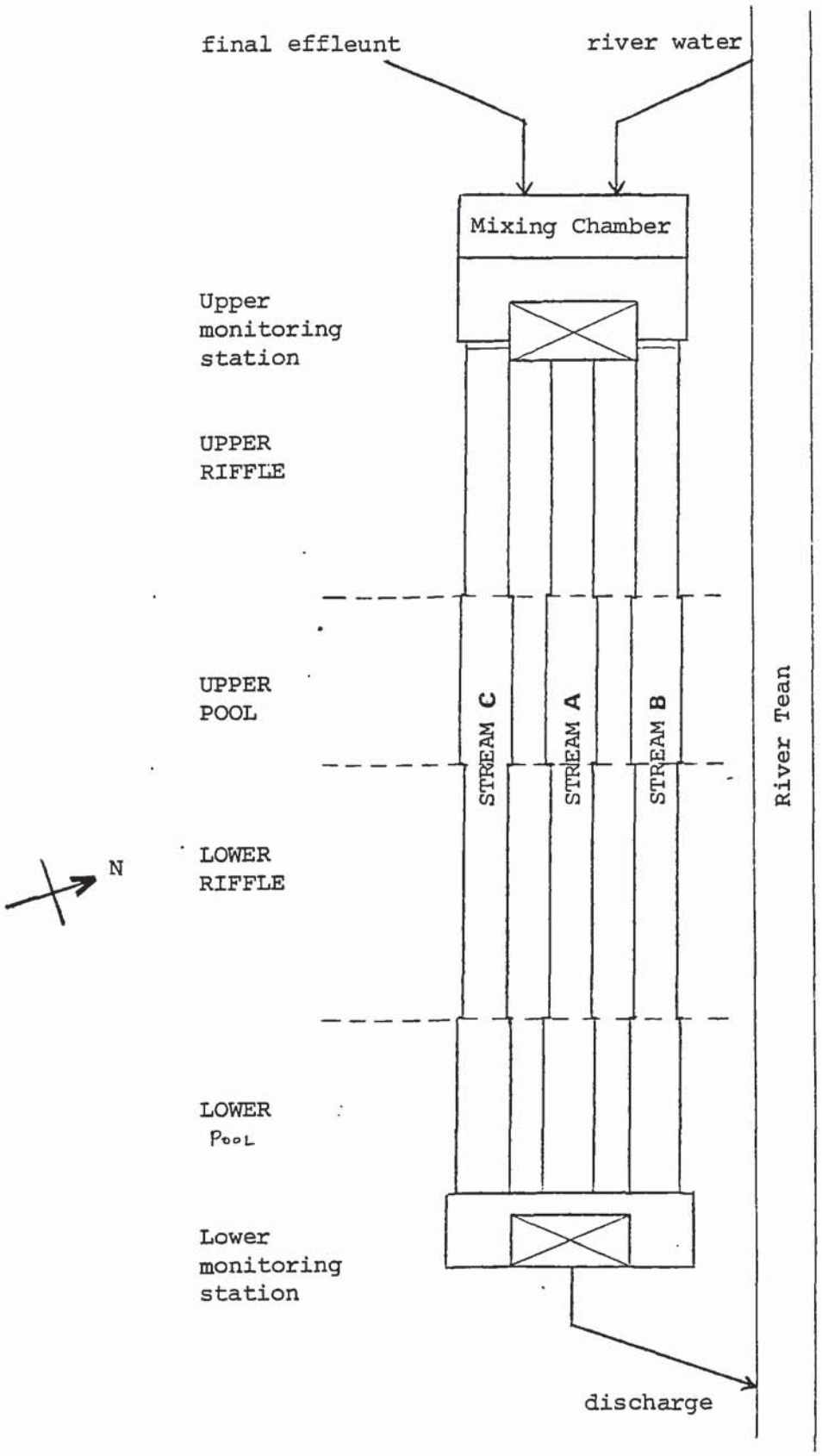


Table 4.1 Physico-chemical analyses carried out by the Checkley research team.

Parameter (Unit)	Method of taking sample	Method of sample analysis
Temperature (mgl^{-1})	Continuously measured by electrode	Model 505 Thermistor Cicuit Simac Inst. Ltd.
Suspended solids (mgl^{-1})	Manual sampling	DOE (1972)
Dissolved oxygen (mgl^{-1})	Continuously measured by electrode	Model 505 DO Meter Simac Inst. Ltd.
Chloride (mgl^{-1})	Manual sampling	DOE (1972)
Conductivity (μ S)	Manual sampling	Type MCl Mark III Bridge Electronic Switchgear Ltd.
pH	Continuously measured by electrode	Model 2836 pH Meter EIL
Total Alkalinity (mgl^{-1} as $CaCO_3$)	Manual sampling	DOE (1972)
Total Hardness (mgl^{-1} as $CaCO_3$)	Manual sampling	BDH Test - modified Schwarzenbach method
NH_3-N (mgl^{-1})	Manual sampling	Chapman *et al.* (1967) using Technicon Auto-Analyser
NO_3-N (mgl^{-1})	Manual sampling	Chapman *et al.* (1967) using Technicon Auto-Analyser
NO_2-N (mgl^{-1})	Manual sampling	Chapman *et al.* (1967) using Technicon Auto-Analyser
PO_4-P (mgl^{-1})	Manual sampling	Technicon Auto-Analyser industrial method 3-68W (1969) or 93-70W (1971)
Total/filterable Cd (mgl^{-1})	Manual sampling	250 ml water sample plus 5 ml atomic absorption grade HNO_3 (approx 70%, SG 1.42) reduced to dryness. Re-dissolved in 2 ml atomic absorption grade HCl (approx 36%. SG 1.18) and made up to 25 ml in volumetric flask. Aspirated directly to atomic absorption spectrophotometer (Model 306 Perkin-Elmer Ltd., Bucks). Soluble metals determined after passing through Whatman GF/C and Oxoid 450 nm filters.
Cr (mgl^{-1})	Manual sampling	
Cu (mgl^{-1})	Manual sampling	
Fe (mgl^{-1})	Manual sampling	
Ni (mgl^{-1})	Manual sampling	
Pb (mgl^{-1})	Manual sampling	
Zn (mgl^{-1})	Manual sampling	
Total Radiation	Continuously measured	Solarimeter with analogue output. Kipp and Zonen Ltd., Delft, Netherlands.
Maximum Radiation	Continuously measured	
Free CO_2 (mgl^{-1})	Manual sampling	DOE (1972)

Water samples were returned to the laboratory in plastic screw-top containers, except dissolved oxygen (Do) samples which were collected in 250 ml glass DO bottles and samples for ammonia, nitrate, nitrite and phosphate analysis which were taken in glass universal bottles to which six drops of concentrated hydrochloric acid (SG 1.16) were added from a Pasteur pipette to arrest biological activity.

4.3.2 Biological sampling

After consideration of the relevant literature (see section 3.2.1.1) it was felt best to use a small-scale cylinder sampler for sampling the riffle sites : such a sampler was constructed of polyethylene and metal, with a $0.004m^2$ sampling area (about the size of a 'standard' petri-plate lid). The sampler was easily driven into the uniformly small stones comprising the substratum of the riffle sections, and all of the attached flora (excluding encrusting forms) could be removed by hand and collected. After preliminary trials using an Ekman grab sampler (Hydro-bios, West Germany) to sample pool sites it was found that the grab frequently failed to completely sever filaments of filamentous algae such as *Cladophora*. Such filaments were brought up hanging from the jaws of the grab, resulting in a major disturbance of the surrounding flora. The $0.004m^2$ cylinder sampler was thus also adopted for sampling pool sites, though the thick masses of algae and dense growths of vascular hydrophytes (particularly *Potamogeton crispus*) made such operation difficult and likely introduced a greater source of sampling error at these sites.

Sampling was carried out at monthly intervals, taking 9 samples per riffle or pool i.e. 109 samples/month. The choice of such sampling frequency was largely determined by operational constraints. However the data from sampling the first month was used to provide an estimate of the numbers of samples required to obtain a specified degree of precision. As described in Elliott (1977) and Southwood (1966)

$$\eta \simeq \frac{s^2}{D^2 \bar{x}^2}$$

where η = number of sampling units required
s^2 = variance
$\bar{x}$ = arithmetic mean

D^2 = index of precision (ratio of standard error to arithmetic mean)

For a standard error of 20% of the mean (considered by Elliott (1977) to be a reasonable error in most bottom samplers)

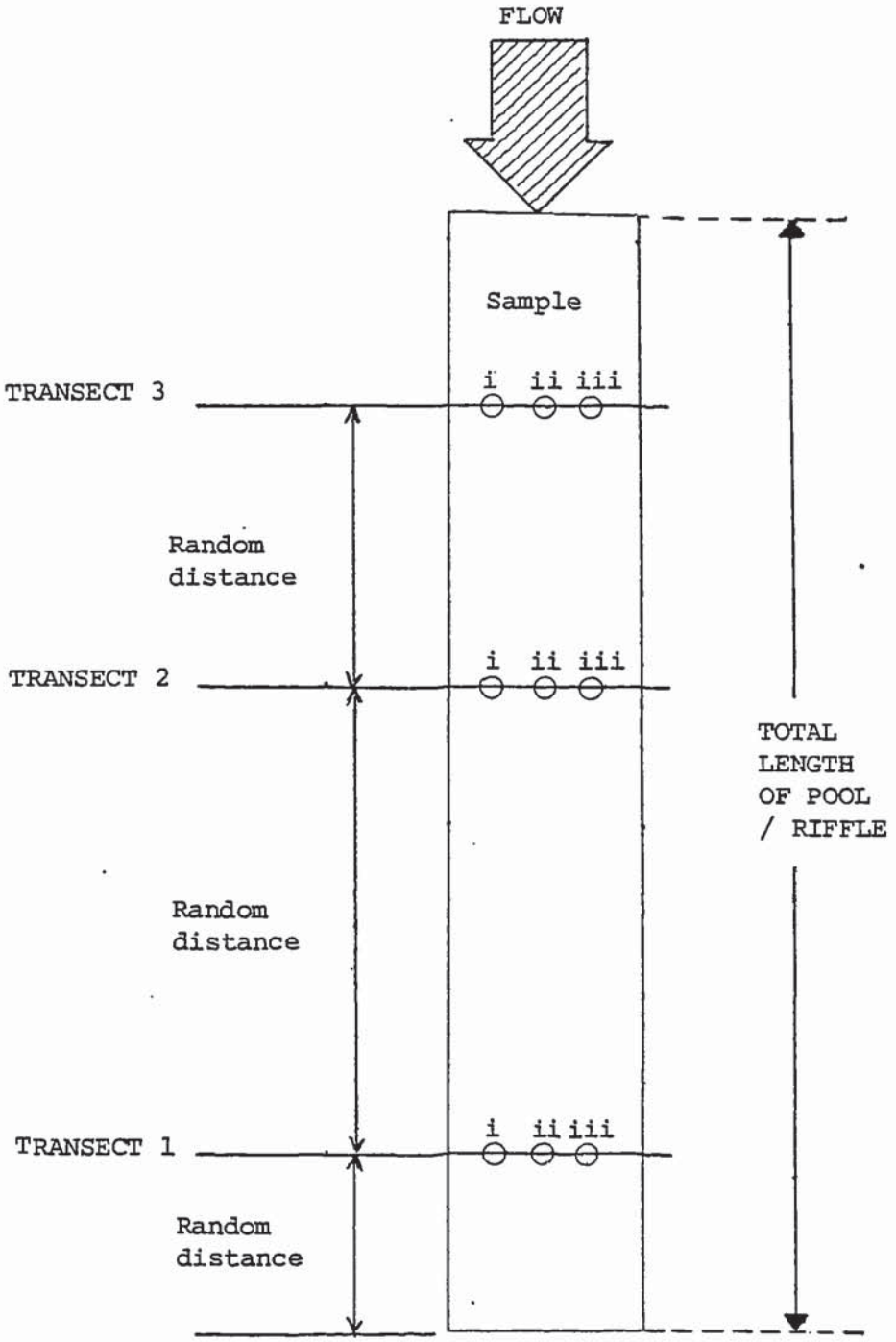
$$\eta \simeq \frac{25s^2}{\bar{x}^2}$$

When applied to the experimental data values of η were found to range from 4 to 255 (for different taxa and different sites) with a mean value of η of 95, indicating that the 9 samples used (per month, per site) were too few. However most of the quantitative analysis from the study was analysed by analysis of variance in a factorial design, providing 24 and 192 degrees of freedom in the main and sub-plot error terms respectively. This was considered to be quite adequate in such a study and sampling frequency was unaltered for the rest of the study.

Using the 0.004m^2 cylinder sampler sampling proceeded upstream so that streamers cut free by the sampler did not interfere with subsequent samples. Samples were taken in groups along three transect lines, the position of each transect up the pool or riffle being determined by random numbers. Along each treatment three 0.004m^2 samples were taken at a distance equidistant from each other and from the stream banks, as shown in fig. 4.2. Each sample was thus located according to stream (A(0), B(25) or C(50)), site (lower pool, lower riffle, upper pool or upper riffle), transect (1, 2 or 3) and position along transect (i, ii or iii). For most purposes such sampling could be described as random. Samples were returned to the laboratory in sealed plastic bags and stored in a refrigerator at 5^{o}C. Sample processing was carried out as soon as possible, since samples were not preserved.

Processing of the samples involved initially washing the material in a sieve (250 µm mesh) under a fine jet of tap water, followed by manual dissection to remove macro-invertebrates (e.g. *Asellus aquaticus* and *Gammarus pulex*) and inorganic material. It was, however, realised that this cleaning procedure would result in the loss of many of the

Fig. 4.2. Positioning of cylinder sampler in pool or riffle (not to scale).



unicellular algae, limiting this study to macro-algal species and the so called macrophytes. Many environmental surveys have estimated total filamentous algal biomass, assuming this to totally comprise *Cladophora*. Preliminary investigations at Checkley, however, revealed the alga to be associated with several other species. Blum (1957) in a study of algae of the Saline River found it easy to separate macro-algal species and dry them separately. In this study, however taxa could only be identified with the aid of a low power dissecting microscope and it was impracticable to manually separate filaments of one species from another. Inevitably a semi-quantitative compromise was made. After cleaning, each sample was placed in a petri-plate, immersed in tap water and teased apart until uniformly dense. Then using a binocular inverted microscope (inverted Lynx microscope, Gillett and Sibert Ltd., London) and/or low power dissecting microscope (Swift 'Stereo 80', Swift Instruments Ltd.) taxa present were identified to the generic or specific level and an estimation made as to the percentage of the sample biomass attributable to each species present. Initially such estimations were made to the nearest 5%; it soon became evident, however, that accuracy to better than 10% was unlikely. The sample was then removed, dried to constant weight at 105°C and ashed to constant weight at 500°C. The volatile solids content (ash-free dry weight) was used in preference to dry weight as an index of total sample biomass since it excluded inorganic contamination. The value for ash-free dry weight was multiplied by 250 (1/0.004) to convert to a one metre sample area, and multiplied by the percentage composition of each taxon present in the sample, to obtain a estimate of biomass for each taxon in gm^{-2} ash-free dry weight. Taxa present in a sample in such small amounts as to be estimated to make up less than 5% of the total biomass were recorded as present by the letter 'P'. Whilst samples comprised only filamentous algal taxa it was felt that such a technique for estimating biomass was quite adequate. The occurrence of vascular hydrophytes such as *Potamogeton*, *Callitriche* and *Elodea* and the bryophyte *Amblystegium* , however, with very different morphology, complicated matters somewhat. It was reasoned that such taxa could not simply be ignored since their presence in a sample could help to explain the absence of other taxa. Estimation of percentage composition, however, was very difficult in samples containing taxa of different morphology and likely resulted in reduced accuracy in estimations. It was fortuitous, therefore, that samples containing *Potamogeton* tended

to contain little or no filamentous algal material, whilst *Callitriche* and *Amblystegium* were only found in small quantities anyway, and it was probably the occurrence of *Elodea* in samples also containing filamentous algae which resulted in the greatest error. One further problem arose from the presence of unidentified plant materials, often decaying and often terrestrial in origin, found especially in samples from pool sites. This material being organic would contribute to the total biomass of a sample and would also tend to prevent floral colonization by 'competing' for space and by shading the benthos : its inclusion was thus felt justified under the 'other macrophytes' category, though the reduction in the precision of the technique owing to the inclusion of such material was realised.

4.4 Results and discussion

The research team at Checkley, as an integral part of their research program, processed physico-chemical data to obtain percentiles. The calculation of percentiles, however, requires a large number of data values which were only collected during periods of experimentation in their program. Percentile values are included as table 4.2. To provide a more complete picture of the fluctuation in physico-chemical variables over the period January-September 1980, raw physico-chemical data were processed to obtain values of the mean and of the variability of the data (appendix 1a). Mean values for the various parameters are presented as fig. 4.3. A list of floral taxa found in the survey is included as fig. 4.4 and the raw quantitative biological data are included as appendix 1b.

4.4.1 Biomass

Data collected from field surveys are rarely in a form suitable for analysis by parametric statistical procedures such as t-tests and analysis of variance (AOV). These tests depend upon one or more assumptions being met concerning the nature of the observations i.e.

1. The data is assumed to be normally distributed.
2. The variance of the sample must be independent of the mean.
3. The components of the variance should be additive.

Initial treatment of the biological data from Checkley showed that for

Table 4.2. Physico-chemical data for the Checkley streams (FEBRUARY - MARCH 1980).
Data courtesy of Checkley Research Team.

VARIABLE (mgl^{-1} unless otherwise stated)	PERCENTILE	STREAM		
		A(0)	B(25)	C(50)
Temperature (^{o}C)	5	3.0	4.0	4.3
	50	4.4	5.4	5.5
	95	5.9	6.9	6.8
Suspended solids	5	3	1	2
	50	9	12	14
	95	89	49	31
D.O	5	10.2	8.2	8.2
	50	12.1	11.5	10.4
	95	13.3	12.6	11.8
Conductivity (µS)	5	275	370	400
	50	440	550	625
	95	500	655	780
pH	5	7.6	7.3	7.1
	50	7.9	7.7	7.2
	95	8.0	7.8	7.5
Total Alkalinity (as $CaCo_3$)	5	90	90	110
	50	130	150	160
	95	150	180	190
Total Hardness (as $CaCo_3$)	5	64	64	60
	50	230	249	260
	95	270	296	308
NH_3-N	5	0.3	0.5	0.8
	50	0.5	1.4	1.7
	95	1.2	2.9	4.1
NO_3-N	5	3.0	4.0	5.0
	50	3.4	5.9	6.9
	95	4.4	7.0	8.1
Filterable Cadmium	5	0.001	0.003	0.004
	50	0.002	0.006	0.007
	95	0.004	0.009	0.014
Filterable Chromium	5	0.001	0.005	0.007
	50	0.003	0.013	0.019
	95	0.005	0.045	0.086
Filterable Copper	5	0.002	0.005	0.012
	50	0.005	0.014	0.018
	95	0.011	0.030	0.032
Filterable Lead	5	0.005	0.020	0.036
	50	0.021	0.048	0.060
	95	0.035	0.108	0.105
Filterable Nickel	5	0.005	0.008	0.010
	50	0.010	0.012	0.014
	95	0.016	0.020	0.019
Filterable Zinc	5	0.015	0.066	0.100
	50	0.030	0.100	0.138
	95	0.058	0.162	0.190

Fig. 4.3. Mean monthly values of physico-chemical variables for Checkley study.

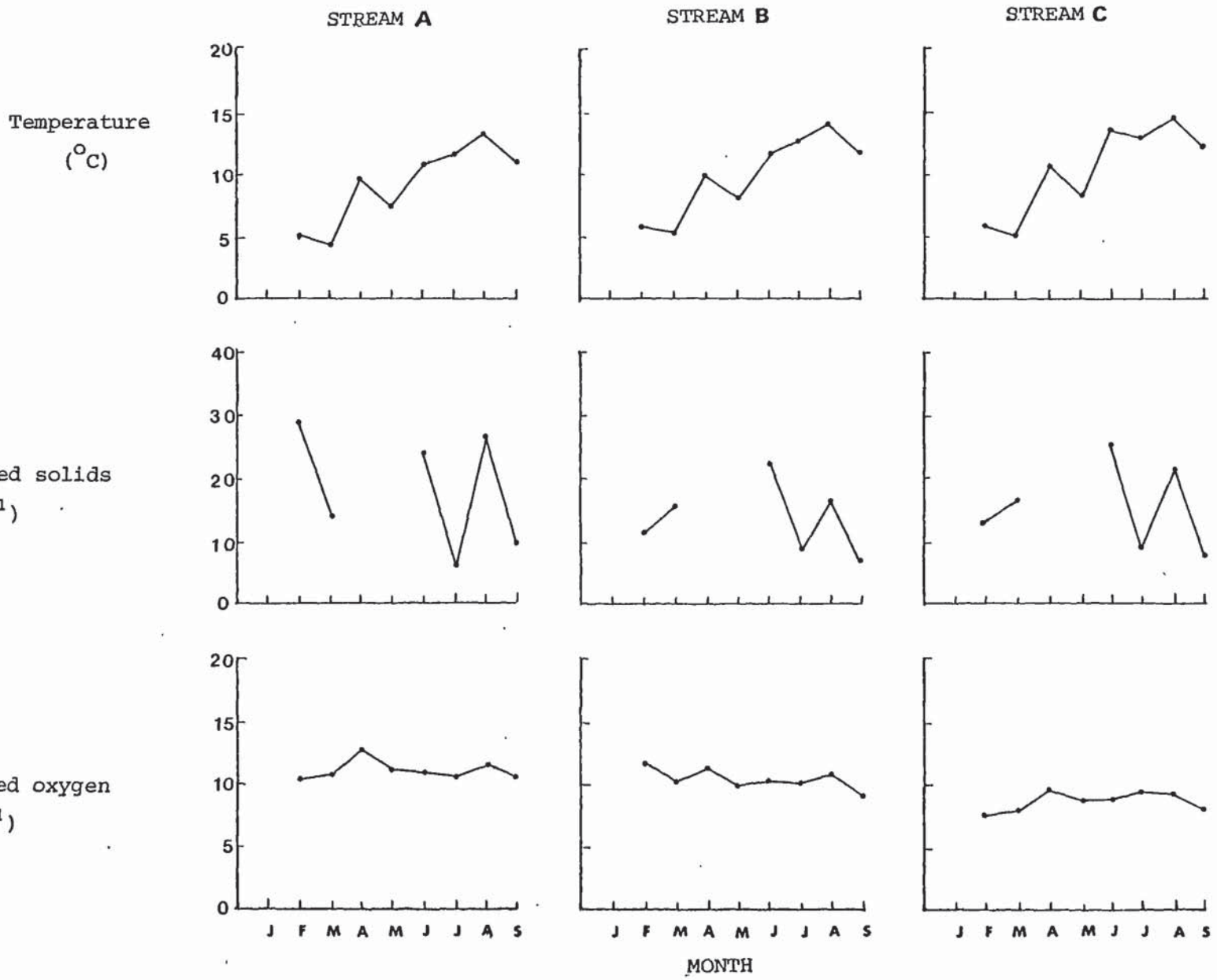


Fig. 4.3. Continued

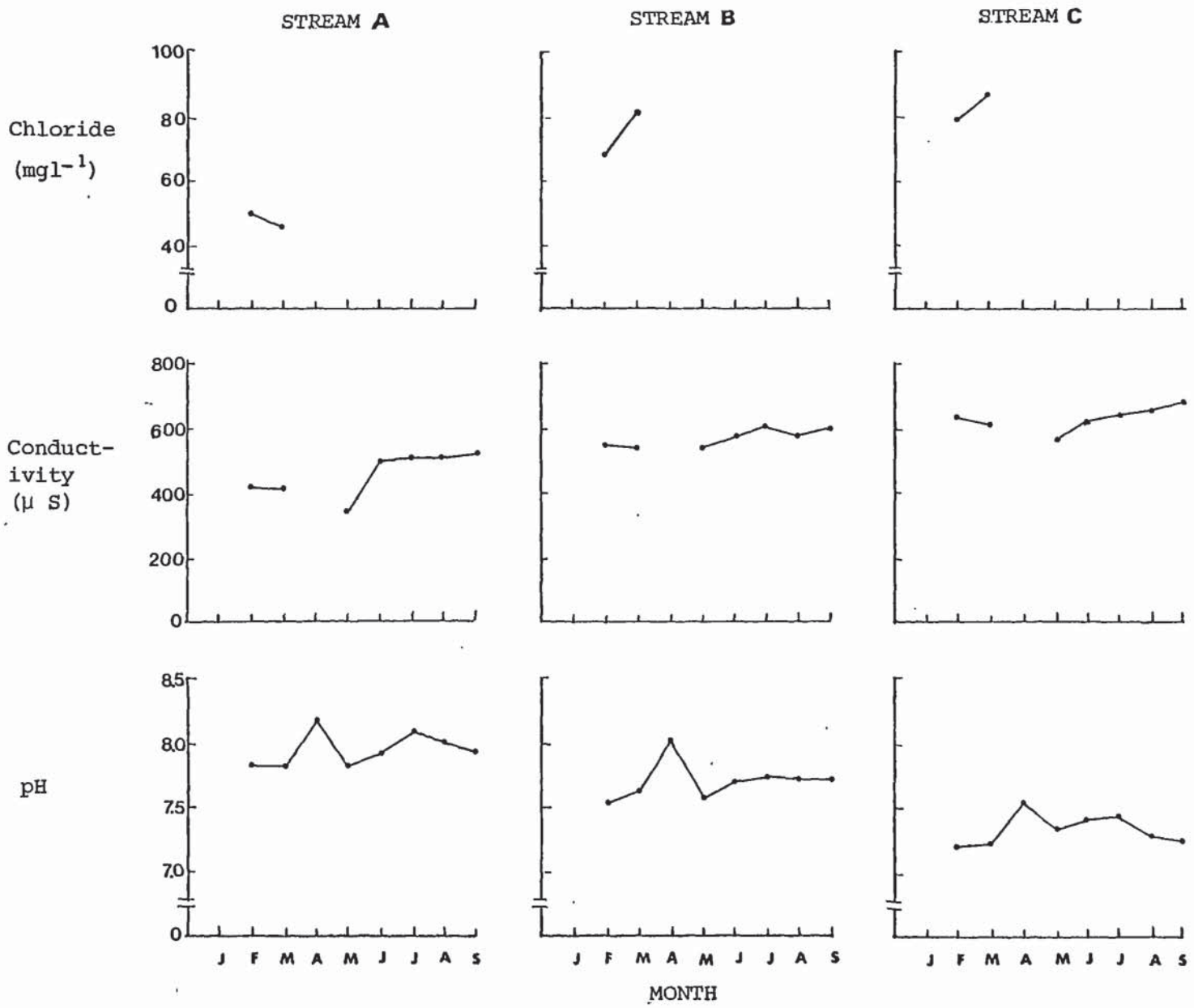


Fig. 4.3. Continued.

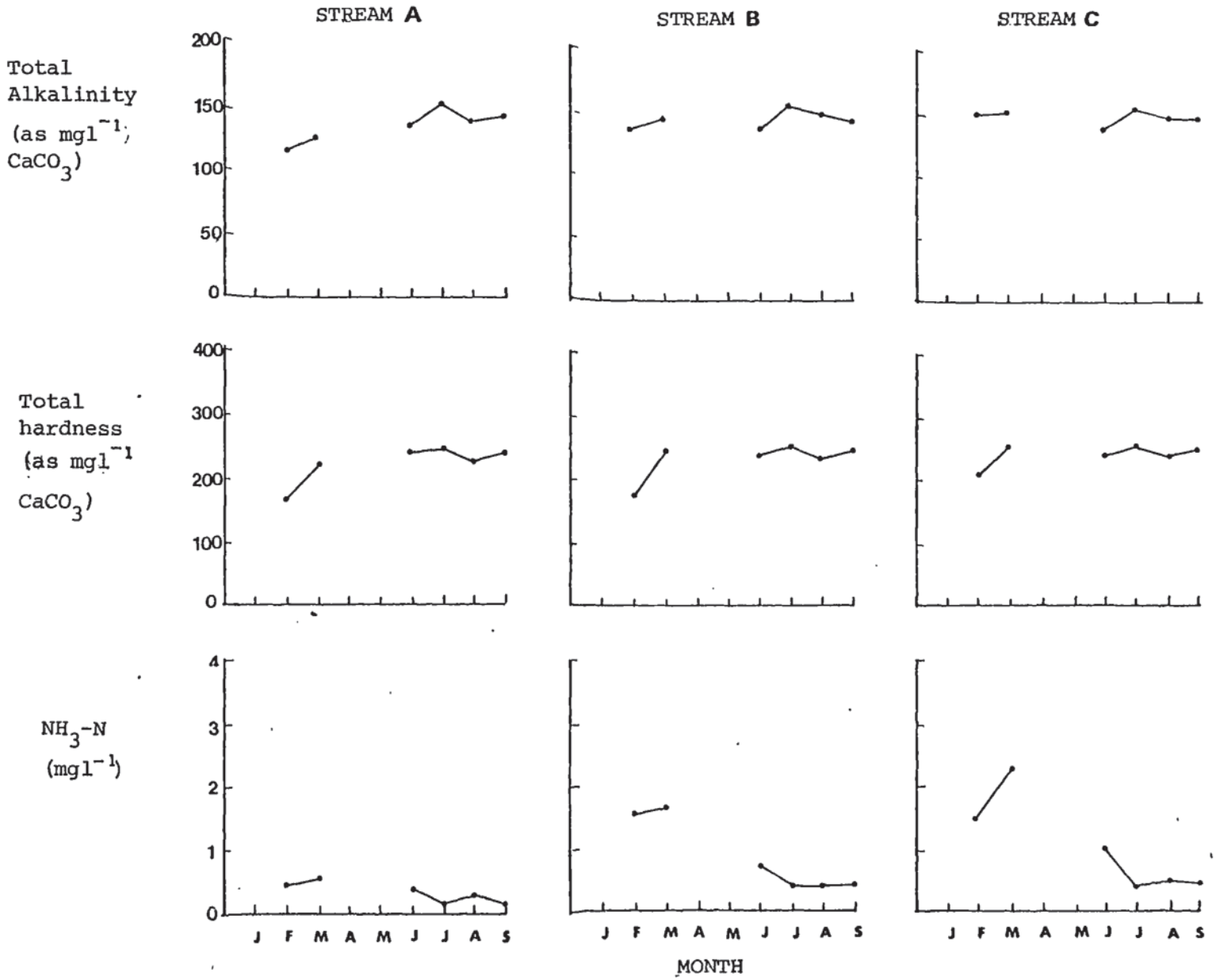


Fig. 4.3. Continued

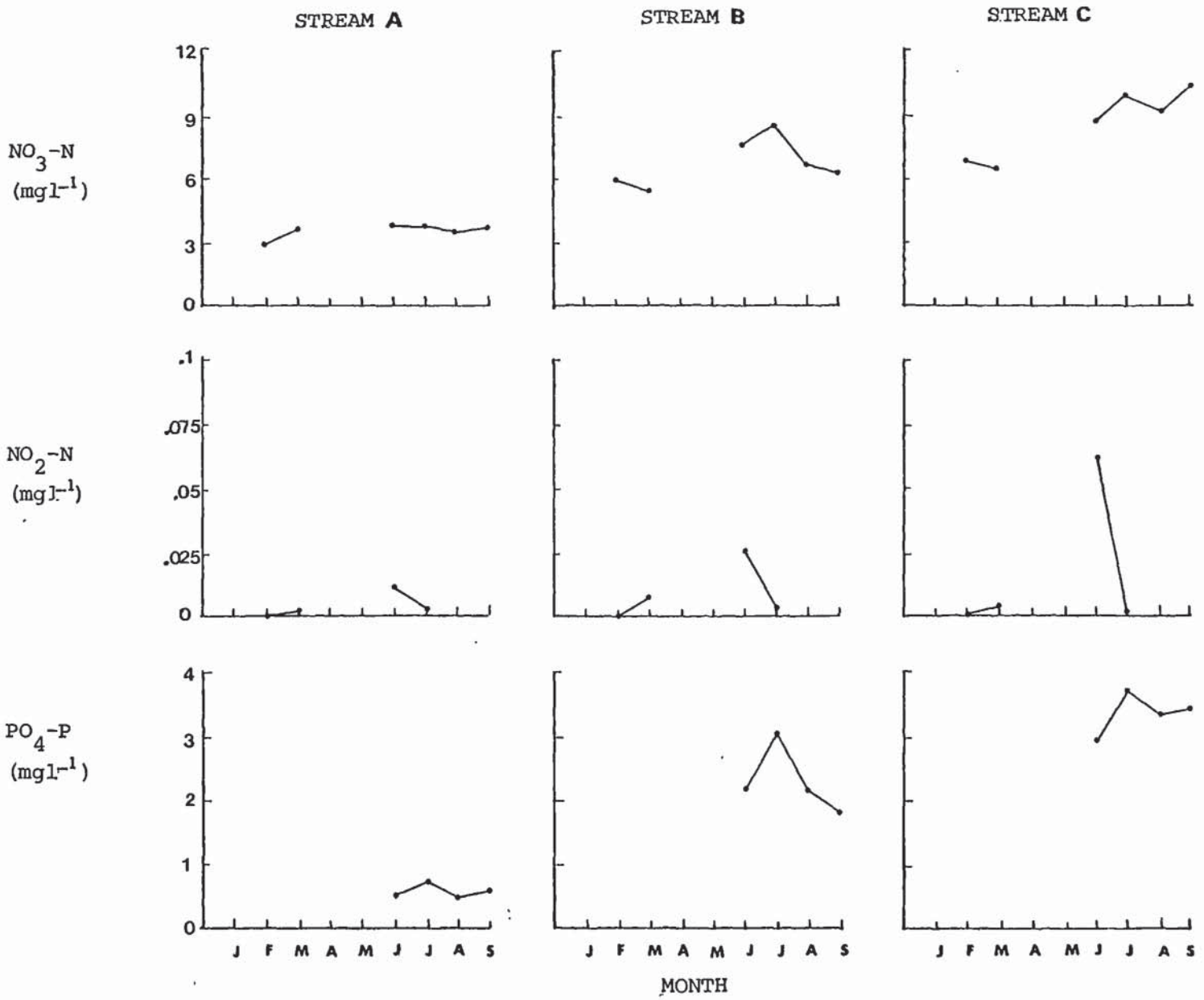


Fig. 4.3. Continued

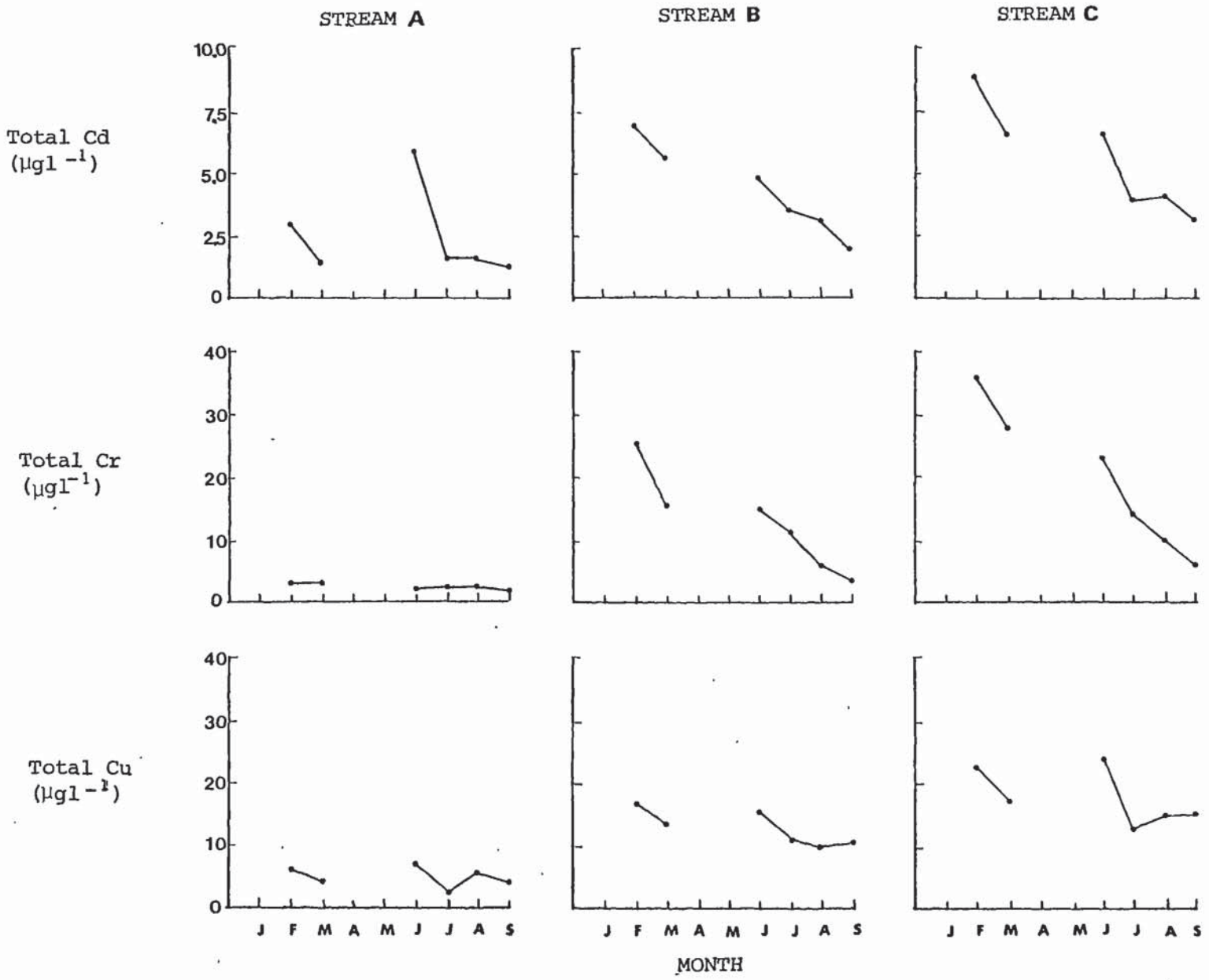


Fig. 4.3. Continued

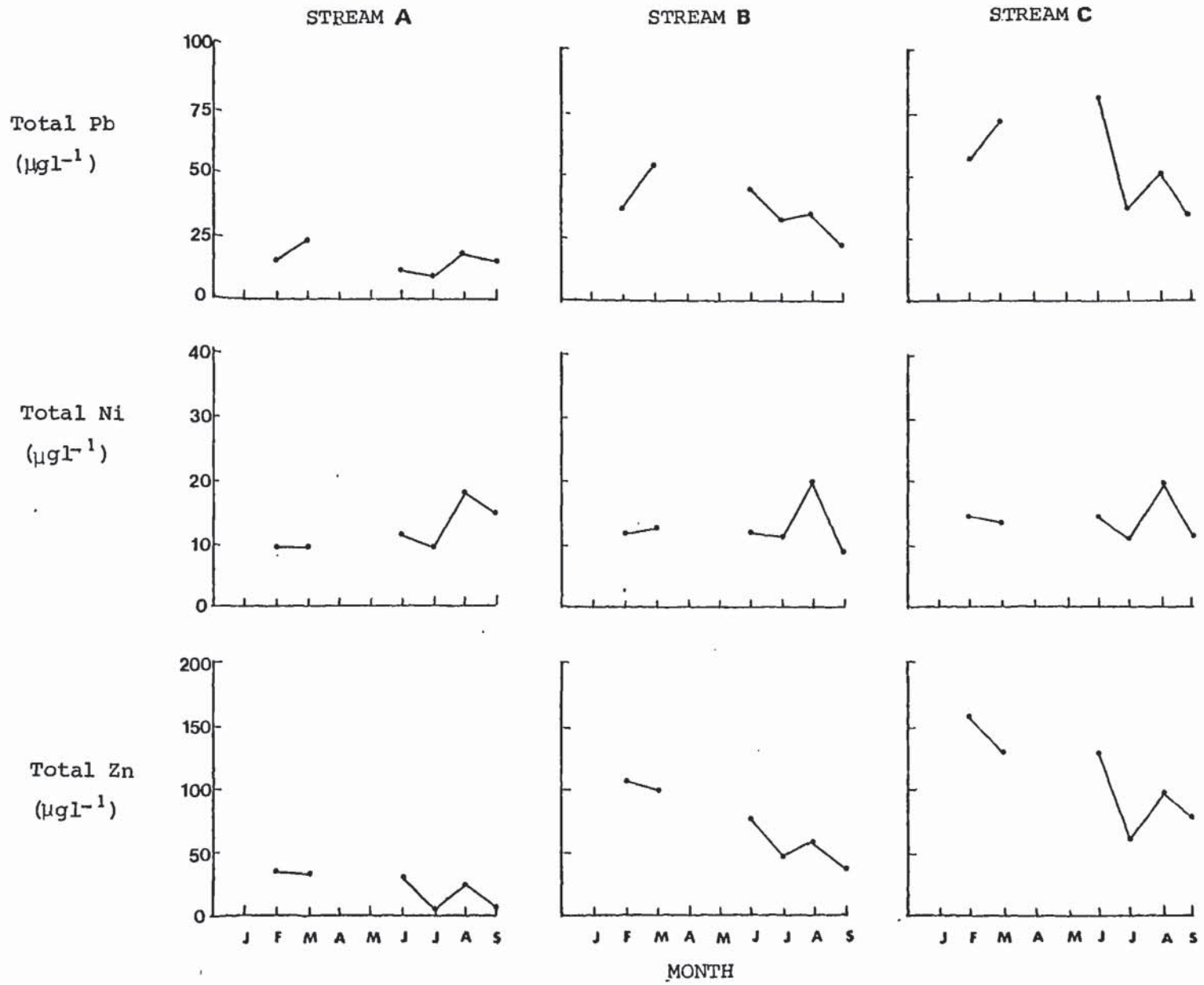


Fig. 4.3. Continued

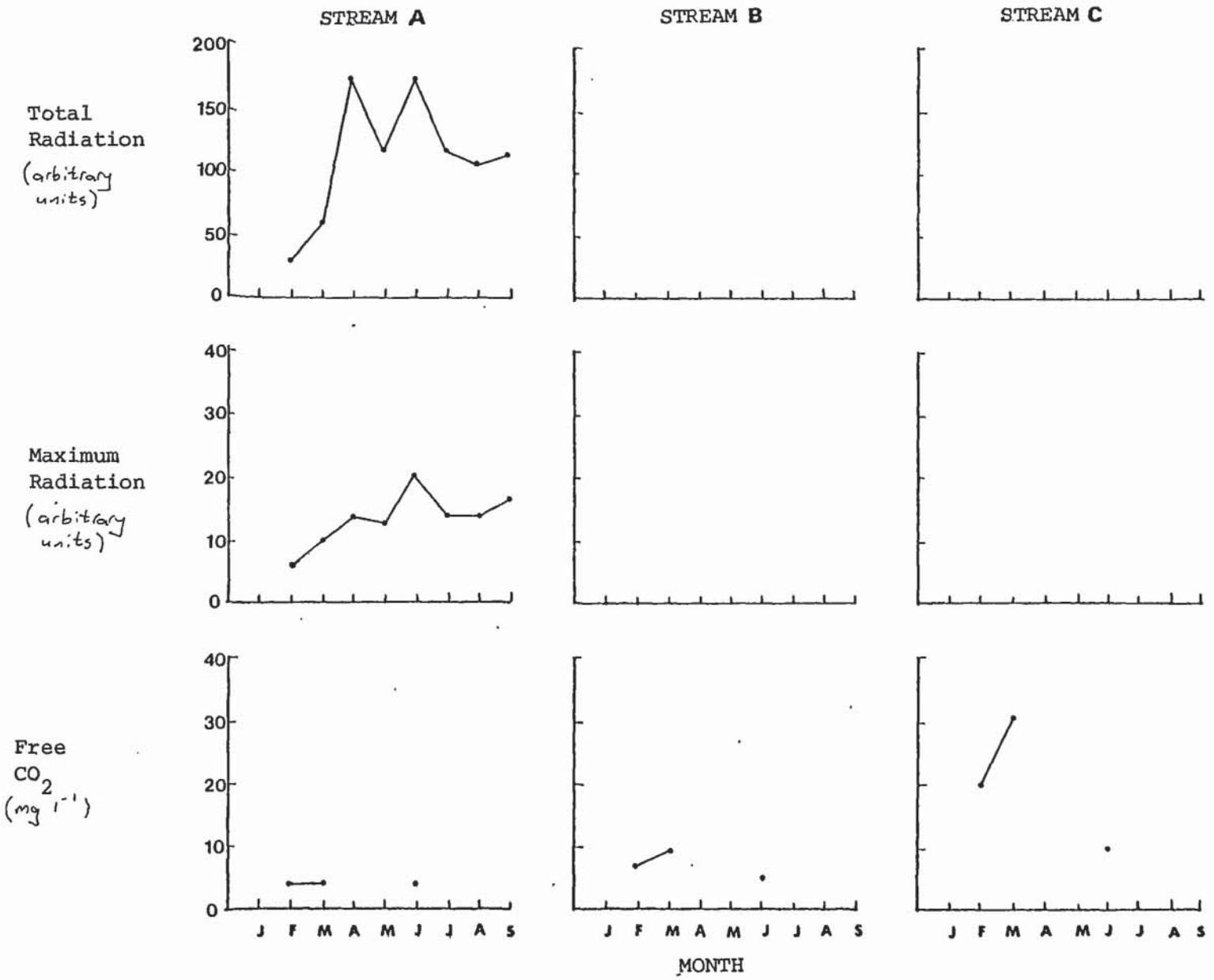


Fig. 4.4 Floral list for Checkley study.

Cladophora glomerata (L.) Kutz.

Vaucheria sp.

Stigeoclonium tenue Kutz.

Oedogonium sp.

Microspora sp.

Spirogyra sp.

Batrachospermum sp.

Rhodochorton sp.

Amblystegium sp.

Elodea canadensis Michx.

Potamogeton crispus L.

Callitriche stagnalis Scop.

Ranunculus sp.

all species the variance exceeded the mean ($s^2 > \bar{x}$) (averaged over all times and sites) indicating some degree of contagion in the data. The volatile solids (ash-free dry weight) data however showed the variance and mean to be approximately equal ($s^2 \simeq \bar{x}$). Unfortunately data which is contagiously distributed tends to involve the variance being dependent on the mean and parametric statistical tests cannot be applied without the risk of considerable errors. Elliott (1977) points out that the difficulty may be overcome by replacing each count by a suitable mathematical function of the count; whilst Quenouille (1966) adds that such transformations also serve to make the distribution more nearly normal so that parametric tests may be applied without any difficulty. To aid in choosing appropriate transformations Taylor's Power Law (Taylor 1961, 1971) was carried out as described in Elliott (1977).

Taylor's Power Law states that the population variance (σ^2) is proportional to a fractional power of the arithmetic mean (μ).

$$\text{i.e.} \qquad \sigma^2 = \log a + b \log \mu$$

Values of a and b may be calculated from regression of log s^2 on log $\bar{x}$. The appropriate transformation is then to replace x with x^{ρ}

$$\text{where} \quad \rho = 1 - \frac{b}{2}$$

Calculated values are included as table 4.3. Having calculated values of ρ Elliott (1977) advocated reading transformed counts from the tables of Healy and Taylor (1962). It was felt, however, that since all but one of the ρ values were close to zero a logarithmic transformation (x^0) would suffice for all taxa, and since the data contained zero values a log (x + 1) transformation was adopted.

Transformed data were analysed in a 3 x 9 factorial split-plot analysis of variance, having 3 main plot treatments (stream A(0), B(25) and C(50)), 9 sub-plot treatments (the months January to September inclusive) and their interaction. A self-written computer program for the Hewlett-Packard HP2000 computer at the University of Aston in Birmingham aided analysis. This program is included in appendix 2 as program SPAN. As well as performing conventional analysis this program carried out two mutally orthogonal contrasts, partitioning the main plot

Table 4.3 Taylor's Power Law calculations for taxa present in the Checkley streams.

Taxon	$\sigma^2 = a\mu^b$	Number of values n	Correlation coefficient r	significance	$p (= 1 - \frac{b}{2})$
Cladophora	$\sigma^2 = 5.9\mu^{1.5}$	75	0.95	***	0.2
Vaucheria	$\sigma^2 = 5.3\mu^{1.9}$	19	0.99	***	0.0
Stigeoclonium	-	0	-	N.S.	-
Oedogonium	$\sigma^2 = 3.9\mu^{1.7}$	29	0.97	***	0.2
Microspora	-	0	-	N.S.	-
Spirogyra	-	0	-	N.S.	-
Batrachospermum	-	0	-	N.S.	-
Rhodochorton	-	0	-	N.S	-
Amblystegium	$\sigma^2 = 6.5\mu^{1.9}$	32	0.98	***	0.0
Elodea	$\sigma^2 = 6.8\mu^{1.7}$	17	0.99	***	0.1
Potamogeton	$\sigma^2 = 26.0\mu^{1.2}$	26	0.93	***	0.4
Callitriche	$\sigma^2 = 8.9\mu^{1.9}$	3	1.00	***	0.1
Ranunculus	-	0	-	N.S.	-
'Other Macrophytes'	$\sigma^2 = 5.9\mu^{1.8}$	53	0.98	***	0.1
Total Biomass (volatile solids)	$\sigma^2 = 0.4\mu^{1.6}$	99	0.96	***	0.2

LEGEND

N.S. = Not significant (P>0.05)
*** = Significant (P<0.001)

sum of squares to compare stream A(0) against the mean of B(25) and C(50), and compare stream B(25) against C(50) : the former contrast testing the null hypothesis that treatment (i.e. the addition of well oxidised sewage effluent) had no effect on growth, the latter testing the null hypothesis that amount of treatment had no effect on growth. These effects were averaged over the nine months and were performed for each taxon present at each site (i.e. upper and lower riffle and pool). The AOV summary tables resulting from these analyses are included as appendix 3; whilst table 4.4 summarises the significance of the effects.

When an analysis has been carried out using transformed data there is usually some difficulty concerning the presentation of results Quenouille (1966). In this case mean values of transformed data (on a log(x + 1) scale) were found to be rather difficult to interpret (fig. 4.5b). Transformation of these values back to the original scale by taking antilogarithms and subtracting one allowed derived mean values to be calculated (fig. 4.5c). Generally, however, these were found to be lower than the untransformed data for which they were calculated. As described in Quenouille (1966) the addition of 1.15 times the variance of each set of observations to the appropriate mean before transformation back to the original scale allows corrected derived means to be calculated (fig. 4.5d). When such calculations were performed, however, it was found that in somes cases the additive effect of the variance prior to re-transformation resulted in many corrected desired mean values being larger than any of the data from which they were calculated. It was felt, therefore, that biomass data was best presented as transformed values on a log (x + 1) axis. This had the additional advantage that the variability was symmetrical about the mean. Fig. 4.6 shows the transformed data for each species.

The findings of the quantitative biomass study are thus summarised in fig. 4.6 and table 4.4 with accompanying physico-chemical data summarised in fig. 4.3. The spatial and temporal differences in the distribution of the macro-algae and macrophytes seem best discussed in turn. The term 'significant' is only used if an analysis has been carried out and yielded a result with ($P \leqslant 0.05$) unless otherwise stated.

Visual inspection of fig. 4.6 reveals the pool sections of streams B(25) and C(50) to be dominated by the alga *Cladophora*, whilst in

Table 4.4 Summary of significance of component effects of analysis of variance for biological data from Checkley study.

TAXON	SITE	SIGNIFICANCE				
		Main Treatment (i.e. Stream)	Av(B+C)/2	BvC	Sub Plot Treatment (i.e. Time)	Main/Sub Plot Interaction
Cladophora	Upper Riffle	***	***	**	***	*
"	Lower Riffle	***	***	N.S.	***	***
"	Upper Pool	***	***	N.S.	***	***
"	Lower Pool	***	***	**	**	***
Vaucheria	Upper Riffle	*	**	N.S.	***	*
"	Lower Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Upper Pool	*	*	N.S.	***	*
"	Lower Pool	N.S.	N.S.	N.S.	*	**
Oedognium	Upper Riffle	***	**	***	***	***
"	Lower Riffle	***	***	N.S.	***	***
"	Upper Pool	**	***	N.S.	***	***
"	Lower Pool	***	***	N.S.	***	***
Microspora	Upper Pool	N.S.	N.S.	N.S.	N.S.	N.S.
Batrachospermum	Lower Pool	N.S.	N.S.	N.S.	N.S.	N.S.
Amblystegium	Upper Riffle	**	**	N.S.	N.S.	N.S.
"	Lower Riffle	**	*	**	*	N.S.
"	Upper Pool	N.S.	N.S.	N.S.	N.S.	N.S.
"	Lower Pool	*	N.S.	*	*	*
Elodea	Upper Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Lower Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Upper Pool	***	***	***	***	***
"	Lower Pool	***	*	***	*	*
Potamogeton	Upper Riffle	N.S.	*	N.S.	*	*
"	Lower Riffle	*	*	N.S.	***	***
"	Upper Pool	***	***	N.S.	**	***
"	Lower Riffle	***	***	N.S.	***	***
Callitriche	Lower Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Upper Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Lower Pool	N.S.	N.S.	N.S.	*	**
'Other Macrophytes'	Upper Riffle	N.S.	N.S.	N.S.	N.S.	N.S.
"	Lower Riffle	N.S.	*	N.S.	N.S.	N.S.
"	Upper Pool	***	*	***	***	*
"	Lower Pool	**	***	N.S.	***	***
Volatile soilds	Upper Riffle	**	**	N.S.	***	*
"	Lower Riffle	**	***	N.S.	***	*
"	Upper Pool	N.S.	N.S.	N.S.	N.S.	**
"	Lower Pool	**	**	N.S.	N.S.	***

LEGEND

N.S. = Not significant (P>0.05)
* = Significant (P<0.05)
** = Significant (P<0.01)
*** = Significant (P<0.001)

Fig. 4.5. Mean values for typical biomass data from Checkley study (*Cladophora* biomass - stream B, lower pool).

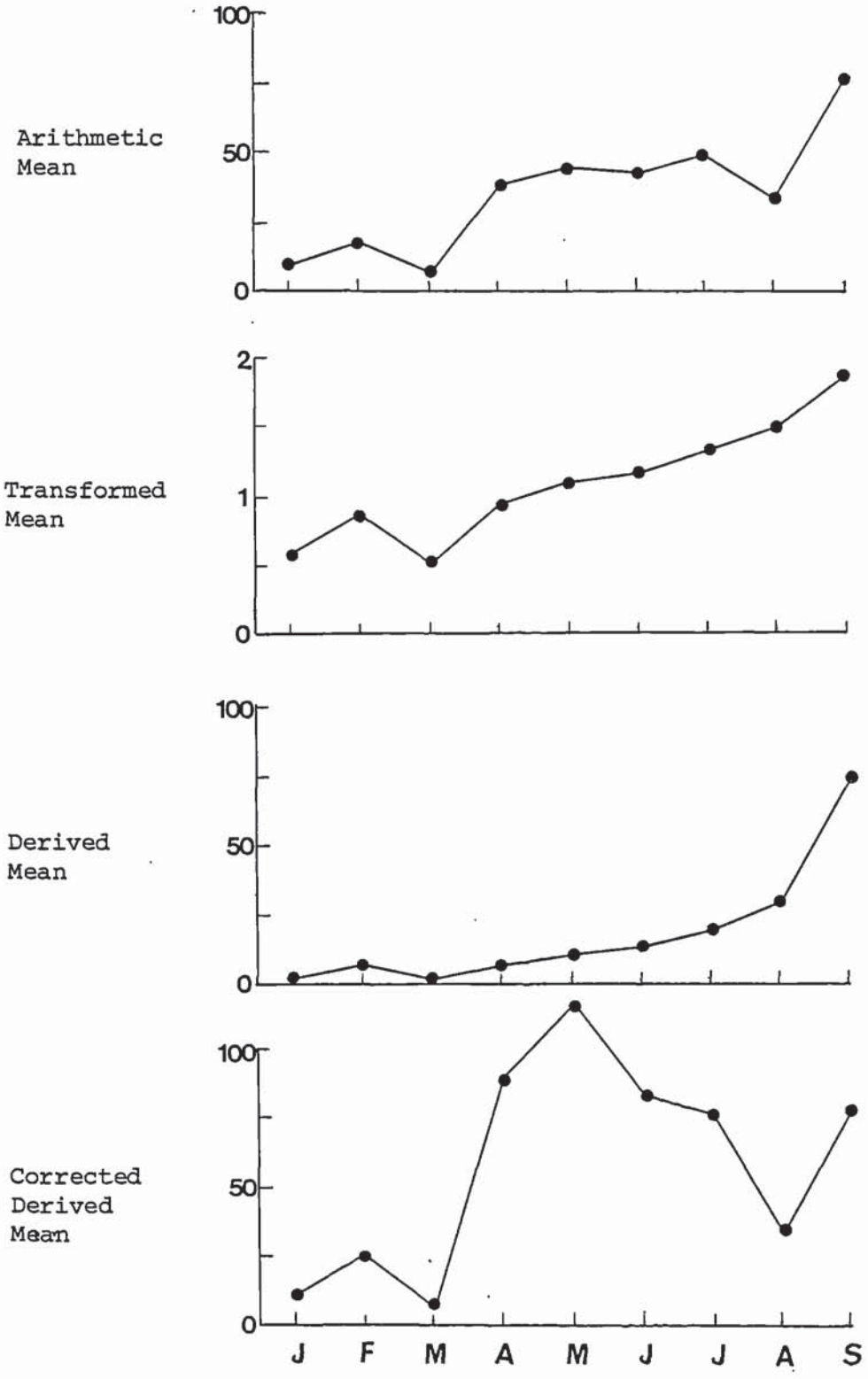


Fig. 4.6. Mean transformed biomass for taxa found in Checkley study. (vertical bar = standard error).

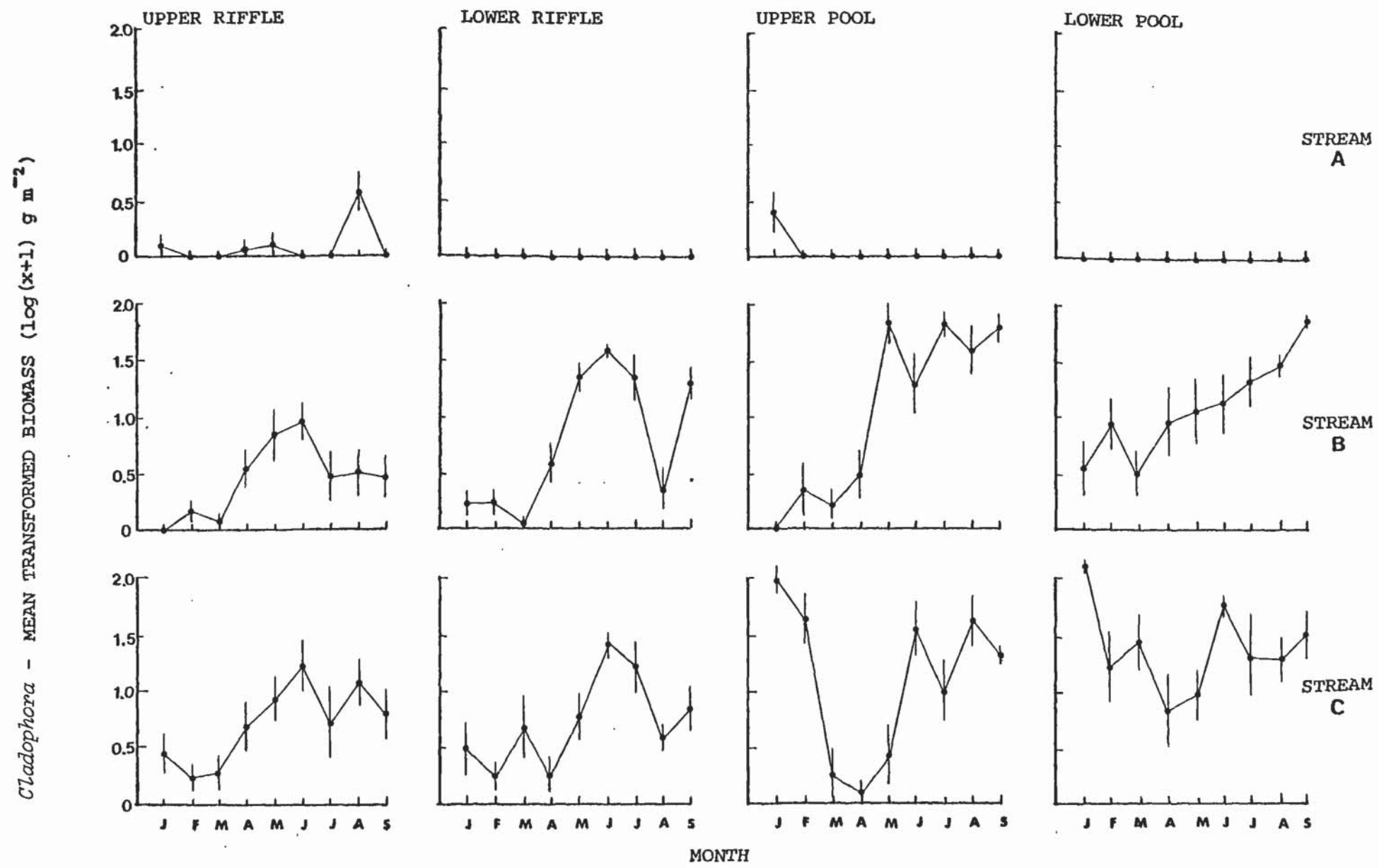


Fig. 4.6. Contined

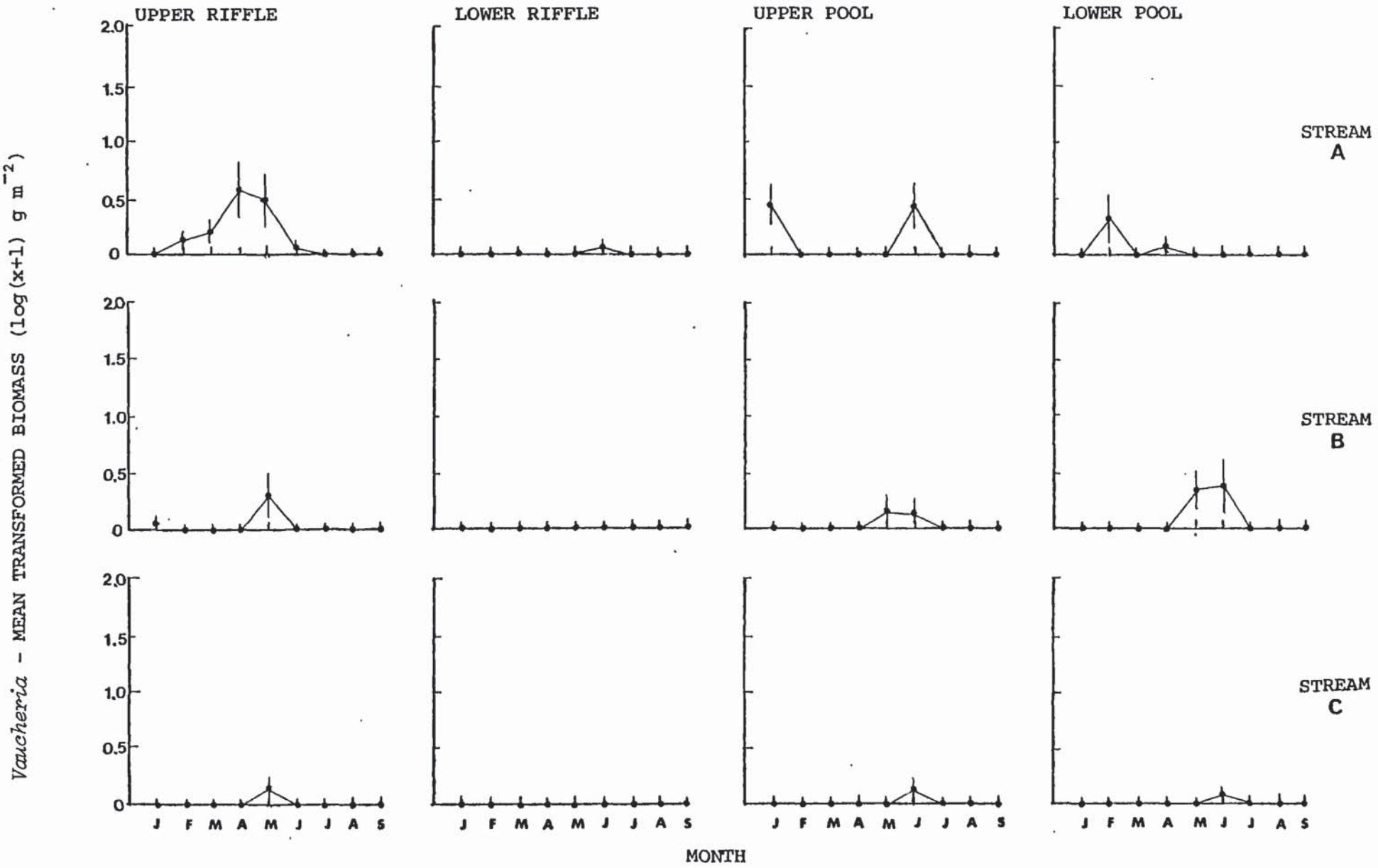


Fig. 4.6. Continued

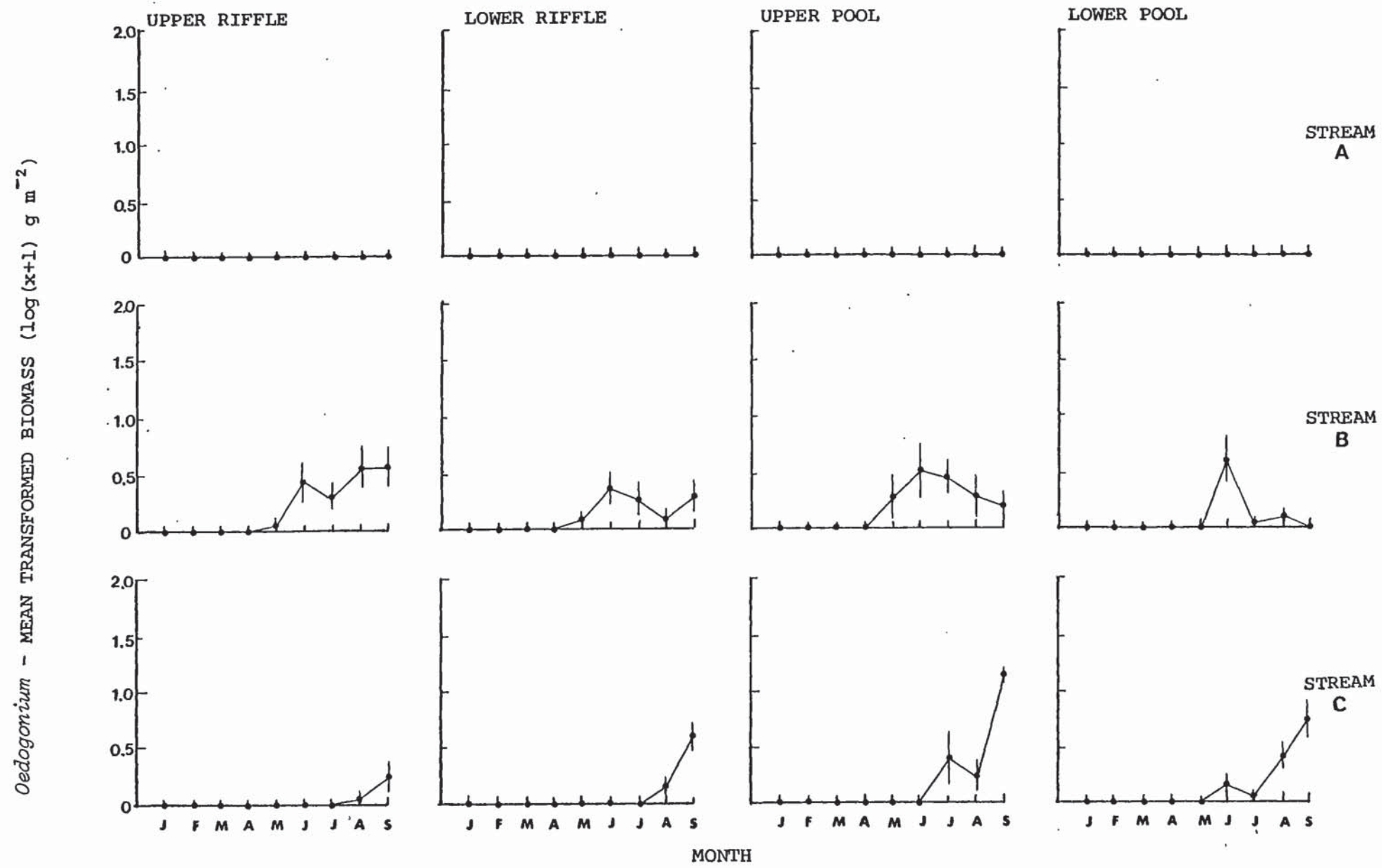


Fig. 4.6. Continued

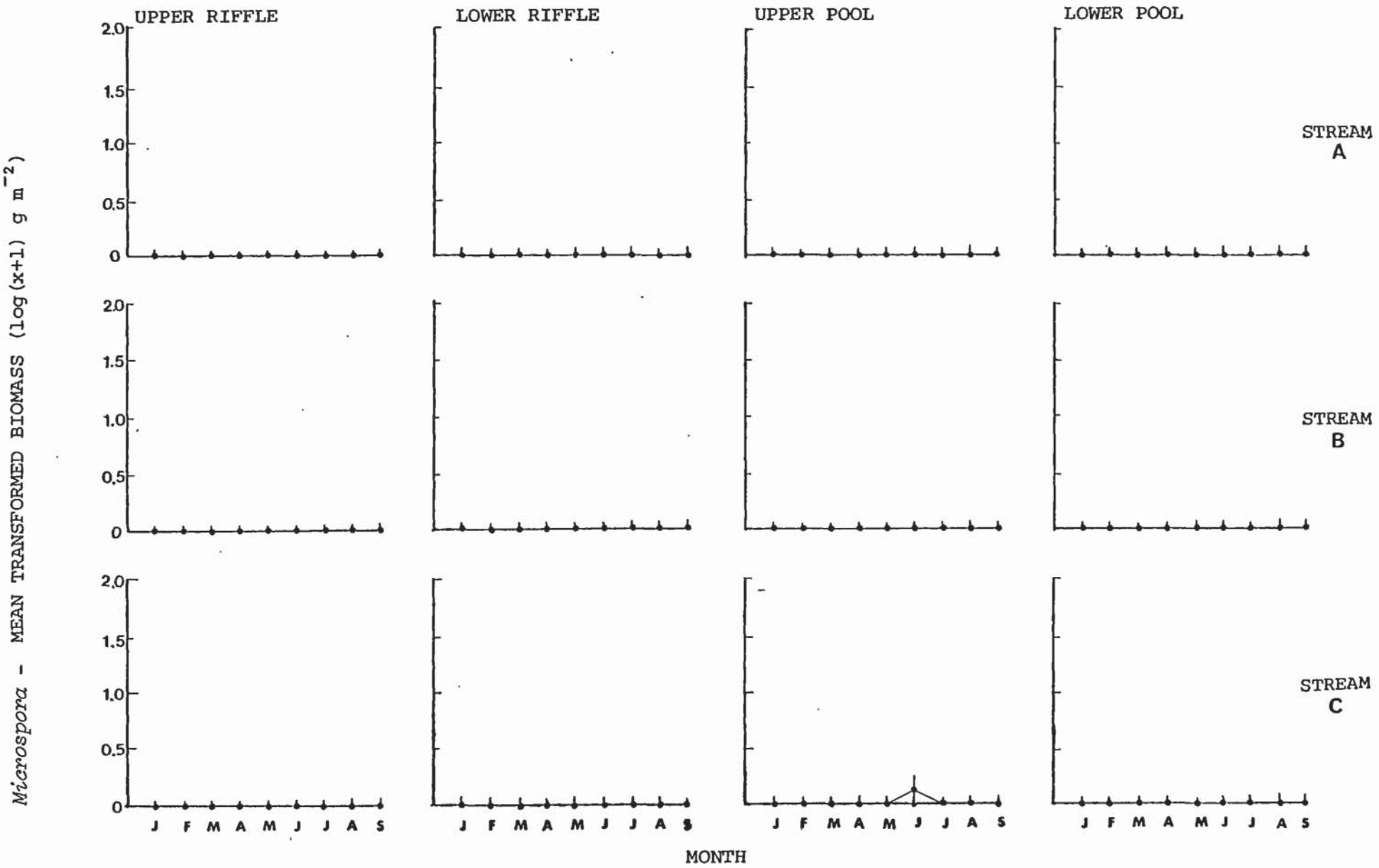
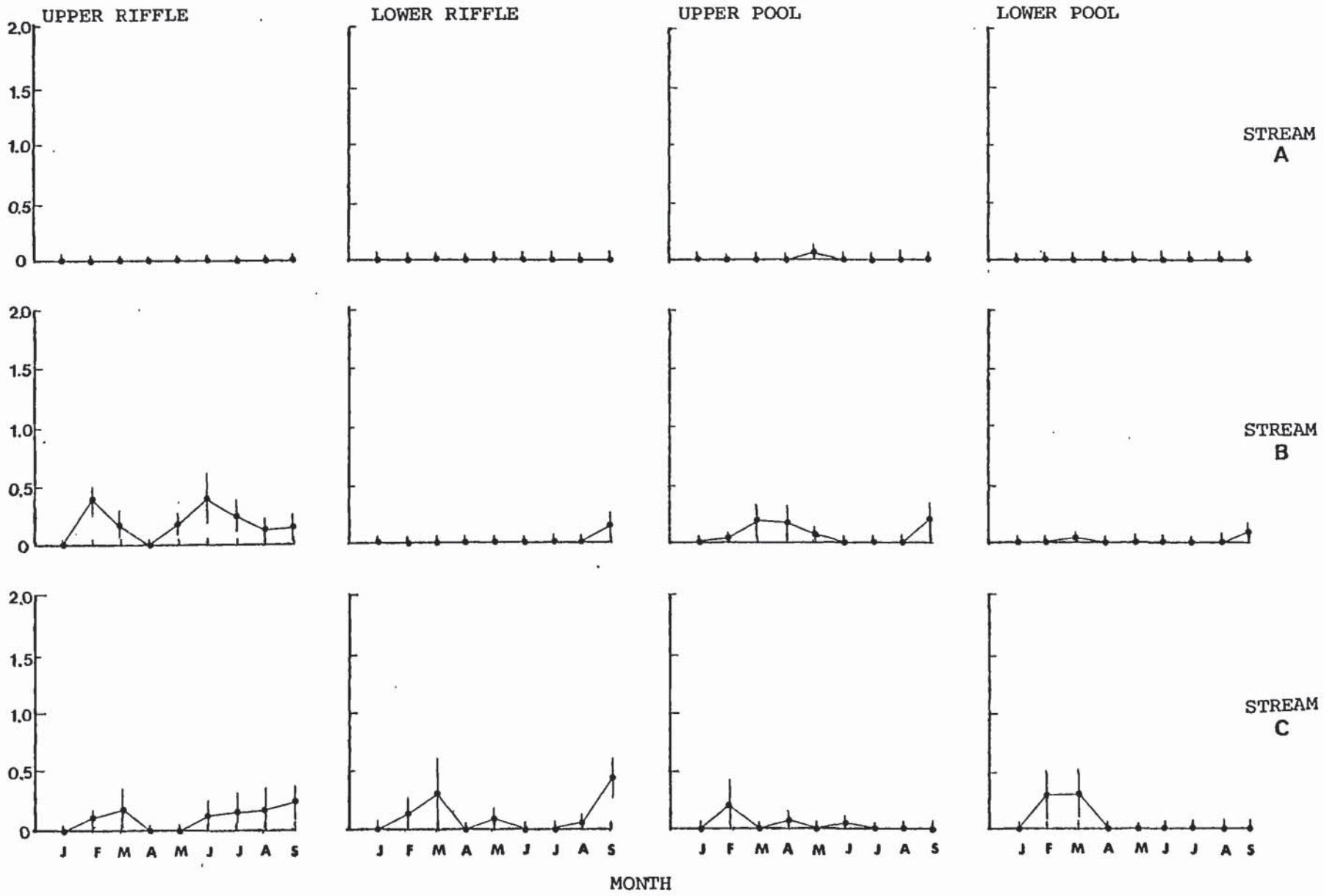


Fig. 4.6. Continued



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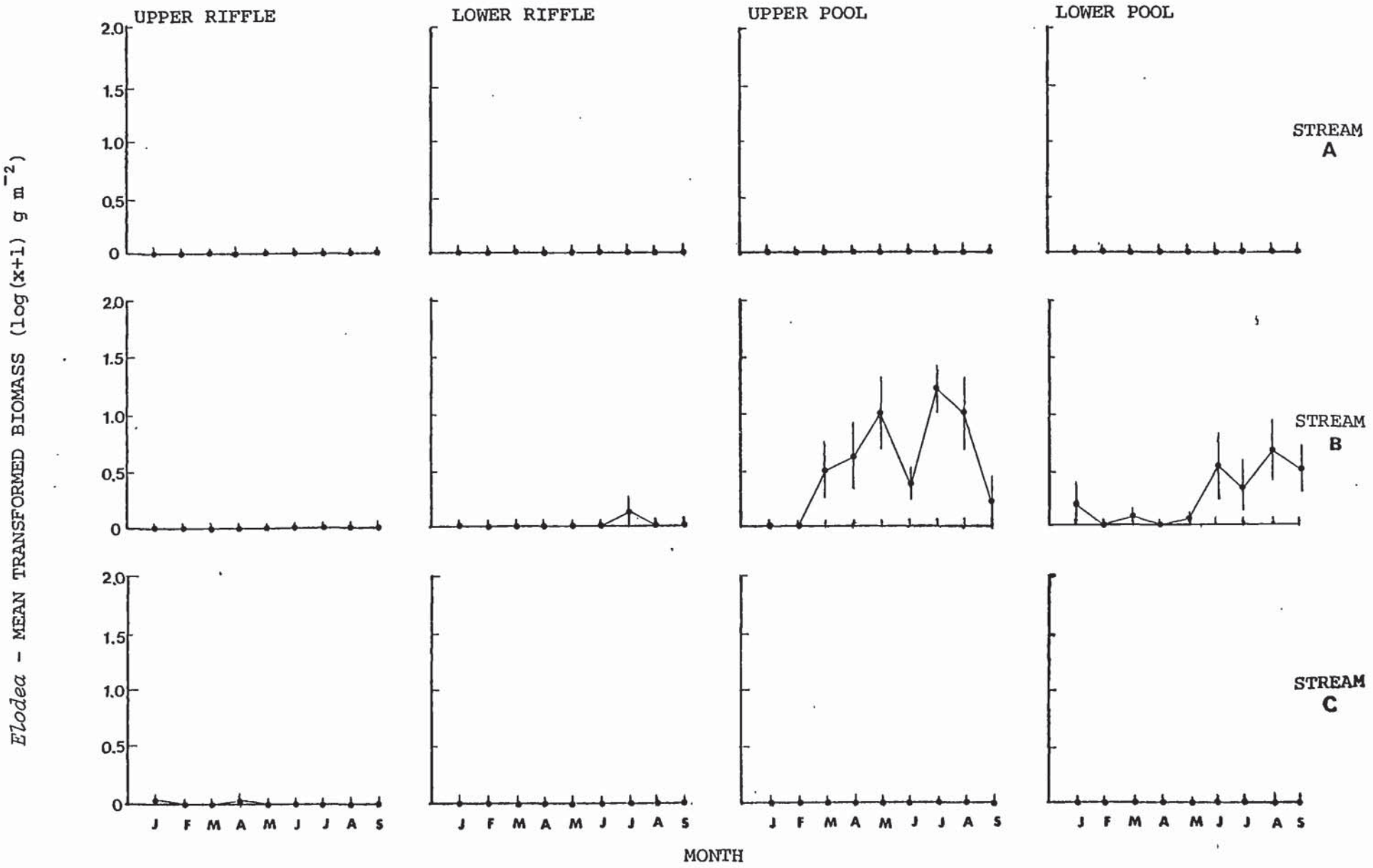


Fig. 4.6. Continued

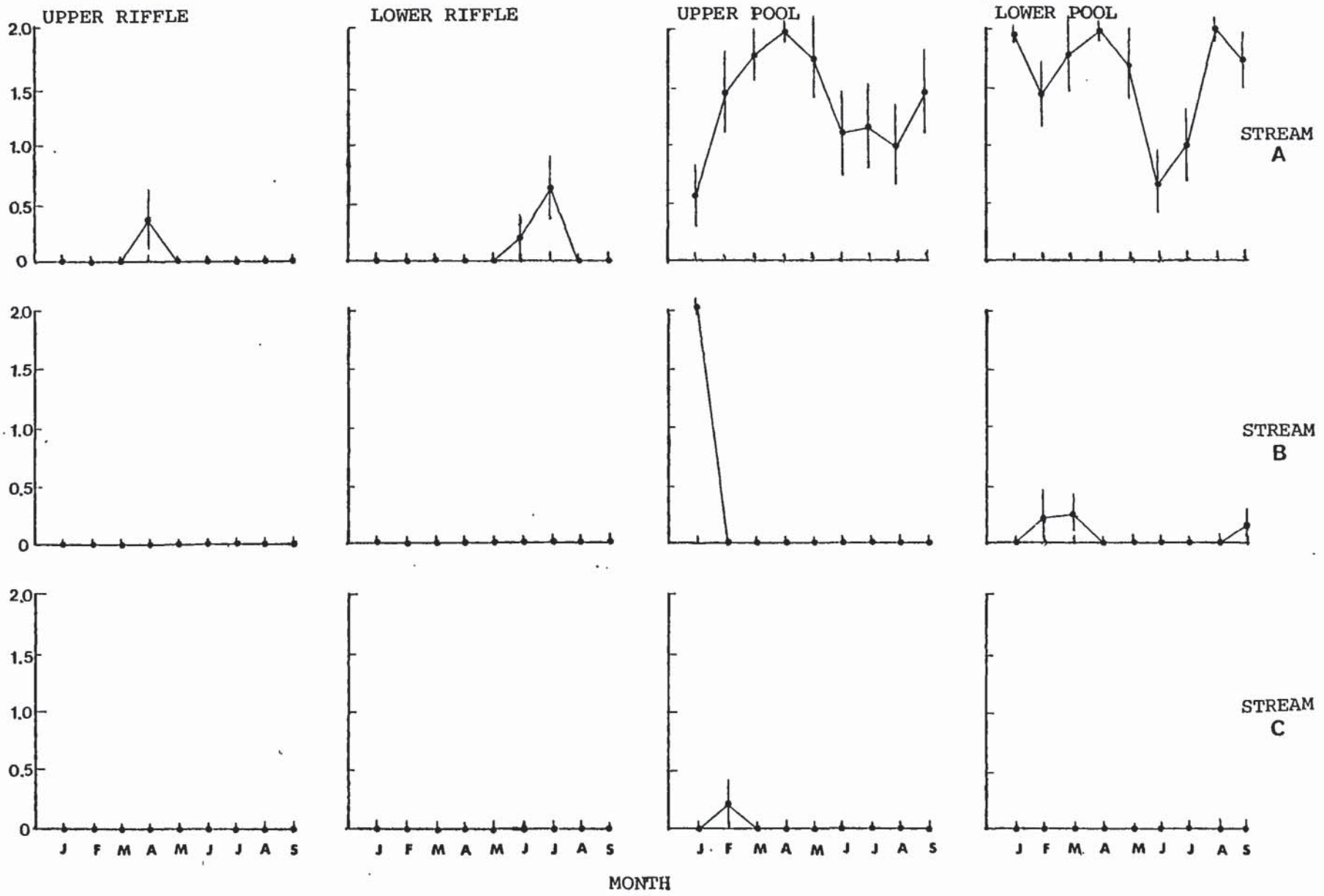


Fig. 4.6. Continued

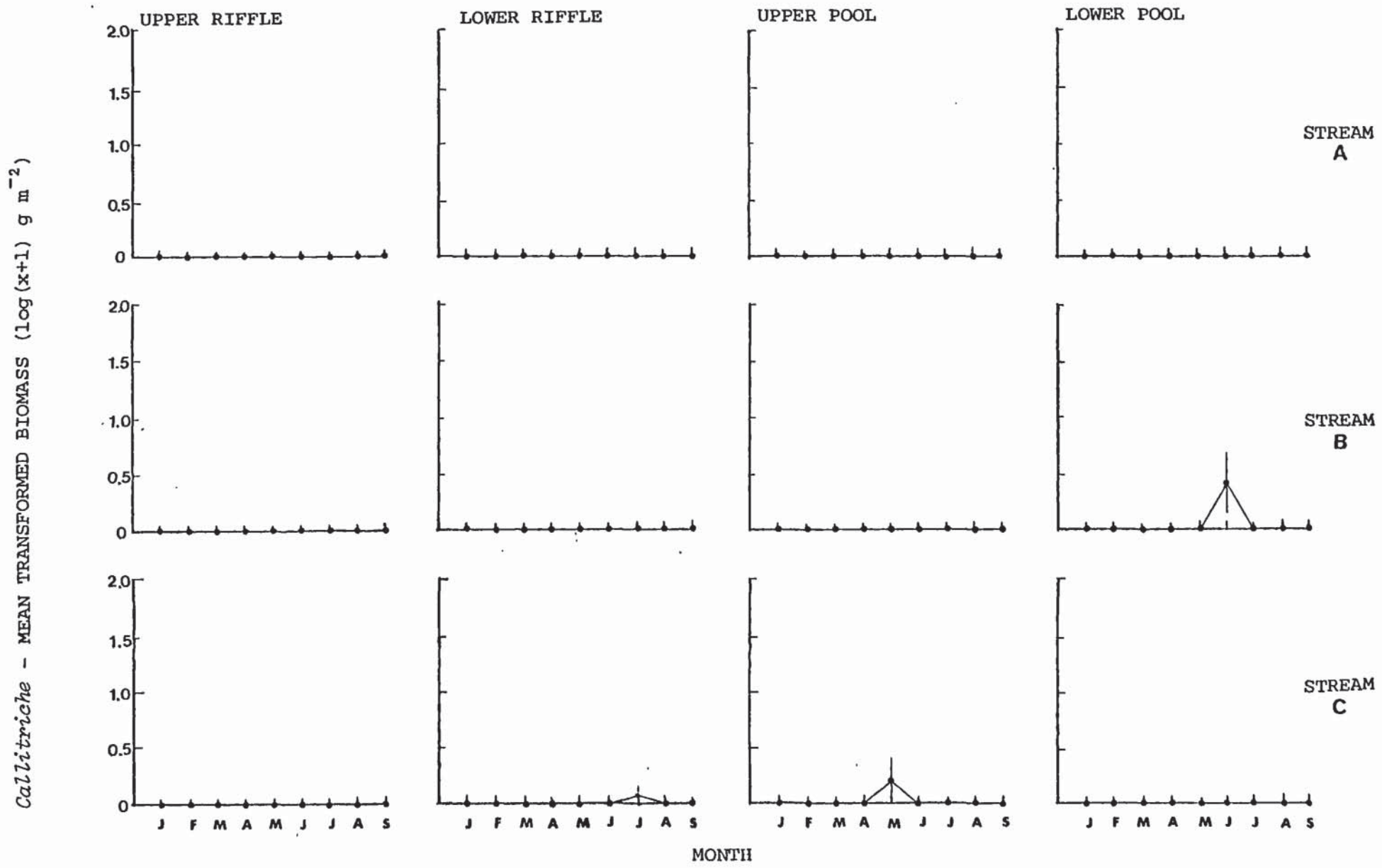


Fig. 4.6. Continued

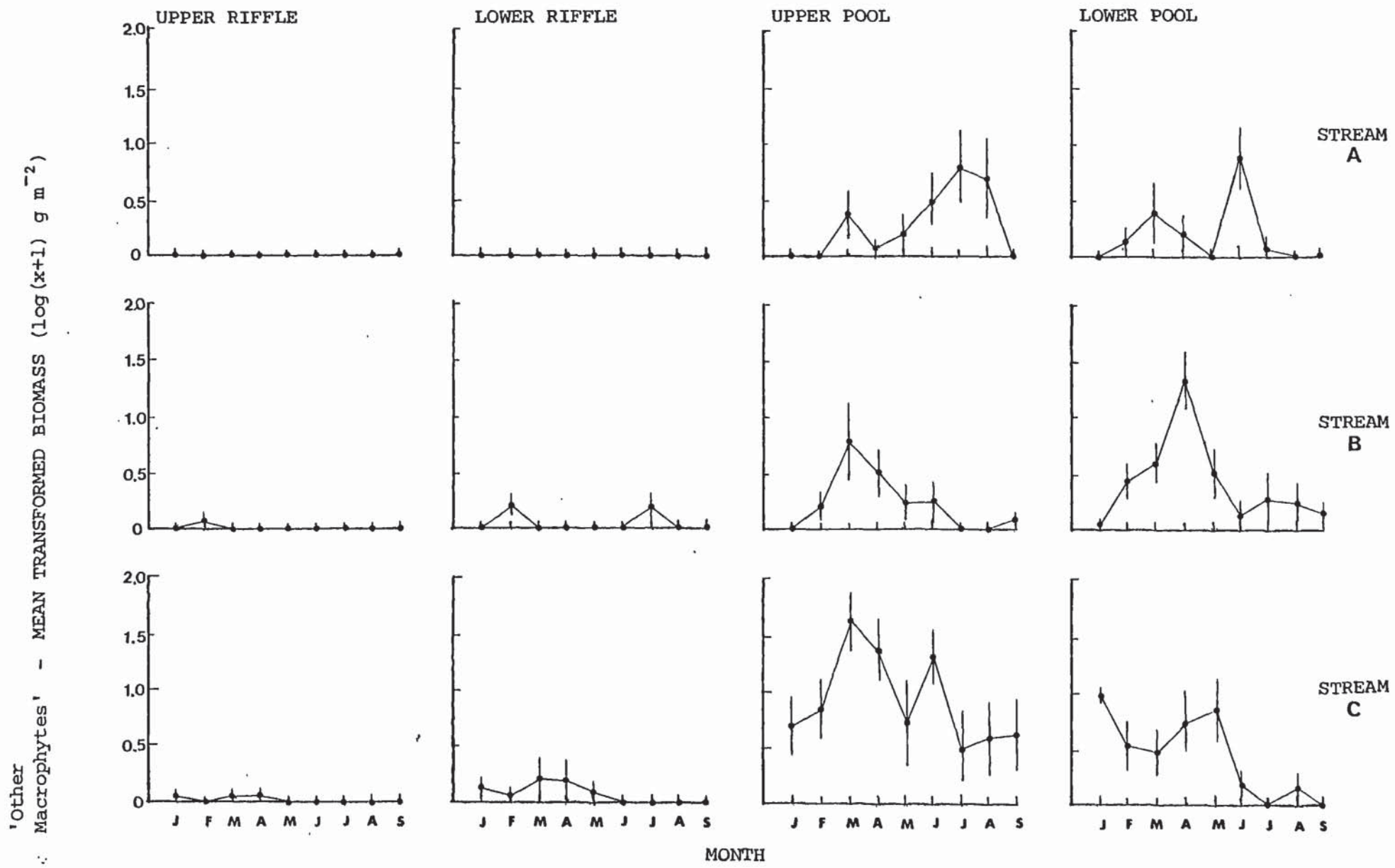
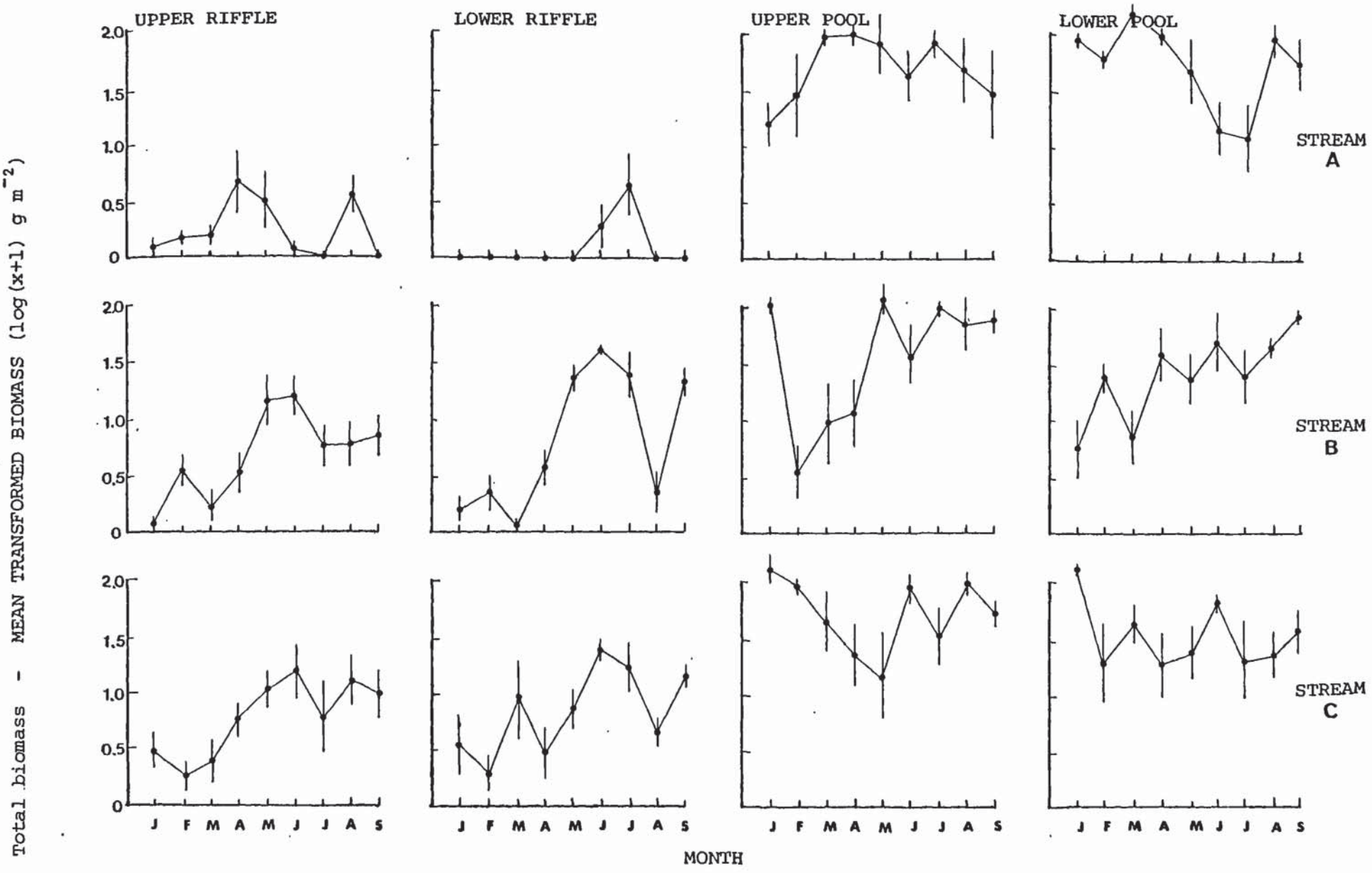


Fig. 4.6. Continued



stream A(O) *Potamogeton crispus* is undoubtedly the commonest species. Analysis of total biomass reveals no significant difference between the upper pool sites of the three streams; whilst in the lower pools the addition of sewage effluent significantly reduces standing crop. However, it was not felt that the pool sites, provided a particularly good model of 'normal' riverain conditions. The muddy bottomed pool sections certainly provided a natural substrate for the growth of *Potamogeton* in streams A(O), but it was felt that much of the algal material in streams B(25) and C(50), especially *Cladophora* and *Oedogonium*, had floated down from the riffle sections. The absence of *Potamogeton* in these two streams could therefore have not only arisen as a consequence of the more polluted nature of these waters but also from the inability to compete with masses of macro-algae for substrate and light. The presence of *Potamogeton* in samples from streams B(25) and C(50) low in *Cladophora* (e.g. samples taken in January in the upper pool of stream B(25)) tends to support this latter hypothesis. *Elodea canadensis*, however, seemed quite able to grow in stream B(25) in close association with large growths of *Cladophora*, but was not found in stream C(50). It seems more likely that this absence arose from variations in the physico-chemical quality of the sites than the case of *Potamogeton*.

Inspection of the table 4.4 reveals that biomass in the riffle sections is generally lower than the pool sections. It thus seems probable that competition between species for substrate and light at such sites would be lesser and changes in the spatial distribution of species in riffle sections is thus more likely to reflect tolerances to ambient physico-chemical conditions. In the upper riffle section of stream A(O) much of the total biomass is attributable to *Vaucheria*, which is significantly reduced by the addition of effluent. There is no significant difference in standing crop of *Vaucheria* between stream B(25) and C(50) - the alga being virtually absent from both sites. In the lower riffles *Vaucheria* was almost absent from all three streams. *Cladophora* and *Oedogonium* were both found in riffle sites of stream A(O), but significantly higher crops were found upon addition of sewage effluent. In the upper riffle the crop of *Cladophora* was significantly larger in stream C(50) than B(25); whilst that of *Oedogonium* was significantly larger in stream B(25) than C(50). *Callitriche*, *Elodea* and *Potamogeton* were all found in riffle sections, but only in low quantities and rather rarely. The bryophyte *Amblystegium* was virtually

absent from stream A(O) but found in significantly larger quantities upon addition of effluent.

The causal factor for the significant spatial differences in flora may be indicated by the accompanying physico-chemical data (fig. 4.3). The addition of effluent to streams B(25) and C(50) noticably increases the mean concentrations of filterable cadmium, chromium, copper, lead and zinc; increasing the temperature, alkalinity, conductivity, and NO_3-N, PO_4-P and chloride concentrations : DO and pH are noticably reduced. Speculation as to which (if any) of the factors stimulates growth of *Cladophora* is difficult owing to the shortage of experimental evidence. Pitcairn and Hawkes (1973), however, suggest phosphate (with an interaction with nitrate) to be highly influential on growth, a hypothesis favoured by many workers (Whitton, 1970b). It is however possible that some factor (such as concentration of certain vitamins) which was not measured in the experimental streams could be the causal, stimulatory, substance.

At most sites where taxa are present significant temporal (i.e. monthly) changes in biomass were found. The biannual growth cycle of *Cladophora* has already been well documented (e.g. Bellis and McLarty, 1967), standing crop generally peaking in spring and autumn with a sag in the summer months. In this study, however, pool sites showed little overall seasonal pattern in variation of biomass. It was felt that this was a result of the *Cladophora* growing in the riffle sections detaching and settling in the pool sections. Riffle sections did exhibit a seasonal pattern of growth : a peak in standing crop of *Cladophora* is found in May/June followed by a sag in July/August and a second peak in August/September. *Vaucheria* biomass exhibits a peak in April/May : whilst *Oedogonium*, apparently absent earlier in the year, peaks in July/August/September. Many of these 'trends' are, however, based upon the deviation of single set of values (i.e. one mean value) and it was felt that more frequent sampling would have aided analysis and interpretation of the overall pattern of the temporal changes in biomass. The significant main/sub-plot interaction found in nearly all cases where taxa are present further complicates matters in that it indicates that the growth response of the taxa (as indicated by standing crop) to the addition of effluent is significantly different at different times of the year, and conversely the temporal

responses of the taxa are significantly different in the three streams.

Causal physico-chemical factors responsible for temporal changes in the biomass of taxa include temperature and solar radiation, both of which were measured over the period of this study. Concerning *Cladophora*, it has been suggested that high summer temperatures lead to the sag in biomass by inhibiting growth, whilst lower temperatures in spring and autumn are conducive towards growth causing the biannual growth cycle. A curscry look at the data from this study certainly reveals a maximum in mean temperatures in August when the fall in *Cladophora* biomass was also found. This peak, however, was only around 15°C and laboratory studies have shown *Cladophora* to grow well up to an optimum of 25°C (Zuraw, 1969) or 30°C (Bellis, 1968a). Wong *et al.* (1978), however, suggested that the tolerance limit of *C. glomerata* at fixed values of daily mean temperature changes with the daily temperature range,which may in part explain the inhibition at lower temperatures reported in field studies. In this study, however, mean August temperatures were 13.4, 14.2 and 14.6°C (for stream A(0), B(25) and C(50) respectively), and extrapolation of the calculations of Wong *et al.* (1978) would suggest that a 12-13°C daily water temperature fluctuation would be required for temperature to limit (i.e. inhibit) growth. Personal communication with the Checkley research team and other workers at the experimental facility suggested that daily temperature fluctuations of this magnitude did not occur. A plot of mean transformed biomass against mean monthly temperature, with data from each stream, indicated no apparent curvilinear relationship. The association between *Cladophora* biomass in the riffles and temperature was thus examined by linear regression. Accompanying correlation coefficients (table 4.5) show that only in stream C(50) are temperature and *Cladophora* biomass significantly correlated, and here only 43% (= r^2) of the variability of algal biomass is attributable to its linear regression on temperature.

The trend of both mean total and maximum radiation show peaks in June and fall in July/August, in close agreement with the *Cladophora* standing crop data. The necessity for light energy in the photosynthesis process and the dependence of growth on photosynthesic activity is established. Correlations, accompanying linear regressions

Table 4.5. Correlation coefficients (r) for the correlation between mean transformed biomass of *Cladophora* in the Checkley riffles and mean monthly temperature, total radiation and maximum radiation.

STREAM	n		Temperature (°C)	Total Radiation	Maximum Radiation
A(0)	16	r	0.33 N.S.	0.03 N.S.	0.02 N.S.
		r^2	0.11	0.00	0.00
B(25)	16	r	0.42 N.S.	0.59 *	0.66 **
		r^2	0.18	0.35	0.43
C(50)	16	r	0.65 **	0.57 *	0.77 ***
		r^2	0.43	0.32	0.59

LEGEND

N.S. = Not significant (P>0.05)

* = Significant (P<0.05)

** = Significant (P<0.01)

*** = Significant (P<0.001)

Table 4.6 Correlation coefficients (r) for the correlation between net *Cladophora* growth in the Checkley riffles and mean monthly temperature, total radiation and maximum radiation.

STREAM	n		Temperature (°C)	Total Radiation	Maximum Radiation
A(0)	16	r	0.148 N.S.	0.000 N.S.	0.130 N.S.
		r^2	0.022	0.000	0.017
B(25)	16	r	0.230 N.S.	0.263 N.S.	0.138 N.S.
		r^2	0.053	0.069	0.019
C(50)	16	r	0.095 N.S.	0.259 N.S.	0.305 N.S.
		r^2	0.009	0.067	0.093

LEGEND

N.S. = Not significant (P>0.05)

of *Cladophora* biomass in the riffles and solar radiation are included in table 4.5 These show significant positive correlation between both mean total and mean maximum radiation and mean transformed biomass in streams B(25) and C(50). The lack of correlation in streams A(0) is likely explained by the near absence of the alga from the sites at any time of year. Such correlations, however, do not necessarily imply a causal relationship between the two variables but certainly merit further consideration and investigation.

4.4.2 Growth

Section 4.4.1 revealed various significant correlations between mean transformed *Cladophora* biomass at riffle sites and mean monthly temperature, total radiation and maximum radiation. It must be noted, however, that although biomass (= standing crop) is obviously related to growth rate the two terms are in no way synonymous : biomass may be considered as the cumulative product of growth and decay. Thus although the three physical variables are significantly correlated with standing crop (at certain sites) conclusions may not be drawn as to their effects on growth. However, by calculating a value for monthly change in biomass (by substracting the mean transformed *Cladophora* biomass of the previous month from that in question) an estimate of net monthly growth may be made ; this may be a positive or negative value. Plots of net growth rate against mean monthly temperature, total radiation and maximum radiation showed no visible trend and correlation coefficients, accompanying linear regression were all low ($P>0.10$) - see table 4.6. Results therefore suggest that mean monthly temperature, total radiation and maximum radiation are not significantly correlated with net *Cladophora* growth at riffle sites. In stream A(0) this lack of correlation is likely explained by the near absence of the alga from sites at any time of year. In streams B(25) and C(50) lack of correlation is likely attributable to losses of algal material from the riffle sites, by fragmentation, particularly in mid-summer. Such losses result in negative net growth, at such times, even though 'real' growth may still be occurring.

4.4.3 Species richness

Species richness - the number of species present a particular site and particular time - is one of the components of the term species diversity. Diversity is perhaps best defined as a measure of variety in a community. Species diversity, however, is usually defined as a function of the number of species present (species richness) and the evenness with which the individuals are distributed among the species (species equitability). Thus although species diversity and species richness are often positively correlated such positive correlation is neither a biological nor mathematical necessity (Hurlbert, 1971). It has been suggested (with some experimental evidence) that species diversity and community stability are associated in that greater species diversity leads to higher stability, and that pollution tends to reduce diversity. These views are, however, rather controversial and have resulted in the extensive and complex literature surrounding the subject.

In this study it was not felt justified or necessary to evaluate species diversity owing to the low numbers of macro-floral taxa present, the problems caused by identification of taxa to varying degrees of specificity (i.e. some to the specific and some to the generic level), and the difficulty of interpretation of diversity index evaluations. The number of taxa (species richness) could however be easily calculated and provide information to complement the quantitative biomass study. Indeed Ricklefs (1979) points out that results of most studies are relatively insensitive to which index of diversity is applied, or whether any index is used in place of a simple count of species present. The number of taxa present at each site and at each month were therefore calculated and are included as fig. 4.7. The 'other macrophytes' grouping was not included in this evaluation, as it could in no way be considered a 'natural' group.

Fig. 4.7. Number of taxa present in the Checkley streams.

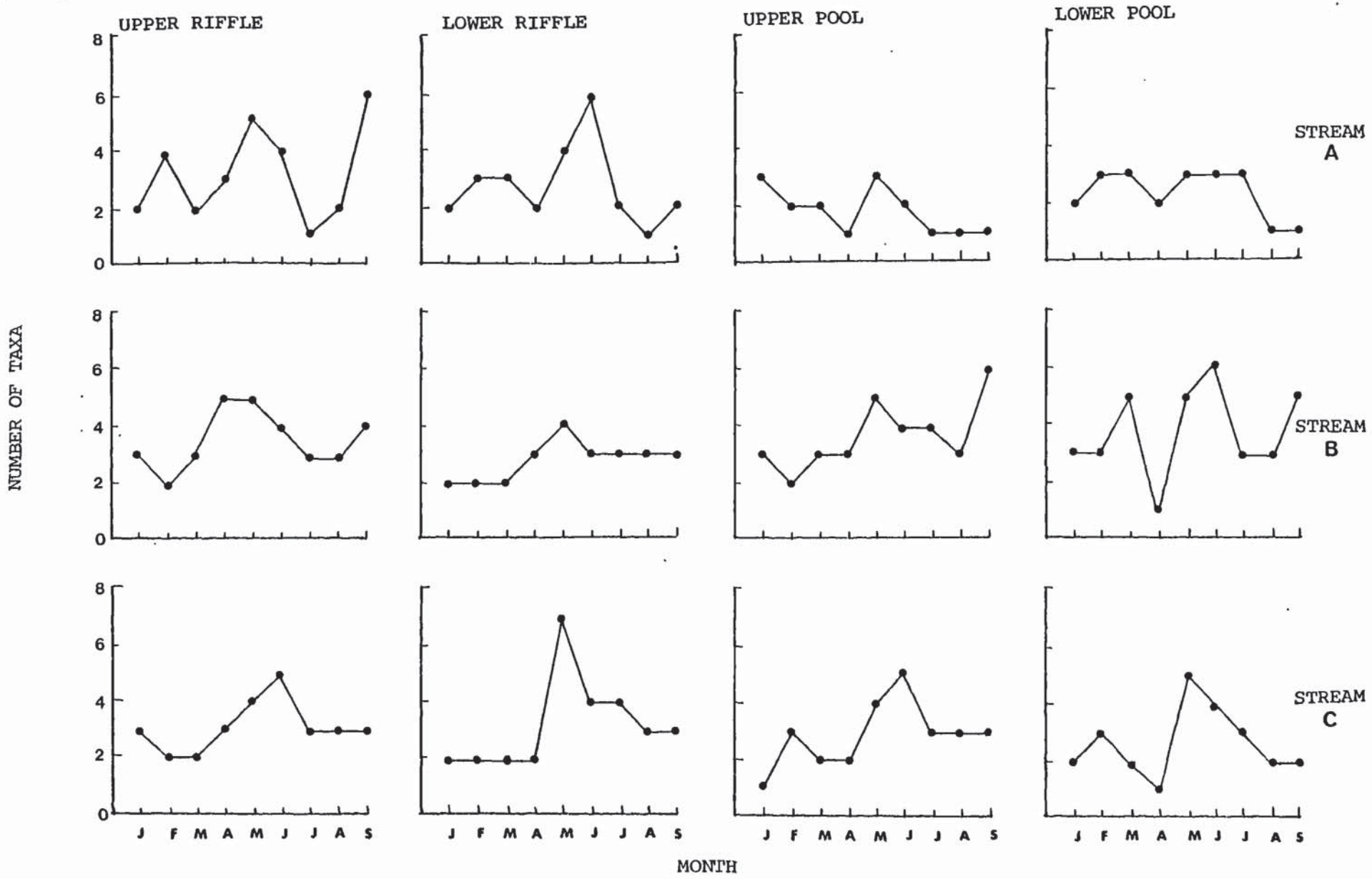


Fig. 4.7 reveals that peak numbers of taxa occur in nearly all sites in May/June; and averaged over the three streams and four sites it is found that the maximum number of taxa (i.e. 5) occur in the month of May. This corresponds closely to the first peak in growth of *Cladophora* (also reflected in the total biomass) to within a month or so, being particularly noticable in the riffle section where temporal biomass changes are most marked. Little evidence is available to support the hypothesis that the two factors are inversely related i.e. high diversity (as indicated by species richness) is found when total biomass is low, and as total biomass increases (largely due to *Cladophora* in the riffles) the number of taxa fall. Indeed regression between the two variables yields a correlation (r) of +0.30 for the six riffle sites ($n = 54$) which is significant ($P<0.05$), indicating number of taxa and total biomass to be significantly positively correlated.

Taken over the nine months sampling duration all streams averaged (to the nearest integer) 3 taxa per site per month : pool sites and riffle sites containing the same number. No evidence was therefore found to support the hypothesis that the addition of 25 or 50% well oxidised sewage effluent leads to a reduction in diversity, as indicated by species richness. This is likely explained by the loss of intolerant taxa being balanced by the gain of tolerant taxa in the 'polluted' sites.

4.4.4 Species association

Throughout the course of the investigation it was apparent that certain taxa were frequently found together in the same sample. This species (or taxa) association was felt worthy of investigation.

Investigation was effected adopting analytical techniques similar to those of Agnew (1961), described in Kershaw (1964). Presence or absence of each pair of taxa were considered in turn and data entered into 2×2 χ^2 contingency tables. The correlations obtained are included as fig. 4.8. The reciprocal of χ^2 values for pairs of taxa which were significantly positively associated ($P<0.05$) were then used to construct a 'species constellation' (fig. 4.9). Taxa positively

Fig. 4.8. Complete χ^2 matrix showing positive and negative species relationships (and significance) present.

LEGEND

* = Significant at the 5% level

** = Significant at the 1% level

*** = Significant at the 0.1% level

Cladophora	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	'Other Macrophytes'
-5.72*													
0.15	0.13												
165.82***	-0.82	-0.08											
11.78***	5.08**	0.69	0.34										
0.34	12.83***	-12.64***	4.04*	-2.15									
-0.15	0.13	-5.86**	-0.08	-0.69	-12.64***								
3.47	0.26	-2.02	-0.03	3.31	-4.91*	-2.02							
29.94***	-10.07***	0.17	-0.58	2.28	-0.02	-0.17	-0.13						
23.62***	-1.80	-0.13	37.79***	-0.62	-0.82	-0.13	0.08	-2.19					
-270.42***	-1.31	0.28	-76.37***	-4.23*	-0.0004	-0.28	-1.68	-13.62***	-4.91*				
0.05	-0.002	-9.24***	0.10	-1.41	-19.40***	-9.24***	-3.46	-0.008	-0.45	-0.04			
-	-	-	-	-	-	-	-	-	-	-	-		
0.58	-5.21**	-0.03	-23.34***	0.67	0.20	-1.18	-4.25*	9.29***	-0.70	-12.15***	-0.49	-	

Fig. 4.9 'Species constellation' for macro-flora in Checkley streams.

SPECIES

C = *Cladophora*

V = *Vaucheria*

O = *Oedogonium*

M = *Microspora*

S = *Spirogyra*

A = *Amblystegium*

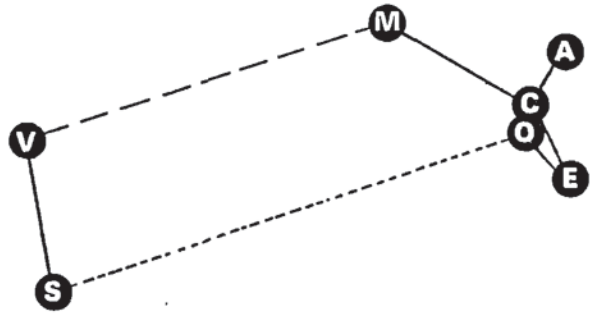
E = *Elodea*

ASSOCIATION (level of significance)

..... = 5%

— — — = 1%,

_____ = 0.1%



associated, having high χ^2 values would have low $^1/\chi^2$ values and would therefore be positioned close together , taxa less positively associated could be positioned further apart. Negatively correlated χ^2 values were used without reciprocation to aid construction of the constellation. Those species with only a single significant positive correlation ($P<0.05$) were excluded. The 'other macrophytes' group was excluded from the constellation since it did not form a natural grouping. The arrangement of fig. 4.9 involved a multi-dimensional arrangement of taxa being condensed into two dimensions, some degree of compromise was therefore necessary in the construction.

Inspection of the resulting species constellation reveals a closely associated group of taxa, vis. *Cladophora*, *Oedogonium*, *Elodea* and *Amblystegium*. These may be thought of as the 'polluted water' community common in streams B(25) and C(50). Whitton (1970b) comments that the literature indicates a considerable uniformity in the floristic composition of epiphytes associated with *Cladophora*, and lists *Oedogonium* as one of the organisms frequently mentioned in this context. In this study, however, whilst the close association between the two algae is evident, no evidence was found of *Oedogonium* being directly epiphytic (i.e. attached) to filaments of *Cladophora*. The alga *Stigeoclonium* was found to be largely absent from all three streams. In rivers receiving gross organic effluents *Stigeoclonium* characteristically dominates the flora upstream of the *Cladophora* dominated zone (e.g. Hawkes, 1964). It may be envisaged, therefore, that *Stigeoclonium* would be present in stream C(50) rather than stream B(25) and A(0). No such pattern was evident, however, and it was considered that even in stream C(50) water quality was good enough to allow colonization of *Cladophora* which through competitive action would prevent colonization of *Stigeoclonium*. Balloch (1977) found this situation to persist even when streams received 75% effluent and only 25% River Tean water. In support of this hypothesis it was found that water samples, taken from the influent inlet of all three streams, would develop growths of *Stigeoclonium* if incubated in the laboratory, proving the existance of spores of the alga in the waters. The source of these spores in stream B(25) and C(50) water was felt to likely be the percolating filters of the sewage treatment works. The spores found in stream A(0) water, however, indicate that

some *Stigeoclonium* was undoubtedly sporulating in the River Tean upstream of the inlet. However, whatever the source of the spores the failure of their development is likely explained (at least in part) by the dense growths of *Cladophora* and/or macrophytes found in the streams. It must also be considered, however, that the dilution of sewage with river water providing the range of conditions in the Checkley simulated stream ecosystem is not directly analogous to the changing passing down an organically polluted watercourse and such differences may also help explain the absence of *Stigeoclonium* in the more 'polluted' streams.

Species association reveals a 'clean water' community comprising *Vaucheria* and *Spirogyra*.. *Microspora* seems to be an intermediary taxon between the 'clean' and 'polluted water' communities. The vascular hydrophyte *Potomogeton crispus* undoubtedly also belongs to the 'clean water' community. The presence of this species in samples, however, frequently excluded all other species. Consequently species association shows the species to be significantly negatively associated to most of the other taxa present.

4.5 Conclusions

1. Analysis of species associations revealed a 'clean water' community dominated by *Vaucheria* which, upon effluent addition, was replaced by a 'polluted water' community, comprising *Cladophora*, *Oedogonium*, *Elodea* and *Amblystegium*. Pool sites of stream A(O) were dominated by *Potamogeton* to the almost total exclusion of other taxa.

2. The presence of *Potamogeton* in the pool sites of stream A(O) and its absence in streams B(25) and C(50) may be explained by its inability to compete with the macro-algal 'masses' found at the latter sites, as well as intolerance to the ambient physico-chemical conditions.

3. Total biomass in the upper pool sites was not significantly altered by effluent addition, averaged over the sampling period. Over the same period, the lower pool sites showed significant reduction in total biomass, upon effluent addition, with no significant difference between the two 'treatment' streams (i.e. B(25) and C(50)).

4. Total biomass in the riffle sites was noticably lower than in the pool sites in all three streams, and seasonal effects were more pronounced. Riffle sites showed a significant increase in total biomass upon addition of effluent and no overall significant difference between the total biomass in the two 'treatment' streams.

5. *Cladophora* biomass exhibited an overall significant increase upon effluent addition. No significant overall trend in the comparison of the two 'treatment' streams could be detected.

6. Factors responsible for the spatial differences in *Cladophora* biomass remain unclear, although the literature suggests phosphorus to be intimately involved. Mean concentrations of PO_4-P over the course of the study were 0.6 mgl^{-1} for stream A(0), 2.5 mgl^{-1} for stream B(25), and 3.5 mgl^{-1} for stream C(50).

7. Seasonal trends in biomass change were generally found difficult to interpret owing to the low frequency of sampling.

8. Maximum mean monthly temperatures ($<15^{o}C$) were considered to be too low to account for the reduction in *Cladophora* biomass in the summer months, even taking into account the effects of diurnal temperature fluctuation. Mean monthly temperature and mean transformed biomass were, however, significantly correlated for stream C(50) data ($r = 0.65$, $P<0.001$).

9. Both mean monthly total and maximum radiation were significantly correlated with mean transformed *Cladophora* biomass in streams B(25) and C(50).

10. Correlation of net *Cladophora* growth, at riffle sites, against monthly mean temperature, total radiation and maximum radiation was not significant ($P>0.10$) in any stream.

11. No evidence was found to support the hypothesis that domination of the floral community by *Cladophora* in streams B(25) and C(50) reduced species richness. To the contrary, a significant positive correlation was found between mean transformed total biomass and number of taxa ($r = 0.30$, $P<0.05$).

5. *CLADOPHORA* BATCH CULTURE STUDIES

5.1. Introduction

The growth of organisms in batch culture has been dealt with in section 3.5.1; whilst the literature dealing with methods of studies specifically concerned with *Cladophora*, and their results, is reviewed in section 3.2.1.4. From the literature it was felt necessary to initially investigate aspects of the growth of *Cladophora* in batch culture prior to applying a standardized methodology to investigate the growth response of the alga to specific growth promotors and toxicants. The study is therefore divided into two sub-sections ; the first (Phase 1) dealing with the preliminary studies on culture techniques whilst the second (Phase 2) deals with the growth response of the alga to various herbicides.

5.2. Phase 1 - Preliminary studies on culture techniques

Phase 1 of the study involves a total of five separate experiments. The dependence of each experiment on the results of the preceding experiment (or experiments) necessitates that each be considered in turn.

5.2.1. Experiment 1 - Contamination investigation

5.2.1.1. Objectives

Experiment 1 was carried out to investigate the effect on the subsequent development of contaminants of using unialgal and non-unialgal inocula and using sterile and non-sterile natural water as medium.

5.2.1.2. Materials and methods

All experiments were performed using 100 ml conical flasks containing 50 ml medium and covered with Oxoid aluminium caps. All glassware was cleaned in detergent and dilute (approximately 5%) hydrochloric acid, and rinsed thoroughly in distilled water prior to use, as recommended by the E.P.A. (1978); then dry sterilised at 160°C for one hour. Flasks were gently shaken (approximately 60

oscillations/minute) on a Griffon orbital shaker. Illumination was supplied continuously (24 hours/day) by one 5 ft 65/80W and two 2 ft 30W Thorn white fluorescent tubes providing an intensity of 4000 lux, measured at the level of the cultures. Light intensity was measured using an EEL portable photometer. The apparatus was placed in a constant temperature room at 15°C, the heating action of the fluorescent lights, however, locally raised the temperature to 17-19°C even though a nearby fan was positioned to blow cooler air over the apparatus.

A moderately eutrophic local pond water was obtained from Langley Pool (Nat. grid ref. SP153968) ; this will be further referred to simply as Langley water. Water was collected at intervals, kept in plastic courtesy tanks and aerated. The chemical composition of Langley water is included as table 5.1. Non-unialgal *Cladophora* (identified a *C. glomerata* according to the criteria of van den Hoek, 1963) was taken from a moderately eutrophic site on the River Cole (Nat. grid ref. SP198896) and cleaned as prescribed by Pitcairn and Hawkes (1973). The unialgal culture of *C. glomerata* was obtained from the Culture Collection of Algae and Protozoa, Cambridge (ref. 505/3) and grown in the continuous culture system described in Chapter 7. The experiment involved three treatments with 5 replicates of each treatment. Into each of one set of flasks was placed 50 ml Langley water and a 2 cm long, well branched, tuft of *Cladophora* (as prescribed by Pitcairn and Hawkes, 1973). The second treatment was similar except that the Langley water was first filtered through a 0.25µm membrane filter. The third treatment similarly using membrane filtered water but using a unialgal *Cladophora* inoculum. The presence and extent of contamination was assessed by regular visual inspection. After 20 days the experiment was terminated. The crop produced in each flask was removed by retention in a 250µm sieve, and dried to to constant weight at 105°C. By necessity the initial dry weights of inocula had to be ignored.

5.2.1.3. Results and discussion

Visual inspection of the flasks revealed that in both sets of flasks receiving non-unialgal inocula contaminants were visible by day 20.

Table 5.1 Physico-chemical analysis of Langley water used in batch culture experimentation (for methods of analysis see table 6.1).

PARAMETER ($mg\ l^{-1}$)	Mean ($\bar{x}$)	Standard Error (S.E.)	Number of Samples (n)
pH	8.3	0.0	15
Total Alkalinity	136	7	15
Phenolphthalein Alkalinity	5	2	15
Total Hardness	313	13	15
Calcium Hardness	190	9	15
NH_3-N	0.3	0.1	10
NO_3-N	6.5	0.8	13
NO_2-N	0.030	0.006	13
PO_4-P	0.2	0.1	13
Na^+	19	2	11
K^+	6	0	11
Chloride	32	2	15
Total Cd	0.002	0.000	12
Cr	0.000	0.000	12
Cu	0.021	0.002	12
Fe	0.109	0.009	12
Ni	0.012	0.001	12
Pb	0.020	0.002	12
Zn	0.020	0.002	12

Except pH. Alkalinity and hardness mg l^{-1} as $CaCO_3$.

The greater contamination in the flasks receiving non-membrane filtered water indicated that contamination occurred from the medium (i.e. natural water) as well as the inoculum. No contamination was observed in the flasks receiving membrane filtered water and unialgal inocula. Table 5.2 contains the results of the dry weight analysis. Analysis of this data by a single classification analysis of variance (AOV) was performed producing the AOV summary table included as table 5.3.

Table 5.2. Results of Experiment 1.

Inoculum	Water	Dry wt (mg) after 20 days					
		Replicates					Mean
unialgal	filtered	30.0	30.0	12.4	23.0	21.3	23.3
non-unialgal	filtered	20.3	12.4	19.5	23.8	14.7	18.1
non-unialgal	non-filtered	29.3	8.7	6.1	15.4	7.4	13.4

Table 5.3. AOV Summary Table for Experiment 1.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
Treatment	2	248.166	124.083	2.24181 (P>0.10)
Error	12	664.192	55.3494	
Total	14	912.358		

Results indicate that at the 5% level of significance there is no significant difference in the growth of *Cladophora* in the three treatments.

It was however felt that a uni-algal inoculum and sterile medium would be the best combination for further studies since contamination from other algal species would be avoided. Great difficulty in growing sufficient uni-algal *Cladophora* for inocula for batch culture experiments was encountered, however, and as a result it was thought best in practice to use *Cladophora* obtained from the River Cole and cleaned prior to use, together with a membrane filtered medium, in further work. Contamination in cultures thus treated was very small upto 10 days incubation, and was felt 'acceptable' in most cases beyond this time.

5.2.2. Experiment 2 - Medium replacement investigation

5.2.2.1. Objectives

The final crop of *Cladophora* produced in the flasks in experiment 1 was felt to be very small - in the order of 20 mg. This indicated that growth in batch culture may be limited before 20 days in Langley water. The objectives of this experiment were to find if this was indeed the case.

5.2.2.2. Materials and methods

As in experiment 1, using cleaned alga from the River Cole as inoculum. One set of 5 flasks had their membrane filtered Langley water replaced every 3 days; whilst a second set of 5 flasks (controls) had no such change of medium. Growth was measured as dry weight of alga produced after 20 days.

5.2.2.3. Results and discussion

Table 5.4. presents the results of this experiment, and table 5.5. the analysis of the results.

Table 5.4. Results of Experiment 2.

Water	Dry wt (mg) after 20 days					
	Replicates					Mean
changed every 3 days	56.6	65.0	55.5	40.6	50.3	53.6
not changed	20.3	12.4	19.5	23.8	14.7	18.1

Table 5.5. AOV Summary Table for Experiment 2.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
Treatment	1	3143.53	3143.53	61.9734 ($P<0.001$)
Error	8	405.791	50.7239	
Total	9	3549.32		

Analysis of variance reveals a significant difference at the 0.1% level between the dry weight of alga produced in the two treatments. Replacement of water every three days provides a mean algal biomass approximately three times greater than that produced in the control flasks. Undoubtedly then, by day 20 growth of *Cladophora* has been inhibited, possibly by the lack of some nutrient (or nutrients).

5.2.3. Experiment 3 - Growth medium investigation

5.2.3.1. Objectives

Experiment 2 had revealed that by day 20 growth of *Cladophora* had possibly been limited by lack of nutrients in the natural Langley water.

However, many artificial media have been developed for the growth of algae having higher concentrations of nutrients than may be found in natural waters. It was reasoned that such media should therefore be able to support a greater growth of *Cladophora* over a 20 day period than could Langley water. Since several workers at the University of Aston had used the growth medium of Pitcairn and Hawkes (1973) with good results it was felt appropriate to use this as a typical inorganic growth medium in comparison with natural water.

5.2.3.2. Materials and methods

As experiment 1. Five flasks received membrane filtered Langley water, five flasks received the nutrient medium developed by Pitcairn and Hawkes (1973) and five flasks received the medium of Pitcairn and Hawkes but without the 10% membrane filtered natural water which the medium normally contains. Growth was measured as dry weight after 20 days incubation.

5.2.3.3. Results and discussion

Table 5.6 presents the results of this experiment and table 5.7 the analysis of the results.

Table 5.6. Results of Experiment 3.

Medium	Dry wt (mg) after 20 days					
	Replicates					Mean
filtered natural water	9.8	7.6	5.1	11.0	6.1	7.9
soln. of Pitcairn and Hawkes (with 10% natural water)	12.9	2.9	6.6	8.1	6.8	7.5
soln. of Pitcairn and Hawkes without 10% natural water	6.4	9.1	8.2	3.8	6.8	6.9

Table 5.7. AOV Summary Table for Experiment 3.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ration
Treatment	2	2.8252	1.4126	.182758 (F>0.20)
Error	12	92.7518	7.72932	
Total	14	95.577		

Results indicate that at the 5% level of significance there is no significant difference in the growth of *Cladophora* in the three media.

Interpretation of these results must be somewhat tentative, however three explanations seem likely. First, it may be that the high within-treatment variability found in using the technique may be masking truly significant differences between the treatments. Second, the 'limiting nutrient' (or nutrients) is found in the same concentration in all three media and limits growth to similar amounts in all treatments. Thirdly, the limitation in growth found in batch culture is not a result of nutrient limitation but that the growth of the alga in the medium changes some physical and/or chemical feature of the medium making it unfavourable for further growth. This third hypothesis would best describe the results of both experiment 2 and experiment 3. It was felt that a pH drift may explain such results ; initial investigations supported this hypothesis.

5.2.4. Experiment 4 - pH drift investigation

5.2.4.1. Objectives

The drift in pH in the flasks was felt worthy of investigation, since experiments 2 and 3 had indicated the possibility of pH acting so as to limit growth in 20 day culture experiments.

5.2.4.2. Materials and methods

The medium of Pitcairn and Hawkes (1973) was used but without the 10% natural water addition, this solution was found to have the same buffering capacity as the Langley water used in experiments 1 - 3. To achieve different algal growth rates light intensities of 5000, 6700 and 9700 lux were adopted. Flasks were static, but manually shaken daily. Inoculum was obtained from the River Cole as in experiments 2 and 3. Sets of 5 replicate flasks were used for each treatment. Two types of control treatment were adopted. One set of five flasks received no addition of *Cladophora*; whilst in the second set *Cladophora* was added and regularly agitated for one hour, before removal. This second set of flasks therefore received some of the contaminants associated with the *Cladophora* inoculum. Both sets of control flasks were incubated under 9700 lux light intensity. Algal growth was measured as dry weight produced after 21 days. The pH of the initial medium was 8.0 ± 0.1 and the final pH was measured immediately after the crop of alga had been removed from the medium.

5.2.4.3. Results and discussion

Table 5.8 presents the results of this experiment.

Table 5.8. Results of Experiment 4.

Treatment	*Cladophora* dry wt after 21 days.						pH after 21 days					
	Replicates					Mean	Replicates					Mean
Control 1*	-					—	8.0	7.9	7.8	7.8	7.8	7.9
Control 2**	-					—	8.0	8.2	8.6	8.5	8.6	8.4
5600 lux	43	27	24	22	44	32	9.5	9.7	9.2	9.4	9.2	9.4
6700 lux	18	32	33	28	57	34	9.4	9.5	9.6	9.5	9.9	9.6
9700 lux	28	26	26	28	34	28	9.1	10.1	10.2	9.8	9.4	9.7

* = No alga added to flask.

** = Alga added for 1 hour, then removed.

Results show that growth of *Cladophora* in batch culture results in an elevation of the pH of the medium from 8.0 ± 0.1 to 9.2 – 10.2 after 21 days. Such results are in good agreement with those of Sikes (1976) who, using batch culture techniques, observed pH to drift from 7.3 to 10.5 over the duration of his experimentation. It seems likely that this evaluation of pH is a direct result of the removal of CO_2 from solution during photosynthesis. Whether or not elevation of pH levels is the causal factor which inhibits growth of the alga in 20 day batch culture experiments is still a matter for conjecture; there is, however, evidence to support the hypothesis. Sawyer and McCarty (1978), for example, commented that algae may continue to extract CO_2 from water until an inhibiting pH is reached, usually within the range pH 10-11; whilst Bellis (1968b) found growth of unialgal cultures of *Cladophora* to occur only within the pH range 7.0-9.0. It does, therefore, seem likely that the pH levels found in flasks in experiment 4 would be sufficient to retard growth of the alga and could explain (in part at least) the results of experiments 2 and 3.

Worthy of mention at this point are the differences in final pH of the two sets of control flasks. Such a comparison indicates that a sizeable proportion of the pH drift found in the three sets of 'treatment' flasks may be attributable to the activity of epiphytic contaminants.

5.2.5. Experiment 5 - Growth evaluation investigation

5.2.5.1. Objectives

Experiments 1-4 had used a 20 or 21 day growth period and had assessed growth by dry weight of alga at the end of this period. Initial dry weight of inocula were, of necessity, ignored. The choice of this experimental duration had largely been based on values reported in the literature and upon experimental constraints. This investigation was therefore performed to follow the growth of cultures, with time, and to choose a more appropriate index of growth for further investigations.

5.2.5.2 Materials and Methods

As Experiment 1. A set of 5 replicate flasks were set up with Langley water and inoculated with *Cladophora* from the River Cole. On day 0 and every fifth day thereafter the alga in each flask was carefully removed with fine forceps and an inoculating loop and layed on a Whatman No. 1 filter until the free liquid was drawn off (this usually took 10 seconds or so). The inoculum was then quickly weighed on a single pan balance and returned to the medium. Using this technique the growth of five algal cultures should be followed. A second series of flasks were similarly inoculated and every five days five flasks were taken - the cultures removed, wet weighed (as described above) and then dried to constant weight at 105°C. In this group of flasks each measurement was therefore made on a separate algal culture.

5.2.5.3 Results and discussion

Table 5.9 presents the results of this experiment. Mean algal weight increase is shown in Fig. 5.1. The weighing of a different 5 cultures at 5 day intervals (Fig. 5.1b and 5.1c) meant that specific growth rate calculated from the equation specific growth rate $\mu = \frac{\ln Wt - \ln Wo}{t}$ could not be applied for each culture individually. However a mean specific growth rate may be applied. Inspection of the curves revealed that exponential growth certainly did not continue after day 5. The mean biomass for day 0 and day 5 were thus entered into the equation above resulting in values of 0.26 (for wet weight determinations - Fig. 5.1b) and 0.27 (for dry weight determinations - Fig. 5.1c). The weighing of the same 5 cultures at 5 daily intervals, however, meant that the appropriate weight on day 0 could be subtracted from the weight on day 5 for each of the 5 cultures, enabling not only a mean specific growth rate to be calculated but also an estimate of its variability. Calculation revealed the mean specific growth rate constant and 95% confidence intervals to the 0.27 ± 0.12. It was felt, however, that the wet weighing technique used as rather subjective. Whitton (1967) had used a slightly more refined wet weighing technique - removing the alga on an inoculating loop, gently blotting free the surplus

Table 5.9. Growth of *Cladophora* in batch culture using 3 methods of growth assessment.

A Same 5 cultures weighed at 5 day time intervals.

Time (days)	Wet weight (mg)					
	Replicates					Mean
0	30.3	78.0	22.5	53.1	41.0	45.0
5	66.2	219.0	174.5	234.3	172.4	173.3
10	81.1	255.8	291.0	240.5	239.3	221.5
15	95.3	413.9	274.1	250.0	241.3	254.9
20	84.0	563.9	435.4	267.3	270.3	324.2
25	109.0	449.1	329.3	256.3	203.8	269.5
30	130.0	721.5	325.3	265.7	267.7	342.0

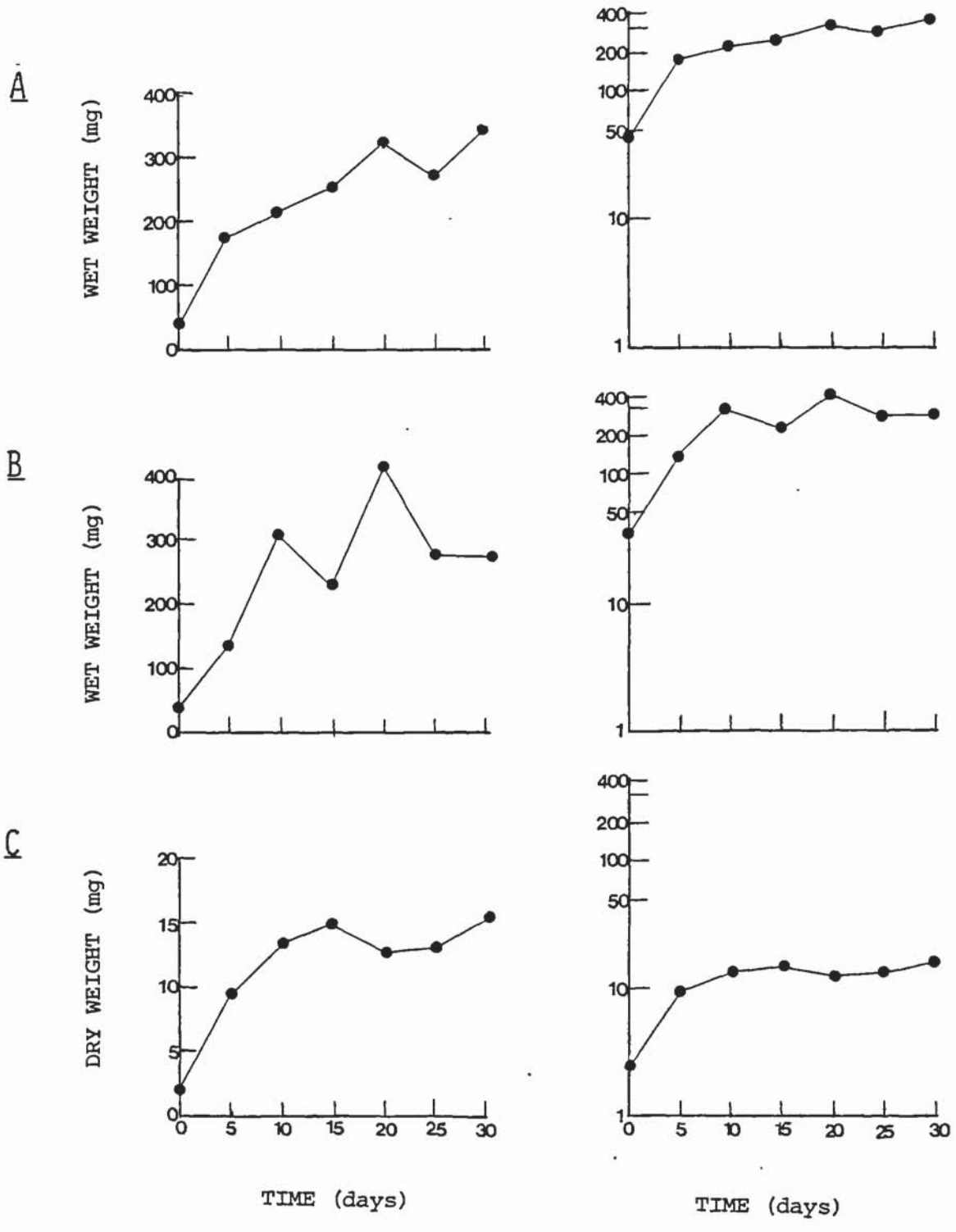
B 5 different cultures weighed at each time interval.

Time (days)	Wet Weight (mg)					
	Replicates					Mean
0	24.0	52.7	26.2	64.1	15.0	36.4
5	150.7	190.8	159.5	16.0	156.3	134.7
10	605.5	88.3	371.6	347.3	140.9	310.7
15	162.1	294.2	294.0	306.8	83.4	228.1
20	328.6	788.0	381.6	316.3	256.3	414.2
25	342.5	482.3	134.4	276.2	154.6	278.0
30	316.9	102.0	112.9	508.3	325.4	273.1

C 5 cultures used for dry weight determination at each interval.

Time (days)	Dry weight (mg)					
	Replicates					Mean
0	2.3	3.1	2.0	3.0	2.0	2.5
5	13.3	11.6	10.7	1.1	11.9	9.7
10	18.4	9.1	13.0	12.6	14.6	13.5
15	10.2	17.5	18.7	22.1	6.6	15.0
20	18.2	4.7	8.2	18.5	14.7	12.9
25	12.6	16.3	11.3	12.6	13.0	13.2
30	15.1	22.9	15.6	10.3	14.2	15.6

Fig. 5.1. Mean weight of *Cladophora* cultures (on linear and logarithmic axes) against time using three methods of weight determination, A) Measuring wet weight of same 5 cultures at intervals B) Measuring wet weight of a different 5 cultures at every interval C) Measuring dry weights of 5 cultures at each interval.



moisture with filter paper, and weighing in a small glass container with water at the bottom. Similarly blotting the culture between two filters under constant pressure for a standard period of time could reduce subjectivity of measurements and likely improve precision. Such techniques would, however, take considerable time to develop and with the apparent inherently high variability of growth of *Cladophora* in batch culture it was felt better to simply use a dry weight determination after a chosen period of time. The reduced variability in using dry weight rather than wet weight (as determined in this study) is indicated by contrasting Fig. 5.1b and 5.1c - the algal cultures which provided these mean values being the same in both cases, differences only arising from the precision of estimation of dry or wet weights. Fig. 5.1c reveals that little further growth occurs after 10 days of incubation, this time was therefore used in further work as experimental duration. The variability of dry weight of cultures may be compared with the variability of specific growth rate by comparisons of their coefficients of variation.

$$\text{Where coefficient of variation (CV)} = \frac{100 \times s}{\bar{x}}$$

s = standard deviation of the sample

$\bar{x}$ = arithmetic mean

Calculation reveals the coefficient for dry weight after 10 days to be 24.9%, whilst the coefficient for specific growth rate is 35.8 %. Clearly then the dry weight value after 10 days is - on the grounds of its lower variability - a better statistic of growth to use.

5.3. Phase 2 - Growth response studies

5.3.1. Introduction and Objectives

The objectives of phase 2 of the batch culture studies were to assess the growth response to various growth promotors and toxicants. The methods used in this section (see section 5.3.2.) were based on the findings of experiments included in phase 1. Since this study

was being carried out concurrently with the continuous culture studies (see Chapter 7), and it was considered that continuous culture was able to provide more information concerning the growth response of *Cladophora* to variation in nutrient levels (e.g. nitrate and phosphate) it was decided to use batch culture to assess the effects of acute toxicants on the alga. Heavy metals were, however, thought better investigated in continuous culture since bioaccumulation in such culture was more easily extrapolated to the natural, lotic, environment in which the alga is found. One group of toxicants which has received little investigation, however, is the herbicides. It was therefore decided to investigate the growth response of *Cladophora* to various herbicides likely to intentionally or unintentionally enter waterways.

The use of herbicides in the United Kingdom is strictly controlled through the Pesticides Safety Precaution Scheme (PSPS) which is a formally negotiated agreement between the Government and the manufacturers Under the scheme manufacturers agree to notify new pesticides and new uses of existing pesticides to the Agricultural and Health Departments and the Health and Safety Executive who are assisted by the independent Advisory Committee on Pesticides. The Government grants clearance for marketing of a pesticide if satisfied that provided the recommended precautions are followed the product can be used without risk to people (whether operators, consumers or those likely to be affected by spray drift), livestock or domestic animals, and with minimal risk to wildlife and the environment in general. In addition to the requirements of the PSPS aquatic herbicides are subject to the following Acts of Parliament - The Rivers (Prevention of Pollution) Acts 1951 and 1965, the Rivers (Prevention of Pollution) (Scotland) Acts 1951 and 1965 and the Northern Ireland Water Act 1972. These Acts apply when aquatic herbicides are used to control weeds growing in or by reservoirs, rivers, streams, ditches and drains, but do not apply when such herbicides are used to control weeds growing in ponds or lakes not discharging to a watercourse. A prerequisite before any application of an aquatic herbicide can take place is that users must consult the appropriate Water Authority to obtain their agreement as to the course of action. Disposal of all types of pesticide are subject to the Deposit of Poisonous Waste Act 1972 and its Regulations and also the Health and Safety at Work etc. Act 1974, the Health and Safety (Agriculture) (Poisonous

Substances) Regulations 1975 and the Control of Pollution Act 1974.

At present there are nine commercially cleared herbicides for use in aquatic situations viz. chlorthiamid, 2,4-D amine, dalapon, dichlobenil, diquat, glyphosate, maleic hydrazide, paraquat and terbutryne. These nine herbicides seemed to be most likely to enter the aquatic environment (either deliberately or accidentally) and were therefore selected for investigation.

5.3.2 Materials and methods

Commercial formulations of herbicides cleared under the PSPS for use in or near waterways were obtained as follows :-

'Prefix' (containing chlorthiamid) from Shell Chemicals (UK) Ltd.

'Dormone' (containing 2,4-D) from Diamond Shamrock Agrochemicals.

'PP Dalapon' '(containing dalapon) from Plant Protection Division, ICI Ltd.

'Casoran G' (containing dichlobenil) from Midox Ltd.

'Reglone 40' (containing diquat) from Plant Protection Division, ICI Ltd

'Roundup' (containing glyphosate) from Monsanto Ltd.

'Chipman Grass Growth Retarder' (containing maleic hydrazide) from Chipman Ltd.

'Clarosan' (containing terbutryne) from Ciba-Geigy Agrochemicals.

'Gramoxone S' the only commercial formulation containing paraquat passed under the PSPS has since been discontinued by ICI Ltd., and was not therefore available for test. 'Chipman Grass Growth Retarder' has similarly been discontinued and replaced by 'Chipman Grass Growth Retarder 50R' which has not yet been approved for use in or near water. A sample of the former compound was obtained, however, and used as an example of a maleic hydrazide containing formulation.

Solutions of each herbicide were made up at the concentration which is the maximum permitted under the PSPS (these values may be found in MAFF (1979)) using membrane filtered Langley water as the

solvent. Various dilutions of these solutions with membrane filtered water allowed a range of concentrations of each herbicide, up to and including the maximum permitted concentration under the PSPS, to be made. 100 ml flasks were each filled with 50 ml solution. 5 replicate flasks were used for each treatment level and between 5 and 7 treatment levels (including the control) were used for each experiment. A 2 cm - long, well branched, piece of *Cladophora*, relatively free from epiphytes, was used as inoculum. Flasks were gently shaken at approximately 60 oscillations per minute, kept at 17 -19°C and continuously illuminated at an intensity of 4000 lux. Growth was measured as algal dry weight after 10 days.

5.3.3 Results and discussion

Table 5.10 presents the dry weight of *Cladophora* produced at different concentrations of the eight herbicides tested. These data were analysed by a single classification analysis of variance (AOV) incorporating partitioning of the treatment sum of squares into component effects (Ridgman, 1975). If any effects were found to be significant at the 5% level a line of best fit and 95% condifence intervals were added. This complete analysis was effected by program ANOV 1 included in appendix 2. The resulting AOV summary tables are included as table 5.11. Table 5.12 summarizes the significance of effects of the eight analyses and Fig. 5.2. shows the growth response of *Cladophora* to those herbicides which cause significant change.

Results show that only diquat (as Reglone 40) and terbutryne (as Clarosan) significantly affect the growth of *Cladophora* at concentrations up to and including the maximum permitted under the PSPS. This is in good agreement with MAFF (1979) who list *Cladophora* spp. as being susceptible to diquat and terbutryne, but present no information concerning the response to the other herbicides. Similarly Spencer-Jones (1981) comments that out of the nine herbicides passed for use in or near waterways only diquat and terbutryne possess algal activity; whilst McLarty (1961) also found diquat to be highly toxic to *Cladophora* in studies in the Great Lakes. Fig. 5.2. reveals that in 10 day batch culture both herbicides prevent growth and/or kill the algal cells at concentrations well below the maximum permitted

Table 5.10. Dry weight of *Cladophora* produced after 10 days in various concentrations of herbicides.

CHLORTHIAMID (mgl^{-1} active ingredient).	Dry weight after 10 days (mg)			Mean dry weight (mg)
	Replicates			
0	15.6	11.7	10.2	12.5
0.1	15.8	9.5	10.8	12.0
0.5	13.5	10.5	9.4	11.1
1	10.6	13.1	13.9	12.5
3	12.3	12.1	12.7	12.4

2, 4 - D (mgl^{-1} active ingredient).	Dry weight after 10 days (mg)			Mean dry weight (mg)
	Replicates			
0	12.7	10.1	7.9	10.2
0.01	10.4	9.4	12.7	10.8
0.1	13.8	8.4	12.3	11.5
0.5	7.7	10.6	7.8	8.7
1	9.1	7.6	8.2	8.3
5	11.7	10.7	6.1	9.5

DALAPON (mgl^{-1} active ingredient).	Dry weight after 10 days (mg)			Mean dry weight (mg)
	Replicates			
0	13.4	9.8	7.6	10.3
1	13.2	10.0	14.8	12.7
2	9.1	13.5	9.0	10.5
5	9.6	10.9	17.5	12.7
10	18.5	10.1	10.1	12.9
20	18.0	9.8	7.7	11.8
30	11.8	12.3	15.4	13.2

DICHLOBENIL (mgl^{-1} active ingredient).	Dry weight after 10 days (mg)			Mean dry weight (mg)
	Replicates			
0	13.4	9.8	7.6	10.3
0.01	12.3	13.6	8.9	11.6
0.1	7.6	9.5	12.7	9.9
0.5	14.1	13.7	11.7	13.2
1	6.0	16.6	9.3	10.6
2	13.5	10.3	9.3	11.0
3	10.7	15.7	5.8	10.7

Table 5.10. Continued.

DIQUAT (mgl^{-1} active ingredient).	Dry weight after 10 days (mg) Replicates			Mean dry weight (mg)
0	10.4	10.4	10.1	10.3
0.05	2.4	5.5	1.3	3.1
0.1	2.4	1.5	1.9	1.9
0.2	2.4	2.4	7.7	4.2
0.5	2.4	3.8	3.8	3.3
1	2.5	2.9	3.7	3.0
2	1.5	2.0	2.4	2.0

GLYPHOSATE (mgl^{-1} active ingredient).	Dry weight after 10 days (mg) Replicates			Mean dry weight (mg)
0	12.1	12.7	15.1	13.3
0.001	10.5	11.5	14.4	12.1
0.005	10.8	14.3	17.8	14.3
0.01	8.4	13.4	10.9	10.9
0.05	17.9	7.6	16.8	14.1
0.1	14.2	11.6	10.1	12.0
0.2	9.3	7.4	13.7	10.1

MALEIC HYDRAZIDE (mgl^{-1} active ingredient).	Dry weight after 10 days (mg) Replicates			Mean dry weight (mg)
0	12.1	12.7	15.1	13.3
0.01	9.0	20.4	4.9	11.4
0.05	9.8	9.3	11.2	10.1
0.1	12.1	9.3	20.1	13.8
0.5	11.5	15.2	8.0	11.6
1	14.4	11.1	11.6	12.4
2	12.7	9.0	17.0	12.9

TERBUTRYNE (mgl^{-1} active ingredient).	Dry weight after 10 days (mg) Replicates			Mean dry weight (mg)
0	5.4	10.2	6.3	7.3
0.001	5.2	6.8	7.5	6.5
0.005	4.3	5.9	8.6	6.3
0.01	1.7	4.0	2.8	2.8
0.02	3.5	4.4	2.8	3.6
0.05	2.2	2.0	2.3	2.2
0.1	1.9	2.9	1.2	2.0

Table 5.11. AOV summary tables - growth response of *Cladophora* to various herbicides.

CHLORTHIAMID

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	4	4.0708	1.0177	.192793
LINEAR EFF.	1	.231478	.231478	4.38512E-02
QUAD EFF.	1	.1126	.1126	.021331
CUBIC EFF.	1	3.70497	3.70497	.70187
RESIDUAL	1	2.17552E-02		
ERROR	10	52.7871	5.27871	
TOTAL	14	56.8579		

2, 4 - D

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	5	23.05	4.61001	.966909
LINEAR EFF.	1	2.12153	2.12153	.444973
QUAD EFF.	1	15.6307	15.6307	3.27839
CUBIC EFF.	1	.202661	.202661	4.25064E-02
RESIDUAL	2	5.0952		
ERROR	12	57.2134	4.76778	
TOTAL	17	80.2634		

DALAPON

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	24.7295	4.12158	.300406
LINEAR EFF.	1	6.68578	6.68578	.4873
QUAD EFF.	1	.934304	.934304	6.80977E-02
CUBIC EFF.	1	6.42523	6.42523	.46831
RESIDUAL	3	10.6842		
ERROR	14	192.081	13.72	
TOTAL	20	216.81		

DICHLOBENIL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	20.7524	3.45874	.297069
LINEAR EFF.	1	8.04044E-02	8.04044E-02	6.90588E-03
QUAD EFF.	1	1.42911	1.42911	.122745
CUBIC EFF.	1	2.4304	2.4304	.208745
RESIDUAL	3	16.8125		
ERROR	14	163.	11.6429	
TOTAL	20	183.753		

Table 5.11. Continued.

DIQUAT

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	151.103	25.1838	11.3222
LINEAR EFF.	1	28.2517	28.2517	12.7015
QUAD EFF.	1	8.02955	8.02955	3.60995
CUBIC EFF.	1	19.6352	19.6352	8.82767
RESIDUAL	3	95.1864		
ERROR	14	31.14	2.22428	
TOTAL	20	182.243		

GLYPHOSATE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	44.8701	7.47835	.728477
LINEAR EFF.	1	15.7401	15.7401	1.53327
QUAD EFF.	1	3.94937	3.94937	.384714
CUBIC EFF.	1	1.51562	1.51562	.147639
RESIDUAL	3	23.665		
ERROR	14	143.72	10.2657	
TOTAL	20	188.59		

MALEIC HYDRAZIDE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	29.3779	4.89632	.260515
LINEAR EFF.	1	1.39607	1.39607	.07428
QUAD EFF.	1	.417583	.417583	2.22181E-02
CUBIC EFF.	1	.176506	.176506	9.39122E-03
RESIDUAL	3	27.3878		
ERROR	14	263.126	18.7947	
TOTAL	20	292.504		

TERBUTRYNE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
TREATMENT	6	90.5915	15.0986	6.88833
LINEAR EFF.	1	50.2958	50.2958	22.9461
QUAD EFF.	1	21.2984	21.2984	9.71683
CUBIC EFF.	1	8.19103	8.19103	3.73694
RESIDUAL	3	10.8063		
ERROR	14	30.6867	2.19191	
TOTAL	20	121.278		

Table 5.12. Summary table of significance of component effects of analysis of variance for herbicide investigations.

Herbicide	Significance of overall treatment effect	Significance of component effects		
		Linear	Quadratic	Cubic
Chlorthiamid	N.S.	N.S.	N.S.	N.S.
2, 4 - D	N.S.	N.S.	N.S.	N.S.
Dalapon	N.S.	N.S.	N.S.	N.S.
Dichlobenil	N.S.	N.S.	N.S.	N.S.
Diquat	***	**	N.S.	*
Glyphosate	N.S.	N.S.	N.S.	N.S.
Maleic Hydrazide	N.S.	N.S.	N.S.	N.S.
Terbutryne	**	***	**	N.S.

LEGEND

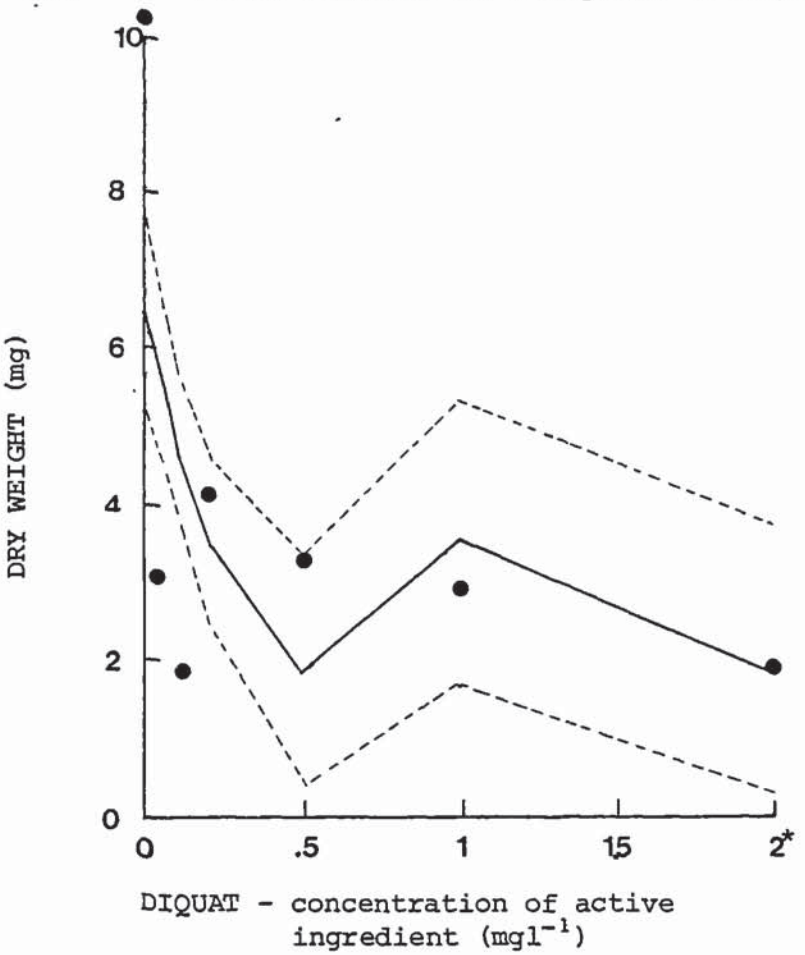
N.S. = not significant at the 5% level

* = significant at the 5% level

** = significant at the 1% level

*** = significant at the 0.1% level

Fig. 5.2. Growth response of *Cladophora* to diquat and terbutryne.



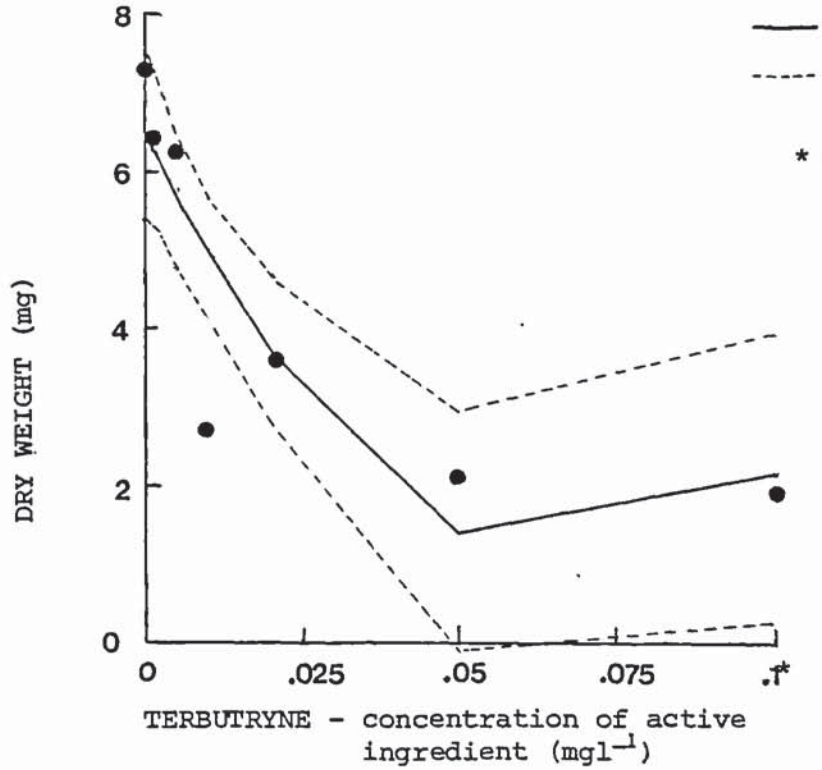
LEGEND

● = Mean (n=3)

——— = Line of best fit

----- = 95% confidence intervals

* = Maximum permitted concentration under the PSPS.



under the PSPS. However it cannot be assumed that similar results may be obtained when similar concentrations are applied to algae under natural conditions. McLarty (1961) pointed out that under natural conditions the chemical properties of the water and the presence of living organisms may have a profound effect on the activity of the algicide; whilst more particularly the problem of maintaining a killing concentration in the vicinity of the algal growth for sufficiently long time is critical in the case of practical control measures.

Diquat is known to be more quick acting (24 hours) than terbutryne (7 days) in its activity (Spencer-Jones, 1981). This experiment, however, with the alga and algicide in contact throughout the 10 day experimental period, did not discriminate between the speed of effect of herbicides. Neil (1962), however, exposed *Cladophora* to various algicides for periodsof 1 or 4 hours and then grew the alga for 6 days to assess the effect. Results indicated that both exposure time and temperature had effects on the damage caused.

It would seem unlikely, therefore, that any of the herbicides which failed to show algicidal activity in this experiment would demonstrate such activity in field situations in which reduced contact time and other factors would be likely to reduce efficacity. The exact effects of diquat and terbutryne would also be rather difficult to predict in field situations for some of the reasons described above, especially in the fast flowing riffles in which *Cladophora* is normally found. However, the value of the laboratory experimental technique, as described, is that it does indicate the relative algicidal merits of the herbicides considered, and shows those worthy of further investigation in the field situation.

5.4. Conclusions

1. Using Langley water as medium in 20 day batch culture it was found that *Cladophora* growth was limited before the end of the experimental period. Regular replacement of medium yielded significantly higher algal biomass.

2. In a 21 day batch culture experiment the pH of the inorganic growth medium drifted from 8.0 ± 0.1 to 9.2-10.2. Evidence from the literature suggests that these pH values may inhibit further growth of *Cladophora*.

3. Yield (dry weight) of *Cladophora* after 10 days growth was considered preferable to specific growth (using wet weight data from day 0 and 5) as an index of growth, on the grounds of lower variability and reduced subjectivity of measurement. Coefficients of variation for the two techniques were 24.9% and 35.8% respectively.

4. Of 8 herbicides tested for activity against *Cladophora* only diquat (as Reglone 40) and terbutryne (as Clarosan) were found to have any significant effect. In 10 day batch culture both reduced growth and/or killed the alga at concentrations well below the maximum permissible under the PSPS.

6. *CLADOPHORA* CONTINUOUS CULTURE STUDIES - DEVELOPMENT OF TECHNIQUES

6.1. Introduction

Initial studies of *Cladophora* growth in small-scale laboratory streams (described in detail by Peters, 1977) unfortunately suffered from problems of contamination, and it was considered that such contamination was a likely result of the recirculation of medium - natural river water - necessary in running such streams. It was reasoned however, that a culture technique in which flow was much slower would allow a 'flow-through' culture method to be employed, and combined with a unialgal culture of *Cladophora*, would prevent problems associated with contamination by eukaryotic organisms. In search for such a technique it became evident that the continuous-flow method used for fungal culture (Hawkes, 1965; Williams, 1971) may also prove useful for the culture of filamentous algal species, such as *Cladophora*, with appropriate modifications.

Hawkes (1965), in his study of the fungi of percolating filters devised a technique of dripping sewage from a Mariotte bottle (described in Brown, 1950) over a basket, upon which a fungal hypha grew. Williams (1971) developed the technique further, devising a culture flask in which the basket was so held as to allow it to be weighed *in situ* thus enabling growth of the fungus to proceed under axenic conditions and be followed by making daily wet weight determinations. Nutrient supply was maintained by peristaltic pumps rather than Mariotte bottles. This experimental technique is summarized schematically in fig. 6.1 and the culture flask used by Williams (1971) depicted in fig. 6.2. Initial findings showed that a unialgal inoculum of *Cladophora* grew well in such flasks if provided with autoclaved river water as medium, and illuminated. Prior to using the apparatus to investigate the algal growth response to variation in various physico-chemical factors, however, it was necessary to carry out some preliminary investigations to define methodology : a series of six experiments was consequently planned.

Fig. 6.1. Schematic representation of culture technique of Williams (1971).

Fig. 6.2 Culture flask of Williams (1971)

LEGEND

A1, A2	Air Inlets (cotton wool plugged)
B	Basket Port
E	Effluent Outlet
F	Feed Inlet
G	Glass Stoppered Port

6.2 Experiment 1 - Investigation of wet weight assessment

6.2.1 Introduction and Objectives

Williams (1971) had made wet weight determinations at daily intervals by draining the basket, upon which the fungus grew, for 10 minutes prior to weighing *in situ*. This study did not attempt to maintain axenic conditions, consequently (with the available apparatus) it was felt better to simply remove the basket carefully from the flask and weigh it on a light plastic and wire framework positioned on an Oertling single pan balance.

It was envisaged that the stainless steel (12 mesh/inch) basket used by Williams (1971) may present problems in later studies, planned to investigate the effect of various metal ions on algal growth. After the evaluation of several materials nylon mesh (precise specification given in section 6.2.2) was adopted for all further work. This satisfied the necessary criteria of being inert, rigid, light and easily fashioned into the required shape.

Williams (1971) had evaluated the relative merits of a variety of basket shapes. In this study, however, Williams' dish-shaped basket was replaced by a simple flat basket enabling a variety of pre-weighing techniques (such as blotting to remove excess moisture) easily operable. Hopefully such techniques would improve the accuracy of the wet weight determinations. The objective of this experiment was to see which (if any) of 3 pre-weighing techniques could improve the wet/dry weight correlation above that found when using a technique similar to that of Williams (1971).

6.2.2 Materials and methods

The culture flask (fig. 6.2) comprised a 1 litre Quickfit culture vessel (FVIL) with a multisocket flange adaptor (MAF 2/32) serving as a lid. The vessel was modified by the addition of an effluent outlet (E - fig. 6.2) and air inlet (Al-fig. 6.2). The four parts in the lid provided for a feed inlet tube in the 10° port (F - fig. 6.2)

(comprising a modified B 19/26 cone to which was added an angled delivery tube), an air inlet tube (A2-fig. 6.2) a basket support in the central port B-fig. 6.2), and a glass stoppered part (G-fig. 6.2). The basket part was fitted with a B19/26 cone with ground upper end over which was fitted an Oxoid test-tube cap; the centre of which was pierced by a length of nichrome wire, sealed in with Araldite epoxy resin. The lower end of the nichrome wire was bent to form a hook to facilitate suspension of the basket. The basket itself comprised a 4.0 cm square of nylon mesh (Nybolt grit gauze No 10GG-2000; aperture 0.2 cm, 3.6 threads/cm; John Staniar and Co. Ltd., Manchester Wire Works, Manchester) to which a 12 cm length of 1mm diameter stainless steel wire was attached with Aquaria silicone rubber sealant, allowing the basket to be hung from the nichrome wire hook under the basket port.

A moderately eutrophic local pond water obtained from Langley mill pool (Nat. grid ref. SP 153968) served as medium. This water, henceforth referred to simply as Langley water, was collected at intervals stored, in plastic courtesy tanks at 15°C, and aerated. Prior to use for experimentation water was filtered through Whatman GF/C filters to remove micro-invertebrates and suspended solids, collected in 3 or 10 litre Pyrex aspirators and 'free-steamed' in an autoclave for 40 or 60 minutes depending on volume. This ensured a medium free from algal contaminants. Prior to the start of an experiment, a sample of the water was removed for chemical analysis. Analyses carried out and methods of analysis are included as table 6.1. Results of analyses for experiments described in this chapter are included as table 6.2. Medium was supplied to the flasks using type-T DCL micropumps, providing a flow rate of 0.5 ml/min.

A unialgal culture of *C. glomerata* (ref 505/3, Culture Collection of Algae and Protozoa, Cambridge-isolated by George, 1950, in England) served as inoculum.

All tubing was silicone rubber and autoclaved (15 lb/in^2, 15 minutes) prior to use. Pyrex culture flasks were dry sterilized at 160°C for 1 hour. Nylon baskets were repeatedly immersed in boiling distilled water.

Table 6.1 Physico-chemical analyses carried out routinely during experimentation.

Parameter (Unit)			Method of sample analysis
pH			Model 292 pH meter, Pye Unicam Ltd./ Model 6320 pH Transmitter, EIL./ Digital 110 expanded scale pH meter Corning-EEL.
Total Alkalinity		(mgl^{-1} as $CaCO_3$)	DOE (1972)
Phenolphthalein Alkalinity		(mgl^{-1} as $CaCO_3$)	DOE (1972)
Total Hardness		(mgl^{-1} as $CaCO_3$)	BDH test - modified Schwarzenbach method
Calcium Hardness		(mgl^{-1} as $CaCO_3$)	BDH test - modified Schwarzenbach. method
NH_3-N		(mgl^{-1})	Chapman *et al.* (1967) using Technicon Auto-Analyser
NO_3-N		(mgl^{-1})	Chapman *et al.* (1967) using Technicon Auto-Analyser
NO_2-N		(mgl^{-1})	Chapman *et al.* (1967) using Technicon Auto-Analyser
PO_4-P		(mgl^{-1})	Technicon Auto-Analyser industrial method 3-68W (1969) or 93-70W(1971)
Na^+		(mgl^{-1})	EEL flame photometer Evans Electroselenium Ltd., Halstead, Essex.
K^+		(mgl^{-1})	EEL flame photometer Evans Electroselenium Ltd., Halstead, Essex.
Chloride		(mgl^{-1})	EEL Chloride Meter, Evans Electroselenium Ltd., Halstead, Essex.
Total	Cd	(mgl^{-1})	250 ml water sample plus 5 ml atomic absorption grade HNO_3 (approx 70%, SG 1.42) reduced to dryness. Re-dissolved in 2 ml atomic absorption grade HCl (approx 36%. SG 1.18) and made up to 25 ml in volumetric flask. Aspirated directly to atomic absorption spectrophotometer (Model 373 Perkin-Elmer Ltd., Bucks).
	Cr	(mgl^{-1})	
	Cu	(mgl^{-1})	
	Fe	(mgl^{-1})	
	Ni	(mgl^{-1})	
	Pb	(mgl^{-1})	
	Zn	(mgl^{-1})	

Table 6.2 Results of physico-chemical analysis - Experiments 1-6.

PARAMETER (mgl^{-1})*	EXPERIMENT						
	1	2	3	4		5	6
			Block 1	Block 2	Block 3		
pH	8.3	8.2	8.2	8.2	8.3	8.5	8.1
Total Alkalinity	140	130	135	140	160	170	120
Phenolphthalein Alkalinity	5	0	5	5	10	20	0
Total Hardness	304	270	298	348	342	348	282
Calcium Hardness	162	144	192	210	204	210	214
NH_3-N	0.3	0.4	-	-	-	0.1	0.3
NO_3-N	5.6	3.0	9.2	7.0	6.4	9.7	10.6
NO_2-N	0.025	0.077	0.025	0.015	0.03	0.035	0.05
PO_4-P	0.1	0.1	0.2	0.8	0.5	0	0.1
Na^+	-	17	-	-	-	28	19
K^+	-	5	-	-	-	6	8
Chloride	29	27	30	38	32	45	34
Total Cd	0.001	0.001	-	-	-	0.001	0.002
Cr	0.000	0.005	-	-	-	0	0
Cu	0.023	0.016	-	-	-	0.015	0.020
Fe	0.094	0.090	-	-	-	0.054	0.140
Ni	0.009	0.008	-	-	-	0.010	0.012
Pb	0.007	0.028	-	-	-	0.023	0.023
Zn	0.0089	0.0154	-	-	-	0.0190	0.0183

* Except pH. Alkalinity and hardness mgl^{-1} as $CaCO_3$

Illumination was supplied from above by 6ft 65/80W Thorn 'white' fluorescent tubes, providing an intensity of 4000 lux at the level of the baskets, on a 16 hour light : 8 hour dark photoperiod. Light intensity measurements were made by using an EEL portable photoelectric photometer (Evans Electroselenium Ltd., Halstead, Essex). The entire apparatus was placed in a darkened constant temperature room at 15±1°C. The heating action of the lights, however, acted so as to locally raise the temperature to 17-19°C in the vicinity of the culture flasks during the light period. Temperature was monitored by continuous recording thermometer.

Baskets were weighed prior to inoculation with a small tuft of *Cladophora* (estimated as being 1-10 mg dry weight). The cultures were then allowed to grow for periods upto 30 days, providing a range of culture sizes for wet weight determinations and subsequent comparison with dry weight. At 1-2 day intervals cultures were weighed by four wet weighing methods - performed in random order, and allowing at least one hour under normal culture conditions between each determination - then removed from the baskets and dried to constant weight at 105°C. The four wet weighing techniques used in the investigation are as follows :-

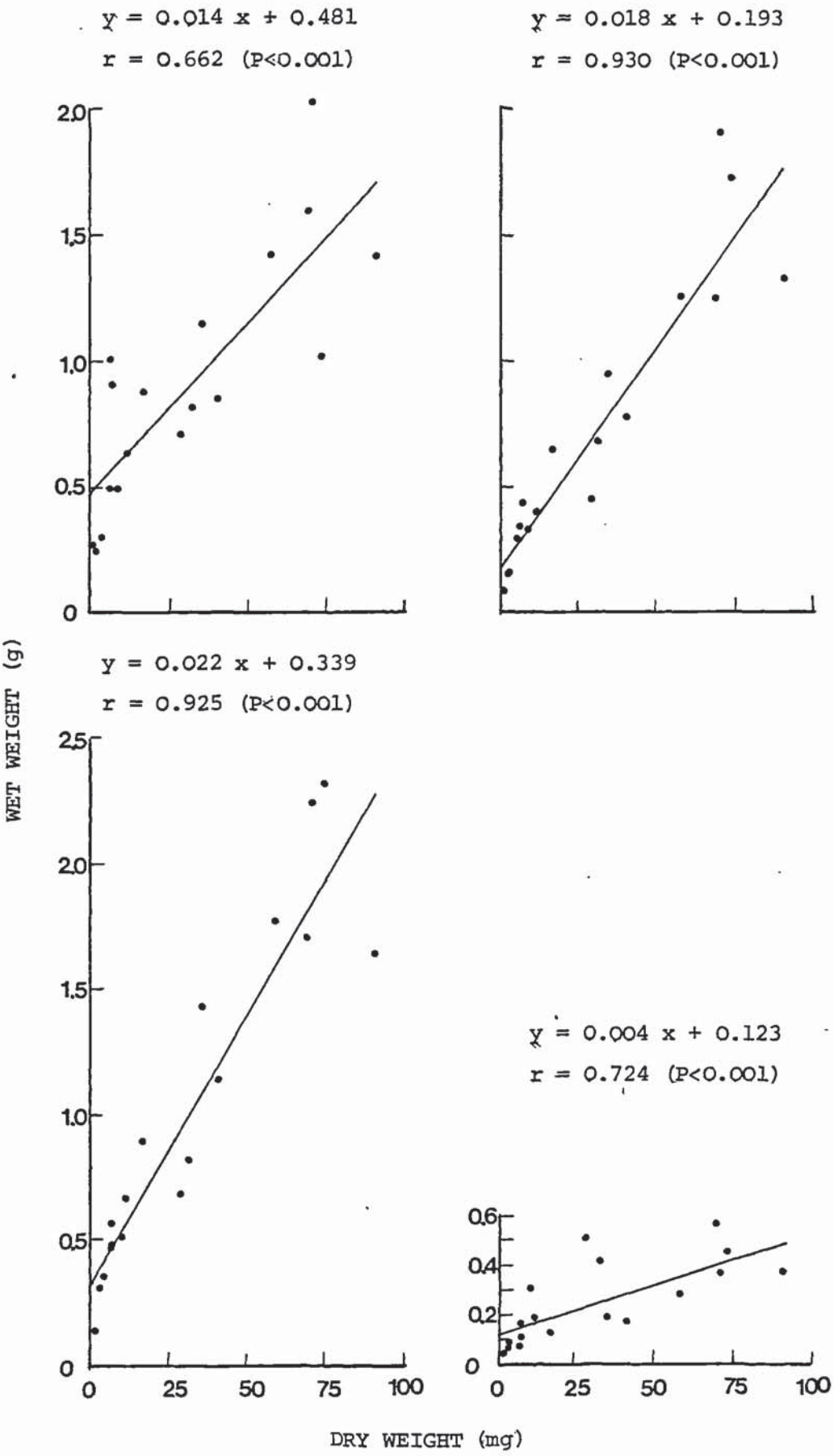
(A) The nutrient flow was stopped, and the basket carefully removed and weighed immediately. (it was not considered necessary to leave the cultures for 10 minutes as did Williams (1971) since no drainage of the cultures was observed over this period : excess medium was most likely to leave the basket upon its removal from the culture flask, if at all.)

(B) As A except that prior to weighing, the basket was gently placed in a 7.0 cm diameter Whatman No. 1 filter, (positioned on a wooden laboratory bench) and firmly held for 5 seconds to remove excess medium. Any remaining drops of medium, held within the mesh by surface tension, were then removed with the edge of the same filter paper, and the basket weighed.

Table 6.3 Wet and dry weight values of *Cladophora* from Experiment 1.

Wet Weight (g)				Dry Weight (mg)
Weighed Immediately	5 second blot	5 second blot plus re-wetting	30 second blot under suction	
0.261	0.084	0.130	0.045	1.6
0.291	0.162	0.339	0.061	2.7
0.496	0.301	0.466	0.170	5.6
0.247	0.156	0.309	0.082	2.4
1.010	0.441	0.563	0.106	6.2
0.886	0.657	0.893	0.133	15.9
0.896	0.342	0.478	0.078	6.8
1.150	0.952	1.431	0.185	34.8
0.852	0.783	1.149	0.170	40.3
1.014	1.723	2.331	0.450	73.6
1.407	1.328	1.637	0.365	90.4
2.026	1.905	2.245	0.367	69.8
1.601	1.255	1.712	0.558	68.5
1.425	1.263	1.770	0.277	57.0
0.816	0.677	0.816	0.407	31.4
0.708	0.456	0.686	0.499	27.7
0.628	0.406	0.663	0.181	11.0
0.486	0.330	0.508	0.312	9.2

Fig. 6.3 Regression of wet weight against dry weight of *Cladophora* cultures from experiment 1.



(C) As B but additionally, following blotting, distilled water was run over the culture from a wash bottle until saturated. Once dripping had ceased the basket was weighed immediately.

(D) As B except the basket was blotted for 30 seconds on a Whatman No. 1 filter placed on a scintered glass filter holder and under suction (better than 26 inHg).

6.2.3. Results and discussion

Results were shown in table 6.3. Regression lines of wet weight against dry weight were calculated and are included in fig. 6.3. The associated correlation coefficients (r) are all significant ($P<0.001$, $n = 18$) : r^2 values evaluate the proportion of the variance of y (wet weight) attributable to its linear regression on X (dry weight). These show that for wet weight determination method B (5- second blot) 86% of the variance of wet weight is attributable to its linear regression on dry weight. Or conversely only 14% of the variance is free from its linear regression on dry weight. On the basis of these results, and after consideration of the probability of contamination and cell damage likely to be caused by each of the four techniques, the 5-second blotting method was adopted in all further studies as the method of biomass determination.

6.3 Experiment 2 - Investigation of the *Cladophora* growth curve

6.3.1 Introduction and objectives.

Using the '5-second blot' method of biomass determination, the objective of this experiment was to investigate the growth curve produced by *Cladophora* under conditions of continuous nutrient flow.

6.3.2 Materials and methods

As experiment 1. A single culture was used. The basket was weighed prior to inoculation with a small tuft of *Cladophora* (approximately 100 mg wet weight). It was then placed in the culture flask for one hour, after which time it was removed and blotted for 5 seconds

on a 7.0 cm diamater Whatman No. 1 filter. Any remaining drops were removed with the edge of the same filter paper and the basket was hung on a light plastic and wire framework positioned on an Oertling single pan balance. A wet weight determination was made and the basket replaced in the culture flask. Growth was followed by making such determinations every 48 ± 2 hours over the 18 day experimental period. Cultures were also photographed at 2 day intervals.

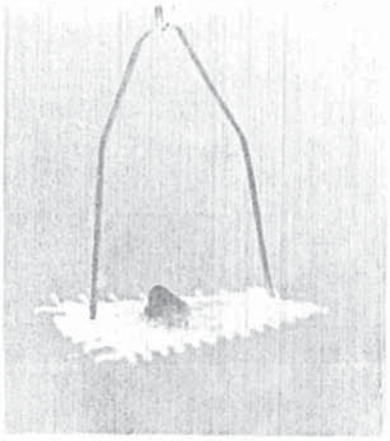
6.3.3 Results and discussion

Raw biological (= wet weight) data are included with 'time-lapse' photographs as plate 6.1. Fig. 6.4a plots cumulative algal wet weight against time to show the growth of *Cladophora* in continuous culture.

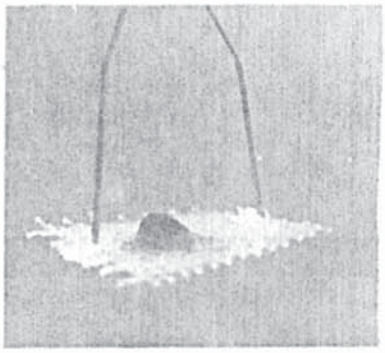
Essentially the culture method may be described as a heterogeneous, two-phase, closed continuous culture, according to the classification of Herbert (1960). This involves growth of the *Cladophora* on a solid phase over which the liquid phase (i.e. nutrient medium) is passed - all, or nearly all, of the biomass produced being retained on the solid phase (= basket). Consideration of microbial growth kinetics indicates that growth, in such a system, would proceed through a lag phase and an exponential phase. Righelato (1975) however comments that when substrates are supplied continuously to a culture exponential growth ceases when the exponentially increasing demand for substrates depletes their concentration in the medium. The rate of supply of substrates to the culture then determines the growth rate, and the growth curve takes the form dictated by the substrate feed rate. If the feed rate is more or less constant the growth rate ($^{\delta W}/\delta t$) would also be constant, but the specific growth rate ($^{1}/W \times {}^{\delta W}/\delta t$) would fall as the organism concentration increases (Righelato, 1975). A phase of linear (or incremental) growth would therefore be expected to follow exponential growth, and would only cease when some component of the reactor became altered or limiting.

The expected growth curve would thus be as fig. 6.5.

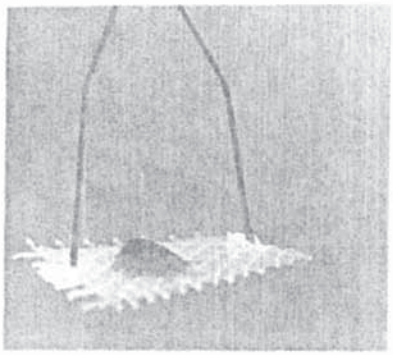
Plate 6.1 The growth of *Cladophora* in closed continuous culture.



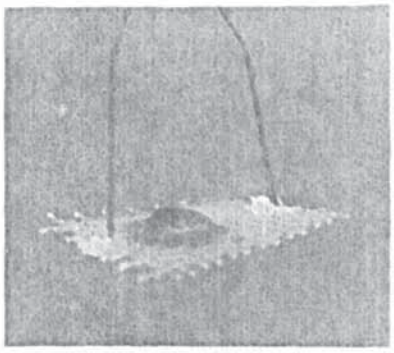
DAY 0 (INOCULUM)
0.131g wet weight



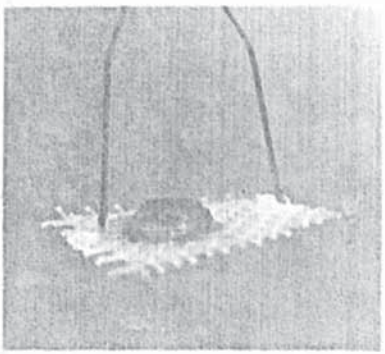
DAY 2
0.164g wet weight



DAY 4
0.171g wet weight

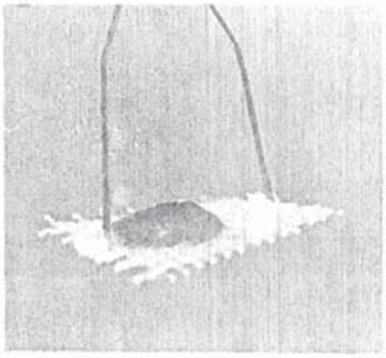


DAY 6
0.265g wet weight

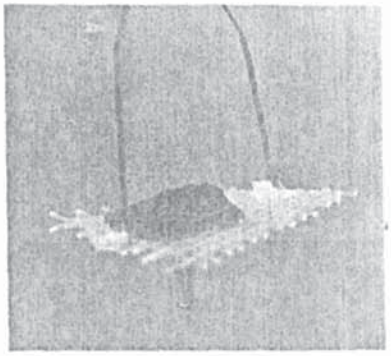


DAY 8
0.420g wet weight

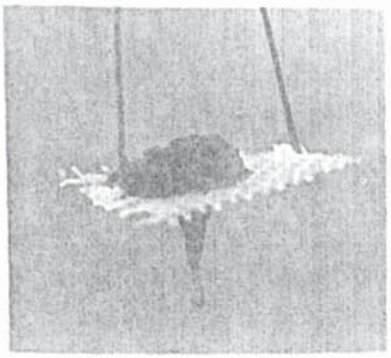
Plate 6.1 Continued.



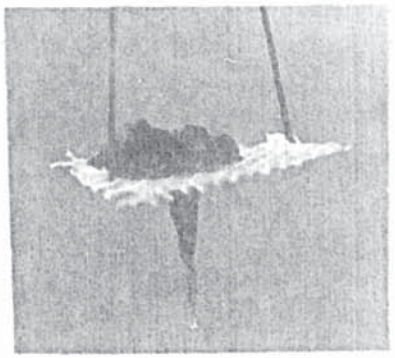
DAY 10
0.680g wet weight



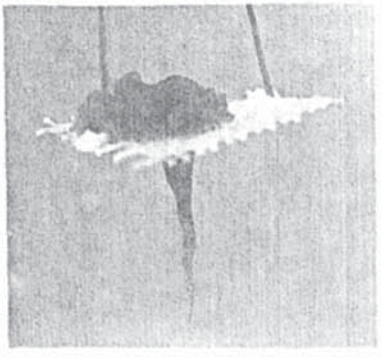
DAY 12
0.881g wet weight



DAY 14
1.123g wet weight



DAY 16
1.378g wet weight



DAY 18
1.433g wet weight

Fig. 6.4 Growth curve of *Cladophora* in closed continuous culture :-

A. y axis untransformed
B. y axis ln transformed
C. y axis $\sqrt[3]{}$ transformed

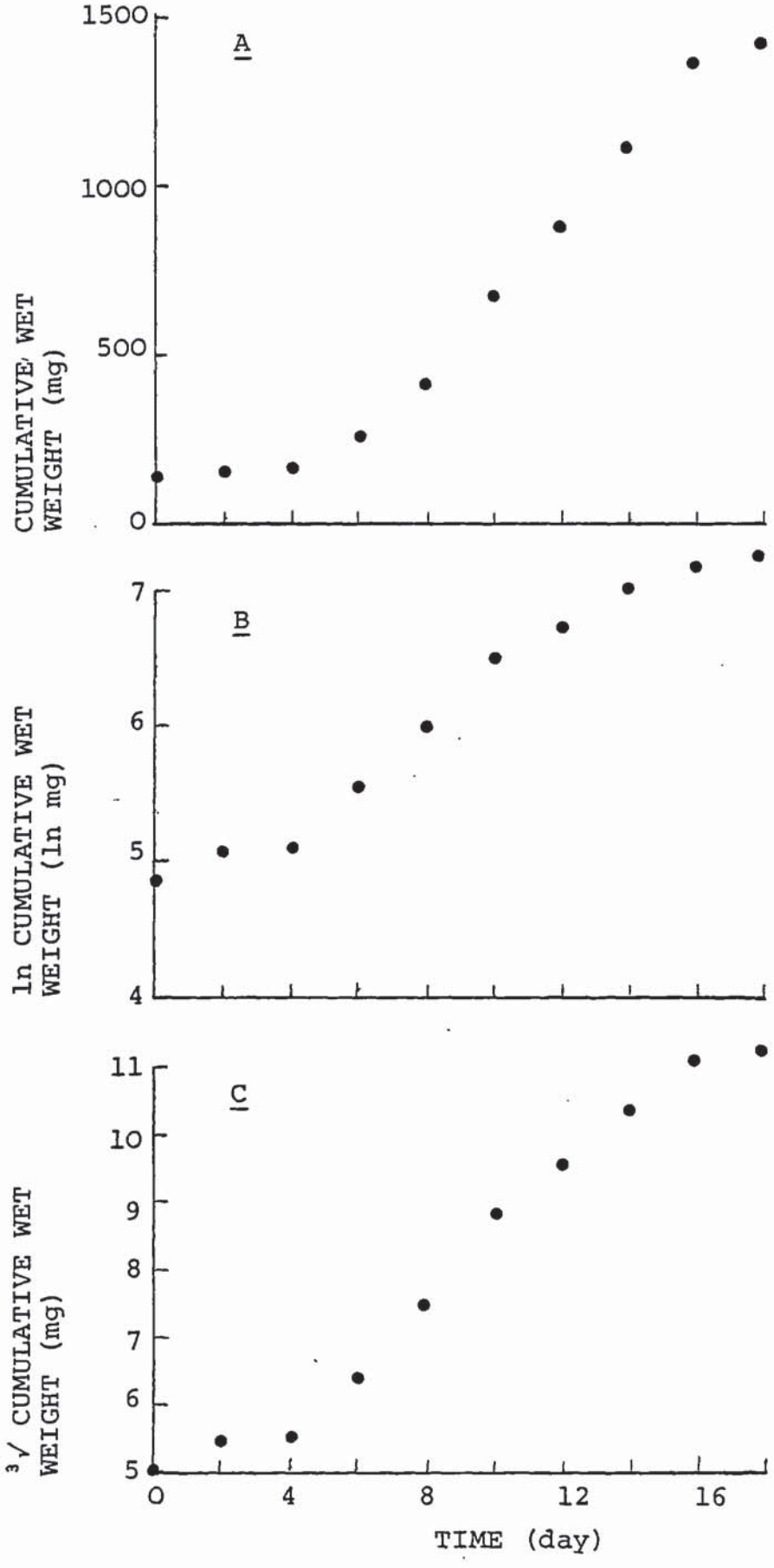
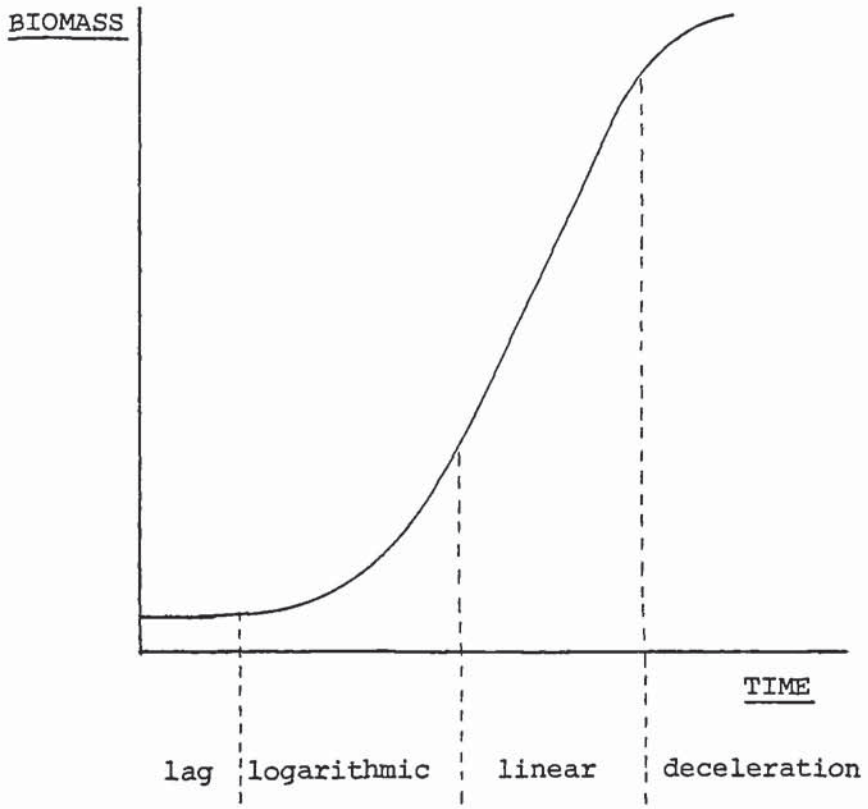


Fig. 6.5 Expected (theoretical) growth curve of *Cladphora* in closed continuous culture.



Comparison of fig. 6.4a and 6.5 reveals good agreement in the shape of observed curves (fig. 6.4) with those expected from consideration of the kinetics of growth (fig. 6.5). Inspection of growth curves produced by the fungi *Sepedonium* sp. and *Ascoidea rubescens* (Williams, 1971) and the freshwater sponges *Ephydatia fluviatilis* and *Spongilla alba* (Poirrier *et al.*, 1981) grown in closed continuous culture reveals curves similar to those produced by *Cladophora*. The growth of *Cladophora* (and indeed any alga) in such closed continuous culture, is however, rather more complex than either of the aforementioned cases owing to its, photoautotrophic habit. Plate 6.1 shows the 'pellet-like' habit of growth, and under such circumstances self-shading would result in the culture becoming heterogeneous - exponential growth would be restricted to the periphery of the pellet, whilst the inner 'core' would be light limited, Such a situation is quite analogous to that of pellet-growth of fungal mycelium which Pirt (1966) considered to result in 'cube-root' rather than true exponential growth. Visual inspection of the growth curve (fig. 6.4a) shows the lag in growth up to day 4.

Growth then proceeds through a period of exponential growth (approximately day 4 - 10) and a period of incremental growth (approximately day 10 onwards) the apparent deceleration on day 18 is not considered to be a 'true' effect since other cultures had grown to greater then 1.5g wet weight without displaying any such signs of deceleration in incremental growth rate. Fig 6.4b and c plot growth data after logarithmic and cube-root transformation respectively. The linear regression of such transformed data against time, over various time intervals, allowed accompanying correlation coefficients to be calculated - these are included as table 6.4.

Table 6.4. Correlation coefficients (r) for the linear regression of transformed data against time.

Time interval over which r calculated	ln transformed data	Cube-root transformed data
DAY 4 - 10	0.9998	0.9960
" 4 - 12	0.9958	0.9973
" 4 - 14	0.9913	0.9970
" 4 - 16	0.9867	0.9962
" 4 - 18	0.9736	0.9874

Best correlation between ln transformed data, and time occurs over the period 'day 4 - 10', with correlation decreasing as the time interval (over which the correlation is measured) is extended. For cube-root transformed data, however, best correlation occurs over the period '4 - 12' and the reduction in correlation as time is extended is not so marked as with ln transformed data. The very high correlation over the period 'day 4 - 10' or 'day 4 - 12', however, indicates that data fits both models with good agreement. However, the slightly higher correlation coefficient found with ln transformed data and the easier calculation of doubling times etc. from such data suggests that growth is better considered as exponential during this phase of the growth curve, even though growth tends to produce a pellet of filaments.

Fogg (1965) considered the situation in cultures of limited volume in which as a culture becomes dense only the cells at the surface receive a light intensity saturating for photosynthesis, the bulk of the culture being light limited and, if the culture is very dense, in virtual darkness. In this situation growth is no longer determined by the size of the population but by the rate of light absorption. The growth curve therefore changes its character from exponential to linear i.e. growth becomes directly proportional to time (Myers, 1953). It is, however, not considered that such an explanation holds true for the change in *Cladophora* growth rate in closed continuous culture since growth in the system is not apparently limited by the volume of the culture flask when growth rate changes from exponential to incremental. It thus seems more likely that this change in growth rate is attributable to the exponentially increasing demand for substrates depleting the concentration of substrates so that the growth curve takes the form dictated by the substrate feed rate, as described by Righelato (1975).

6.4 Experiment 3 - Investigation of experimental stability

6.4.1 Introduction and objectives

Objectives were to assess whether variability of growth in culture was small enough to enable 'realistic' differences to be detected between variously treated cultures. Various concentrations of total ammoniacal nitrogen were used as treatment levels.

6.4.2 Materials and methods

As experiment 1. Preliminary investigation revealed Langley water to contain approximately 0.3 mgl^{-1} NH_3-N. Nominal treatment levels of 0.3, 1, 2, 4, 6, 8 and 10 mgl^{-1} NH_3-N were set by the addition of appropriate small volumes of a 23.6 gl^{-1} $(NH_4)_2\ SO_4$ stock solution. Shortage of apparatus necessitated the experiment to be designed in blocks, with seven treatment levels and three blocks providing the replication. Baskets were inoculated with a small tuft of *Cladophora* (approximately 200 mg wet weight). Cultures were grown for 18 days. Water samples, for chemical analysis were taken three times from the influent of each flask throughout the duration of the experiment, on day 1, 9 and 17. Samples were acidified, with

the addition of six drops of concentrated hydrochloric acid (SG 1.16) from a Pasteur pipette, to arrest biological activity and were placed in a refridgerator at 5°C to await analysis. Total ammoniacal nitrogen (TA-N or NH_3-N) was determined according to the method of Chapman *et al.* (1967) involving the use of a Technicon Auto-Analyser. At the end of the experiment *Cladophora* was manually removed from the baskets with forceps and dried to constant weight at 105°C.

6.4.3 Results and discussion

Chemical analysis of the Langley water used in the experiment is included as table 6.2. *Cladophora* growth data is included as table 6.5. Treatment (NH_3-N) levels in table 6.5 are mean levels resulting from the analysis of total ammoniacal nitrogen described in section 6.4.2. Fig. 6.6 shows the mean growth of the algal cultures over the 18 day experimental period. Data for algal dry weight (after 18 days growth) was entered into an analysis of variance incorporating partitioning of the treatment sum of squares using orthogonal polynomial coefficients (Ridgman, 1975). The resulting analysis of variance summary table is included as table 6.6; whilst fig. 6.7 plots *Cladophora* dry weight against the total ammoniacal nitrogen concentration in the medium, and includes line of best fit and confidence limits.

Visual inspection of fig. 6.7 clearly shows the growth inhibiting (= toxic) effect of increasing total ammoniacal nitrogen concentration. To assess the variability of growth in the experiment the coefficient of variation was calculated.

Where coefficient of variation (cv) $= \frac{s}{\bar{x}} \times 100$

and s = standard deviation of the sample

$\bar{x}$ = arithmetic mean

Using the error mean square from table 6.6 as an estimate of variance the equation becomes

$$cv = \frac{\sqrt{(\text{error mean square})}}{\text{general mean}} \times 100$$

Table 6.5 *Cladophora* growth data for Experiment 3 : growth response to variation in NH_3-N concentration.

Treatment (NH_3-N mgl^{-1})	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)										Final dry weight (mg)
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18	
0.3	0.118	0.000	0.028	0.081	0.157	0.250	0.371	0.571	0.665	0.702	0.725	16.8
	0.291	0.000	0.095	0.231	0.341	0.663	0.953	1.228	1.538	1.530	1.520	71.4
	0.309	0.000	0.076	0.080	0.525	0.758	0.792	0.927	1.108	1.283	1.677	65.0
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.066	$\bar{x}$ = 0.131	$\bar{x}$ = 0.341	$\bar{x}$ = 0.557	$\bar{x}$ = 0.705	$\bar{x}$ = 0.909	$\bar{x}$ = 1.104	$\bar{x}$ = 1.172	$\bar{x}$ = 1.307	
1.5	0.132	0.000	-0.007	0.056	0.072	0.180	0.266	0.276	0.457	0.529	0.572	17.2
	0.276	0.000	0.008	0.042	0.061	0.211	0.411	0.479	0.739	0.837	1.067	38.0
	0.255	0.000	0.038	0.126	0.270	0.582	0.671	0.757	0.958	1.093	1.205	54.4
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.013	$\bar{x}$ = 0.075	$\bar{x}$ = 0.134	$\bar{x}$ = 0.324	$\bar{x}$ = 0.449	$\bar{x}$ = 0.504	$\bar{x}$ = 0.718	$\bar{x}$ = 0.820	$\bar{x}$ = 0.948	
2.5	0.091	0.000	0.034	0.063	0.118	0.158	0.197	0.273	0.402	0.428	0.458	16.9
	0.269	0.000	0.037	0.041	0.071	0.118	0.166	0.185	0.265	0.328	0.551	30.0
	0.171	0.000	0.065	0.065	0.295	0.535	0.655	0.839	0.801	0.825	1.062	54.7
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.045	$\bar{x}$ = 0.056	$\bar{x}$ = 0.161	$\bar{x}$ = 0.270	$\bar{x}$ = 0.339	$\bar{x}$ = 0.432	$\bar{x}$ = 0.489	$\bar{x}$ = 0.527	$\bar{x}$ = 0.690	
4.7	0.131	0.000	0.015	0.048	0.052	0.091	0.124	0.168	0.210	0.191	0.341	10.0
	0.290	0.000	0.095	0.087	0.121	0.230	0.236	0.339	0.384	0.461	0.568	28.7
	0.261	0.000	0.029	0.043	0.067	0.155	0.221	0.289	0.317	0.410	0.446	25.1
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.046	$\bar{x}$ = 0.059	$\bar{x}$ = 0.080	$\bar{x}$ = 0.159	$\bar{x}$ = 0.194	$\bar{x}$ = 0.265	$\bar{x}$ = 0.304	$\bar{x}$ = 0.354	$\bar{x}$ = 0.452	
6.7	0.116	0.000	0.025	0.049	0.067	0.120	0.158	0.187	0.194	0.210	0.228	10.6
	0.218	0.000	0.047	0.085	0.067	0.084	0.221	0.238	0.222	0.381	0.373	21.6
	0.219	0.000	0.025	0.043	0.077	0.179	0.277	0.297	0.344	0.336	0.422	28.7
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.032	$\bar{x}$ = 0.059	$\bar{x}$ = 0.070	$\bar{x}$ = 0.128	$\bar{x}$ = 0.219	$\bar{x}$ = 0.241	$\bar{x}$ = 0.253	$\bar{x}$ = 0.309	$\bar{x}$ = 0.341	
8.2	0.111	0.000	-0.009	0.022	0.029	0.053	0.062	0.055	0.068	0.065	0.103	5.8
	0.171	0.000	0.051	0.023	0.046	0.062	0.096	0.103	0.143	0.186	0.168	15.6
	0.276	0.000	-0.059	-0.033	0.001	0.018	0.012	0.082	0.055	0.101	0.117	16.9
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.006	$\bar{x}$ = 0.004	$\bar{x}$ = 0.025	$\bar{x}$ = 0.044	$\bar{x}$ = 0.057	$\bar{x}$ = 0.080	$\bar{x}$ = 0.089	$\bar{x}$ = 0.117	$\bar{x}$ = 0.129	
10.7	0.113	0.000	0.031	0.008	0.047	0.046	0.054	0.060	0.057	0.075	0.083	5.5
	0.321	0.000	0.019	0.034	0.056	0.082	0.147	0.169	0.198	0.223	0.212	23.0
	0.246	0.000	0.013	-0.014	0.018	0.046	0.025	0.044	0.030	0.066	0.061	16.9
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.021	$\bar{x}$ = 0.009	$\bar{x}$ = 0.040	$\bar{x}$ = 0.058	$\bar{x}$ = 0.075	$\bar{x}$ = 0.091	$\bar{x}$ = 0.095	$\bar{x}$ = 0.121	$\bar{x}$ = 0.119	

Fig. 6.6 Growth of *Cladophora* over 18 day experimental period in response to variation in NH_3-N concentration.

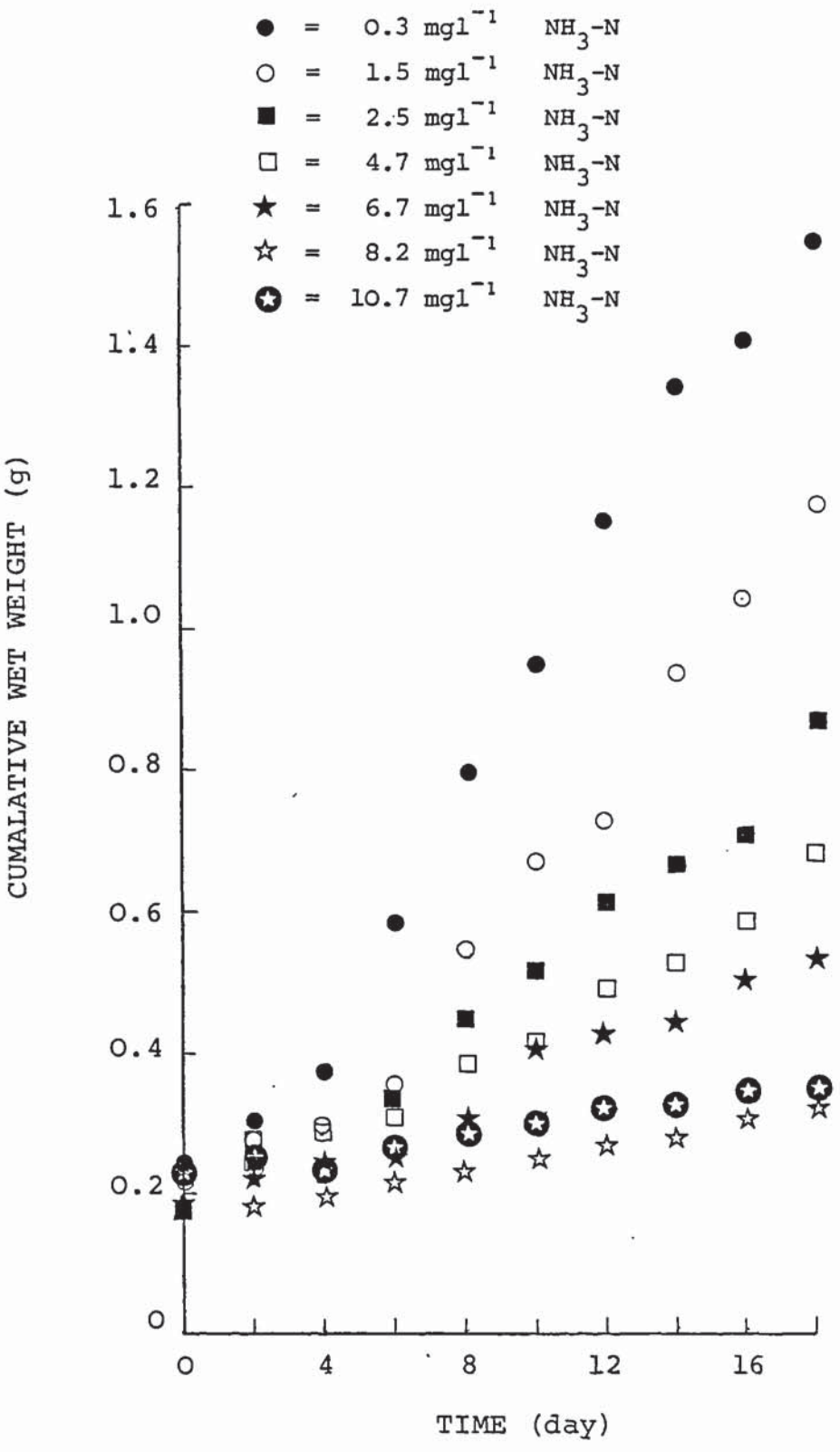
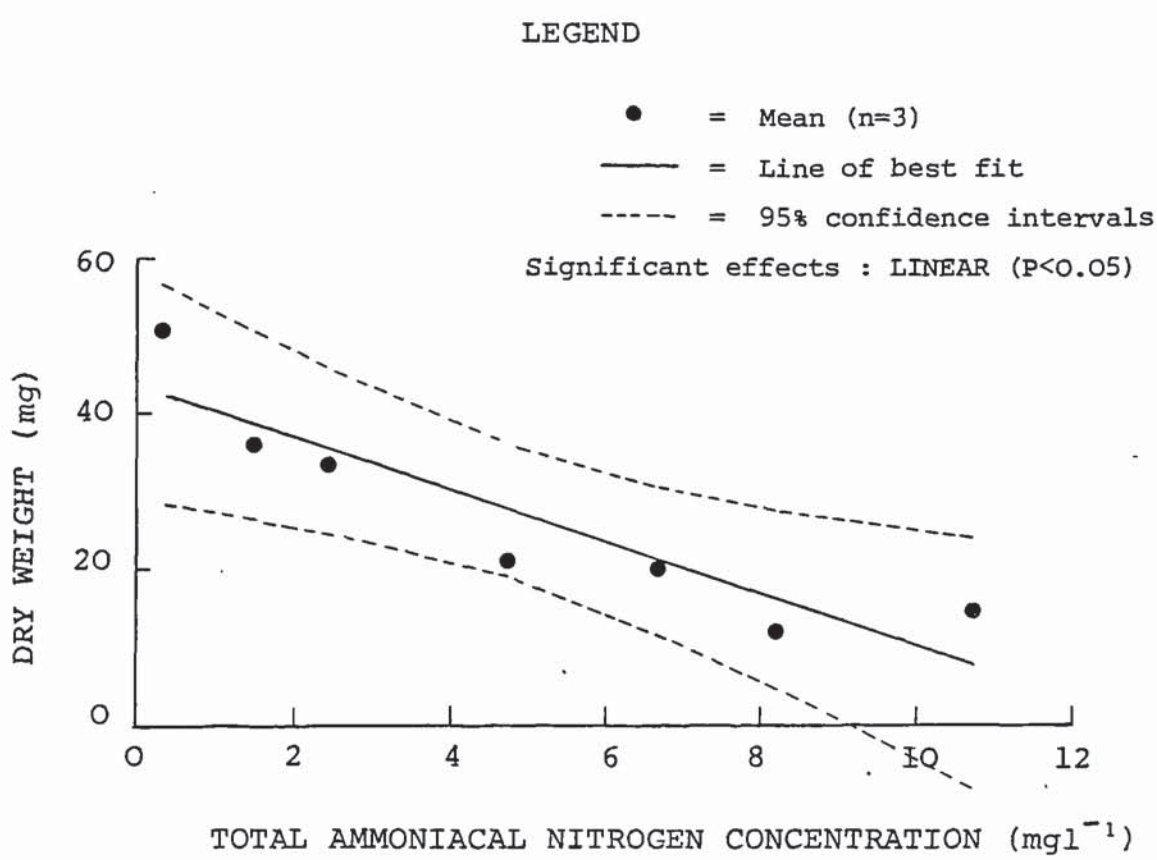


Table 6.6 Analysis of variance summary table for Experiment 3.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	6	3413.62	568.937	1.79265	
Linear Eff.	1	2852	2852	8.98633	(P<0.05)
Quad. Eff.	1	455.911	455.911	1.43652	(P>0.20)
Cubic Eff.	1	17.2067	17.2067	5.42163E-02	(P>0.20)
Residual	3	88.5029			
Blocks	2	2585.29	1292.64	4.07297	(P<0.05)
Error	12	3808.46	317.371		
Total	20	7222.08			

Fig. 6.7. Response curve of *Cladophora* growth (assessed as dry weight after 18 days) to variation in total ammoniacal nitrogen concentration.



Calculation reveals the coefficient of variation of dry weight values to be 65%. Such a value is undoubtedly high and reflects the poor ability of such techniques to detect small differences in growth rate. Considering any two levels of treatment, the growth response (expressed as a percentage of the mean of the two treatment levels under consideration) which would be detected as significant at the P = 0.05 level may be estimated from the equation :-

$$R = \frac{2.\ (cv)\ \sqrt{2}}{\sqrt{r}}$$

Calculation yields R = 107% for values of algal dry weight (produced after 18 days growth). Such high values for R may not, however, be uncommon in biological research. Ridgman (1975) comments that many biologists would be amazed by the number of replicates required to be even 80% confident (P = 0.2) of detecting a 10% difference when coefficients of variation are as high as is common in very detailed studies.

The high F-ratio of 8.99 ($P<0.05$) for the linear effect of treatment on algal growth (see table 6.6), however, indicates that the culture technique is very able to detect differences in growth rate attributable to variation in total ammoniacal nitrogen concentration. This is further demonstrated by the narrowness of the confidence intervals in fig. 6.7. The culture technique was therefore considered worthy of further development.

6.5 Experiment 4 - Investigation of stability of wet/dry weight relationship

6.5.1 Introduction and objectives

Experiment 3 had shown that algal dry weight (produced after 18 days growth) could be used as an index of growth to compare the growth of variously treated cultures. However the evaluation of *Cladophora* biomass by determination of wet weight allows more preferable indices of growth to be calculated (such as the specific

growth rate - μ). Experiment 1 had shown the good correlation between wet weight, determined by a 5-second blot, and dry weight (r^2 = 0.86). The objective of this investigation, however, was to see whether an increase in the total ammoniacal nitrogen concentration of the medium (with its effect in reducing *Cladophora* growth rate) in any way altered the wet/dry weight relationship.

6.5.2 Materials and methods

The investigation made use of 'crops' of *Cladophora* produced in experiment 3 (section 6.4). Materials and methods are therefore as section 6.4.2.

6.5.3 Results and discussion

The ratio of *Cladophora* dry weight (produced after 18 days growth) to *Cladophora* wet weight on day 18 was calculated for each culture. Data were entered into a single classification analysis of variance incorporating partitioning of the treatment sum of squares using orthogonal polynomial coefficients. The analysis of variance summary table for this analysis is included as table 6.7.

Table 6.7 AOV summary table for Experiment 4.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	6	337.121	56.1869	.632488	
Linear Eff.	1	186.67	186.67	2.10132	(P<0.20)
Quad. Eff.	1	6.41022E-02	6.41022E-02	7.21590E-04	(P>0.20)
Cubic Eff.	1	2.50616	2.50616	2.82115E-02	(P>0.20)
Residual	3	147.881			
Blocks	2	878.934	439.467	4.94702	(P<0.05)
Error	12	1066.02	88.8346		
Total	20	1403.14			

Results show no significant difference in wet/dry weight ratio in response to variation in the concentration of total ammoniacal nitrogen concentration ($P>0.05$). In further experiments therefore the ratio was considered constant and the 'crop' of *Cladophora* produced at the end of each experiment could be used for further associated experiments. The significant 'blocks' effect ($P<0.05$), however, indicates that for each experiment the wet/dry weight ratio may vary slightly; it seems best, therefore, to avoid application of a generalized conversion of wet to dry algal weight.

6.6 Experiment 5 - Investigation of the effect of variation in inoculum size on growth

6.6.1 Introduction and objectives

Results of previous experiments and consideration of theoretical growth kinetics both suggested that the size of the inoculum would be an important variable in continuous culture experimentation. The objective of this experiment was therefore to investigate the effect of variation in inoculum size on the growth of *Cladophora* in continuous culture.

6.6.2 Materials and methods

As experiment 1. Inocula of 50, 100, 250 and 500 mg wet weight were chosen for study; these were provided ± 10 mg. Three replicate flasks were used for each level of treatment. Baskets were inoculated with *Cladophora* and growth followed for 18 days by making 5-second blot determinations of algal wet weight every 48 ± 2 hours.

6.6.3 Results and discussion

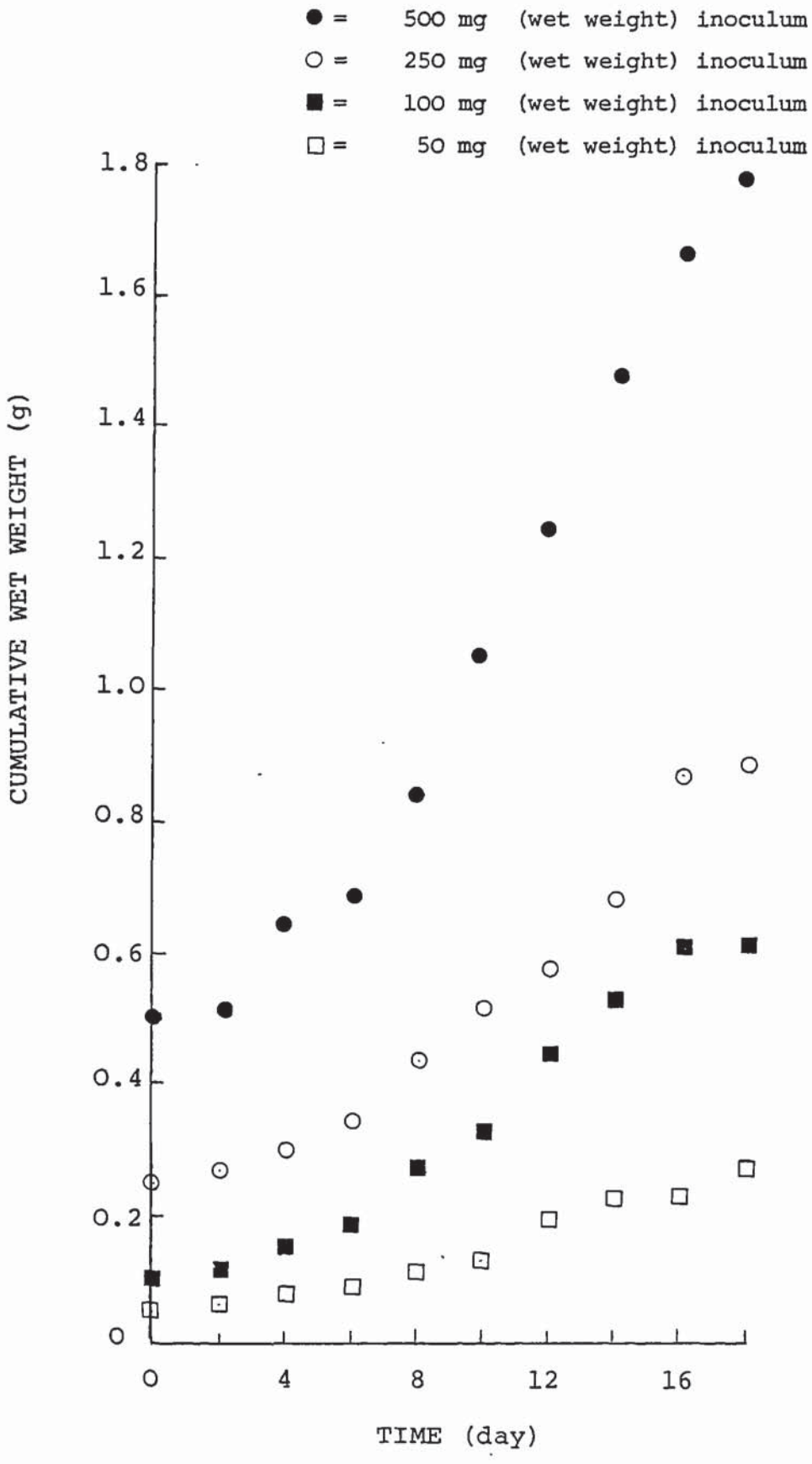
Chemical analysis of Langley water used in the experiment is included as table 6.2. *Cladophora* growth data are included as table 6.8 : whilst fig. 6.8 plots the mean cumulative wet weight of the algal cultures over the experimental period.

Visual inspection of fig. 6.8 reveals a shortening of the duration of the log phase of growth as inoculum size increases.

Table 6.8 *Cladophora* growth data for Experiment 5 : growth response to variation in inoculum size.

Treatment (mg)	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
50	0.048	0.000	-0.001	0.014	0.020	0.059	0.071	0.169	0.167	0.186	0.226
	0.045	0.000	0.038	0.039	0.070	0.084	0.094	0.143	0.140	0.139	0.196
	0.060	0.000	0.001	0.028	0.027	0.043	0.080	0.126	0.221	0.207	0.242
	$\bar{x}$ = 0.051	$\bar{x}$ = 0.000	$\bar{x}$ = 0.013	$\bar{x}$ = 0.027	$\bar{x}$ = 0.039	$\bar{x}$ = 0.062	$\bar{x}$ = 0.082	$\bar{x}$ = 0.146	$\bar{x}$ = 0.176	$\bar{x}$ = 0.177	$\bar{x}$ = 0.221
100	0.104	0.000	0.012	0.063	0.132	0.234	0.351	0.459	0.499	0.672	0.592
	0.102	0.000	-0.001	0.026	0.037	0.152	0.124	0.256	0.369	0.384	0.445
	0.103	0.000	0.043	0.078	0.098	0.141	0.213	0.342	0.426	0.481	0.505
	$\bar{x}$ = 0.103	$\bar{x}$ = 0.000	$\bar{x}$ = 0.018	$\bar{x}$ = 0.056	$\bar{x}$ = 0.089	$\bar{x}$ = 0.176	$\bar{x}$ = 0.229	$\bar{x}$ = 0.352	$\bar{x}$ = 0.431	$\bar{x}$ = 0.512	$\bar{x}$ = 0.514
250	0.248	0.000	0.043	0.057	0.056	0.099	0.158	0.216	0.312	0.498	0.548
	0.245	0.000	-0.006	0.021	0.103	0.255	0.362	0.391	0.512	0.740	0.793
	0.260	0.000	0.028	0.069	0.120	0.217	0.288	0.377	0.482	0.635	0.567
	$\bar{x}$ = 0.251	$\bar{x}$ = 0.000	$\bar{x}$ = 0.022	$\bar{x}$ = 0.049	$\bar{x}$ = 0.093	$\bar{x}$ = 0.190	$\bar{x}$ = 0.269	$\bar{x}$ = 0.328	$\bar{x}$ = 0.435	$\bar{x}$ = 0.624	$\bar{x}$ = 0.636
500	0.498	0.000	0.033	0.159	0.238	0.375	0.678	0.879	1.236	1.205	1.258
	0.503	0.000	0.016	0.124	0.128	0.201	0.303	0.441	0.613	0.676	0.831
	0.500	0.000	-0.010	0.159	0.208	0.443	0.672	0.893	1.085	1.623	1.717
	$\bar{x}$ = 0.500	$\bar{x}$ = 0.000	$\bar{x}$ = 0.013	$\bar{x}$ = 0.147	$\bar{x}$ = 0.191	$\bar{x}$ = 0.340	$\bar{x}$ = 0.551	$\bar{x}$ = 0.738	$\bar{x}$ = 0.978	$\bar{x}$ = 1.168	$\bar{x}$ = 1.269

Fig. 6.8 Growth of *Cladophora* over 18 day experimental period in response to variation in inoculum size.



Growths of 500 mg inocula is noticably incremental from day 6 onwards, supported by the high value for the correlation coefficient - r = 0.997, P<0.001, n = 7 - for the linear regression of mean cumulative wet weight against time. The period over which exponential growth occurs in the 100 and 250 mg inocula cultures is longer : mean cumulative wet weight plots are noticably curved and correlation coefficients accompanying regression of ln transformed data (day 0-18) are 0.988 and 0.993 for 100 and 250 mg inocula cultures respectively (P<0.001, n = 10, in both cases). Growth of the 50 mg inocula cultures is very slow and rather difficult to interpret. Consideration of growth kinetics (section 6.3.3) suggests that growth rate changes from exponental to incremental when a culture attains a 'critical biomass' - inspection of fig. 6.8 indicates this to be in the area of 0.7 g algal wet weight.

6.7 Experiment 6 - Investigation of the effect of variation in flow rate on growth

6.7.1 Introduction and objectives

Results of previous experiments and consideration of theoretical growth kinetics both suggested that the rate of flow of medium over the culture would be an important variable in continuous culture experimentation. The objective of this experiment was therefore to investigate the effect of variation in flow rate on the growth of *Cladophora* in continuous culture.

6.7.2 Materials and methods

As experiment 1. Flow rates of 0.25, 0.5, 1.0 and 2.0 ml min^{-1} were chosen for study; these were maintained to within ± 10% of the nominal rate. Three replicate flasks were used for each level of treatment. Baskets were each inoculated with a small tuft of *Cladophora* (approximately 50 mg wet weight) and growth was followed for 18 days by making 5-second blot determinations of algal wet weight every 48 ± 2 hours.

6.7.3 Results and discussion

Chemical analysis of Langley water used in the experiment is included as table 6.2. *Cladophora* growth data are included as table 6.9, whilst fig. 6.9 plots mean cumulative wet weight of the algal cultures over the experimental period.

Visual inspection of fig. 6.9 reveals the noticable effect of variation in flow rate on the growth of *Cladophora* in closed continuous culture. Analysis of variance of algal cumulative wet weight (including inoculum weight) at the end of the experiment - day 18 - (included as table 6.10) reveals a significant linear effect ($P<0.01$) Fig. 6.10 demonstrates the response of algal biomass to variation in flow rate.

Specific growth rate (μ) was evaluated for each culture, by ln transforming cumulative wet weight data, selecting an appropriate period over which the transformed curve was noticably linear and entering data into a regression equation so that the slope provided an estimate of μ. Table 6.11 includes the results of such calculations as well as the correlation coefficients (r) accompanying regression. The uniformly high correlations demonstrate the 'linearity' of transformed growth data at the periods chosen. The effect on variation in flow rate on algal specific growth rate was assessed by analysis of variance - the summary table of which is included as table 6.12. Results reveal no significant difference in specific growth rate with variation in flow rate ($P>0.05$) although a quadratic trend is apparent but not significant ($P<0.20$).

The lack of effect of variation in flow rate on specific growth rate and the significant linear effect on final agal wet weight appear contradictory. It is conceivable that flow rate does have a significant effect on specific growth rate, but that this was not detected in the analysis of the experimental data. Since the coefficient of variation of both μ and final wet weight are the same (=22%), however, such an explanation would seem unlikely. More likely is the explanation that variation in flow rate has little or no effect on specific growth

Table 6.9 *Cladophora* growth data for Experiment 6 : growth response to variation in flow rate.

Treatment (ml/min)	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.25	0.045	0.000	0.010	0.035	0.046	0.083	0.140	0.157	0.206	0.229	0.238
	0.050	0.000	-0.005	-0.001	0.042	0.044	0.100	0.092	0.112	0.119	0.124
	0.040	0.000	-0.007	0.020	0.022	0.027	0.051	0.053	0.070	0.084	0.075
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.001	$\bar{x}$ = 0.018	$\bar{x}$ = 0.037	$\bar{x}$ = 0.051	$\bar{x}$ = 0.097	$\bar{x}$ = 0.101	$\bar{x}$ = 0.129	$\bar{x}$ = 0.144	$\bar{x}$ = 0.146
0.50	0.061	0.000	0.018	0.081	0.097	0.135	0.106	0.126	0.123	0.177	0.154
	0.049	0.000	0.019	0.033	0.087	0.148	0.186	0.196	0.222	0.222	0.284
	0.041	0.000	0.029	0.058	0.090	0.127	0.177	0.150	0.201	0.242	0.241
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.022	$\bar{x}$ = 0.057	$\bar{x}$ = 0.091	$\bar{x}$ = 0.137	$\bar{x}$ = 0.156	$\bar{x}$ = 0.157	$\bar{x}$ = 0.182	$\bar{x}$ = 0.214	$\bar{x}$ = 0.226
1.0	0.037	0.000	0.007	0.019	0.048	0.103	0.126	0.141	0.260	0.324	0.336
	0.056	0.000	0.009	0.043	0.050	0.137	0.177	0.229	0.275	0.314	0.321
	0.029	0.000	0.023	0.026	0.047	0.075	0.142	0.127	0.151	0.162	0.209
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.013	$\bar{x}$ = 0.029	$\bar{x}$ = 0.048	$\bar{x}$ = 0.105	$\bar{x}$ = 0.148	$\bar{x}$ = 0.166	$\bar{x}$ = 0.229	$\bar{x}$ = 0.267	$\bar{x}$ = 0.289
2.0	0.060	0.000	0.028	0.056	0.069	0.119	0.145	0.186	0.281	0.324	0.390
	0.060	0.000	0.042	0.069	0.116	0.168	0.264	0.259	0.280	0.315	0.374
	0.058	0.000	0.018	0.047	0.092	0.158	0.247	0.263	0.353	0.369	0.442
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.029	$\bar{x}$ = 0.057	$\bar{x}$ = 0.092	$\bar{x}$ = 0.148	$\bar{x}$ = 0.219	$\bar{x}$ = 0.236	$\bar{x}$ = 0.305	$\bar{x}$ = 0.336	$\bar{x}$ = 0.402

Fig. 6.9 Growth of *Cladophora* over 18 day experimental period in response to variation in flow rate.

LEGEND

● = 2.0 ml min^{-1} flow rate
○ = 1.0 ml min^{-1} flow rate
■ = 0.5 ml min^{-1} flow rate
□ = 0.25 ml min^{-1} flow rate

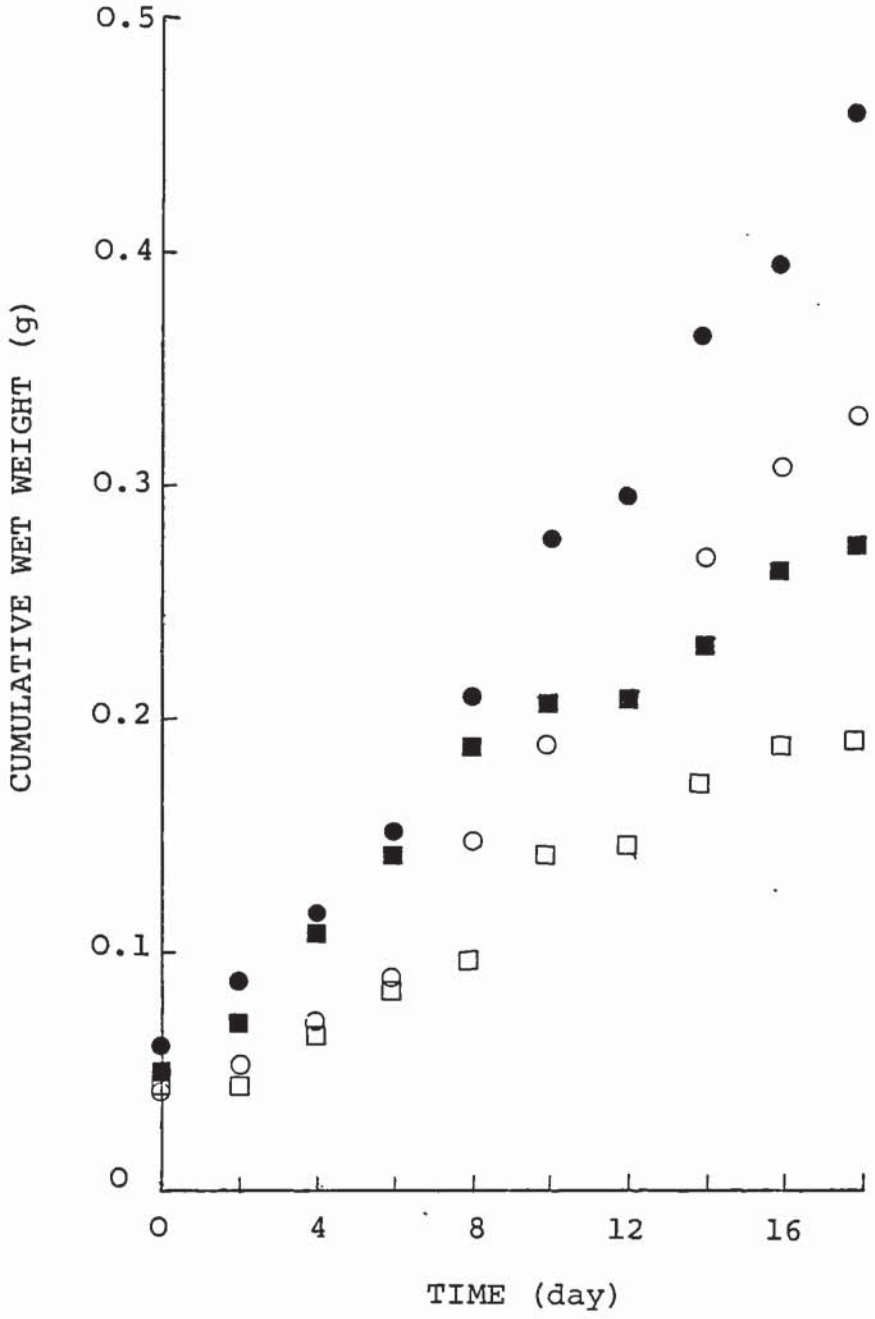


Table 6.10 Analysis of variance summary table for Experiment 6 - Response of final biomass to variation in flow rate.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	3	.115638	3.85462E-02	8.46601	
Linear Eff.	1	.111672	.111672	24.5269	(P<0.01)
Quad. Eff.	1	1.50810E-03	1.50810E-03	.331228	(P>0.20)
Cubic Eff.	1	2.45875E-03	2.45875E-03	.540023	(P>0.20)
Residual	0	-3.87430E-07			
Error	8	3.64244E-02	4.55305E-03		
Total	11	.152063			

Fig. 6.10 Response curve of *Cladophora* growth (assessed as wet weight after 18 days) to variation in flow rate.

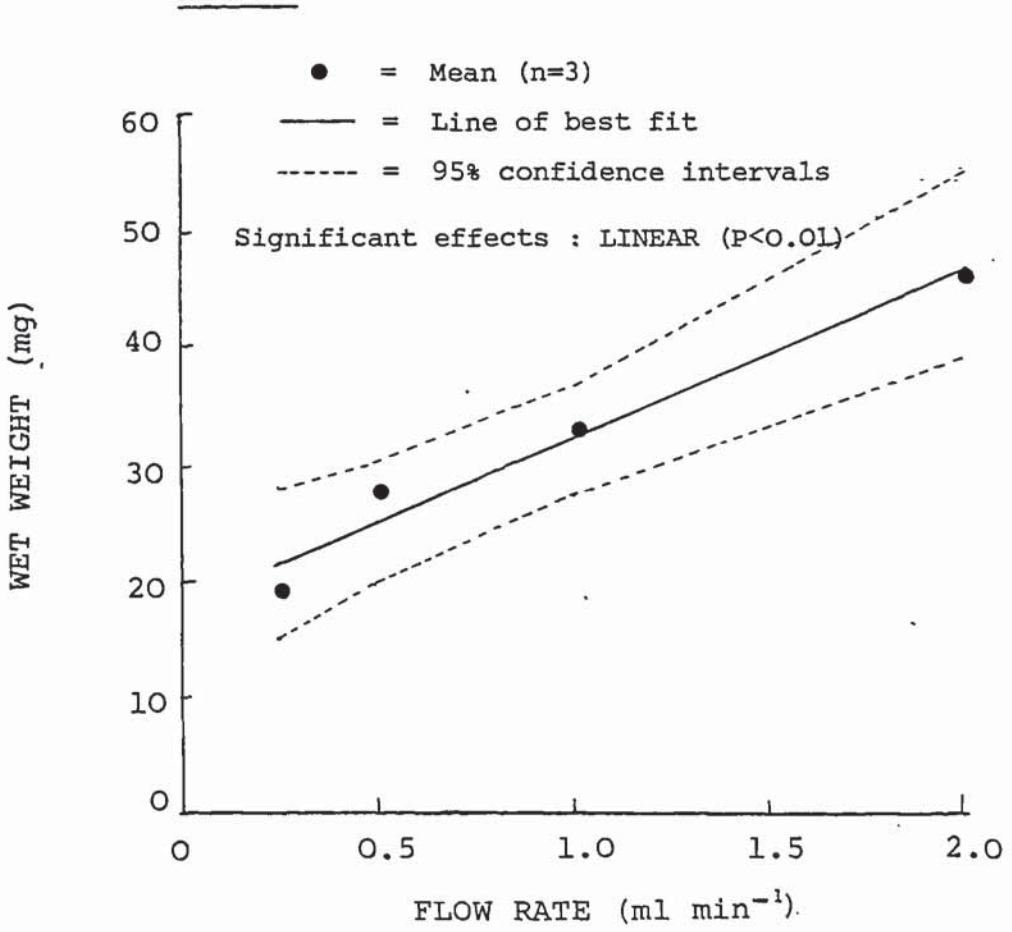


Table 6.11 Calculation of values of μ from growth data of Experiment 6.

Flow rate (ml/min)	Period over which exponential growth evaluated	Regression of ln transformed data		
		Slope (≈ μ)	Correlation Coefficient (r)	n
	0 - 14	0.127	0.993	8
0.25	2 - 10	0.153	0.963	5
	0 - 18	0.071	0.950	10
	0 - 8	0.151	0.971	5
0.5	0 - 10	0.165	0.992	6
	0 - 10	0.161	0.988	6
	0 - 18	0.140	0.988	10
1.0	2 - 10	0.161	0.978	5
	0 - 18	0.111	0.963	10
	0 - 18	0.109	0.994	10
2.0	0 - 10	0.159	0.993	6
	2 - 10	0.175	1.000	5

Table 6.12 Analysis of variance summary table for Experiment 6 - response of μ to variation in flow rate.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
Treatment	3	2.86695E-03	9.55651E-04	1.05489 (P>0.20)
Linear	1	4.00034E-04	4.00034E-04	.441576 (P>0.20)
Quad Eff.	1	2.71139E-04	2.71139E-04	.229296 (P>0.20)
Cubic Eff.	1	2.19590E-03	2.19590E-03	2.42394 (P<0.20)
Residual	0	-1.22935E-07		
Error	8	7.24739E-03	9.05924E-04	
Total	11	1.01143E-02		

rate i.e. growth during the log phase is not limited by nutrient concentrations. As growth proceeds, however, incremental growth begins and growth rate is then proportional to the rate of supply of nutrients (i.e. flow rate).. Results of experiment 5 suggest incremental growth to begin when the wet weight of the culture exceeds 0.7g wet weight. In this experiment, however, no culture exceeded 0.5g wet weight during the 18 day duration, yet results suggest incremental growth to have begun.

6.8 General discussion

In preliminary investigations *Cladophora* biomass (after 18 days growth) had been used as an index of growth. However the employment of wet weight determinations for biomass evaluation allow more appropriate indices of growth to be calculated. Results of experiment 6 suggest that growth in the exponential phase was not limited by supply of nutrients, whilst growth in the incremental phase was limited by the supply of nutrients. It was considered, therefore that by adopting an estimate of growth rate in both phases results would be most easily extrapolated to growth of the alga in the field situation. Whilst growth is in the exponential phase wet weight data may be transformed and the natural logarithm (ln) of the data entered into a linear regression equation : the slope of the regression equation may then be used as an estimate of the specific growth rate - μ. When growth is in the incremental phase however, wet weight data may be entered directly into a linear regression equation and the slope used as an estimate of the growth rate - k. Such indices of growth (i.e. μ and k) may be considered as better than an estimate of algal biomass (determined after an appropriate time) since they are independent of time. However, estimation of both μ and k necessitates selection of appropriate data from the growth curve (i.e, data in which growth rate is exponential or incremental respectively). Results of experiments 5 and 6 were inconclusive in determining the 'critical culture weight' when growth rate changes from exponential to incremental. The practice of evaluation of growth after plotting growth curves on untransformed and ln transformed axes (as performed in experiment 6) was thus felt worthy of continued use. However, in experiment 6 growth curves had been plotted for each culture separately and ln transformed data entered into a regression equation when the growth rate was considered

exponential. Unfortunately the selection of appropriate data was somewhat difficult for certain cultures, especially those in which overall growth was small and those in which wet weight determinations were rather variable. Thus, to reduce subjectivity of estimation to a minimum, it was considered more satisfactory to plot mean cumulative wet weight (on untransformed and ln transformed axes) against time for each treatment level and select the period of exponential growth from these plots. Growth rates could then be calculated by entering ln transformed growth data for each culture into a linear regression equation (over the time period previously selected) and using the slope as an estimate of μ. Difficulties had already been encountered in preliminary studies in discriminating the period over which exponential growth ceased and incremental growth began. Further it was envisaged that any physico-chemical factor acting so as to reduce specific growth rate would extend the period over which growth rate would be exponential. To compare growth in the incremental phase would thus require an 'unrealistically' long experimental duration. It was decided, therefore, only the attempt to evaluate and compare growth in the incremental phase if no significant difference was detectable in the specific growth rate of variously treated cultures.

Experiments 1 - 6 had revealed that *Cladophora* specific growth rates were of the order 0.15 ln mg wet weight day^{-1}. Using the formula $g = 0.69/\mu$ generation time was calculated to be approximately 5 days. In comparison doubling times of 21 - 26 hr and 1.2 days were found by Whitton (1967) and Zuraw (1969) respectively. The long generation time, found in this study, indicated that either conditions for growth were acutely sub-optimal or that only certain cells were actively growing. Consideration of the probable effects of self-shading involved in pellet growth (discussed in section 6.3.3) favours the latter hypothesis. Effectively then, calculated values of μ would be expected to be lower then values calculated if cultures were homogeneous. Comparison of specific growth rate values from heterogeneous cultures is, however, considered valid but in graphical representations of response curves it is considered that the Y axis is best calibrated not only in values of μ but also in % of the maximum specific growth rate.

6.9 Conclusions

1. Results of preliminary experiments indicate the continuous culture technique devised for fungi by Hawkes (1965) and developed by Williams (1971) allows, with appropriate modifications, the study of the growth of *Cladophora* under conditions of continuous nutrient flow.

2. A '5-second blot' method of biomass determination has been found to be very highly significantly correlated with dry weight (r^2 = 0.86, $P<0.001$).

3. The wet/dry weight ratio of *Cladophora* growing on baskets has demonstrated good stability : in an experiment in which the effects of variation in total ammoniacal nitrogen concentration on growth were assessed no significant difference in wet/dry weight ratio was found ($P>0.05$).

4. Using dry weight of *Cladophora* (produced after 18 days growth) as an index of growth the 'within treatment' variability of the culture technique was low enough to enable the linear effect of variation in total ammoniacal nitrogen concentration on *Cladophora* growth to be detected at the $P=0.05$ level.

5. The employment of specific growth rate -μ- and incremental growth rate -k- are suggested as more appropriate indices of the growth of *Cladophora* biomass (determined after an appropriate time period) since they are independent of time.

6. Results are inconclusive in determining the 'critical culture weight' whereupon growth rate changes from exponential to incremental.

7. The period over which growth rate is considered appropriate for evaluation of μ (i.e. exponetial) or k (i.e. incremental)

is considered best chosen from plots of mean cumulative wet weight (untransformed and ln transforned against time. Estimates of μ and k may then be made by entering ln transformed (for μ) or untransformed (for k) cumulative wet weight data into a linear regression equation for each individual culture, and using the slope of the equation as the estimate.

8. Comparison of values of k is only planned if no significant difference ($P>0.05$) is found between values of μ.

7. *CLADOPHORA* CONTINUOUS CULTURE STUDIES - EXPERIMENTAL STUDIES

7.1 STANDARDIZED METHODOLOGY

7.1.1 Introduction

Experimentation described in the previous chapter was mainly performed to investigate the growth of *Cladophora* in closed continuous culture and enable appropriate indices of growth to be chosen so that comparisons between cultures may be made. Various modifications of apparatus were made concurrently with preliminary experimentation, allowing a standardized methodology to be adopted in further experimentation. The objective of this section is thus to describe the apparatus and methodology developed from that of Williams (1971) and Hawkes (1965) and used in further work.

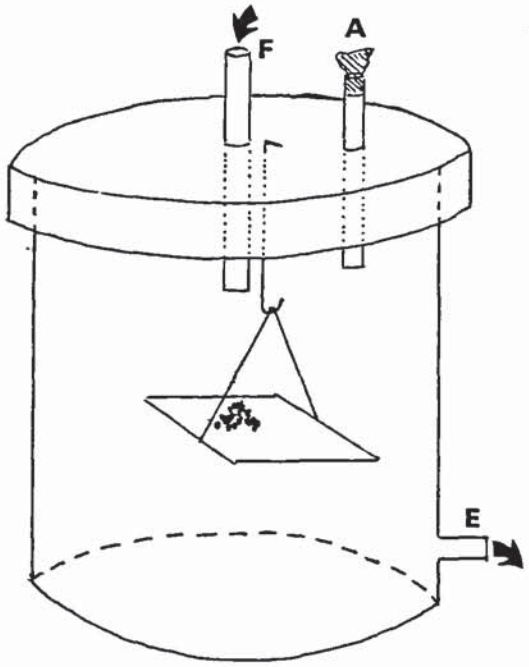
7.1.2 Materials and methods

The apparatus consisted of a 4.0 cm square nylon mesh basket (Nybolt grit gauze No. 10GG-2000; aperture 0.2 cm, 3.6 threads/cm; John Staniar and Co. Ltd., Manchester Wire Works, Manchester) to which a 12 cm length of 1 mm diameter stainless steel wire was attached with Aquaria silicone rubber sealant, allowing the basket to be hung from the culture flask lid. The culture flask (Fig. 7.1) comprised a 400 ml Pyrex beaker modified by the addition of a side arm, with a lid made from a 9 cm diameter plastic petri plate lid to which two 3 cm lengths of glass tubing (4 mm external diameter, 1 mm wall thickness) and a nichrome wire hook were attached with Araldite epoxy resin. A moderately eutrophic local pond water obtained from Langley Pool (Nat. grid ref. SP153968), served as nutrient medium. Prior to the start of each experiment enough water for the entire investigation was collected and aerated in plastic courtesy tanks at 15°C. Water was filtered through Whatman GF/C filters, to remove micro-invertebrates and suspended solids, collected in 10 litre Pyrex aspirators and allowed to 'free-steam' in an autoclave for 60 minutes ensuring a medium free from algal contaminants. Medium was fed to flasks using Watson-Marlow micro metering flow inducers with MC10 or Delta heads (Watson-Marlow Ltd., Falmouth, Cornwall, England) capable of supplying 10 and 20 culture flasks respectively. All tubing was silicone rubber and

Fig. 7.1 Culture flask used in experimentation.

LEGEND

A	Air Inlet (cotton wool plugged)
E	Effluent Outlet
F	Feed Inlet



autoclayed (15lb/in^2, 15 minutes) prior to use. Pyrex flasks were dry sterlized at 160^{o}C for one hour. Flask lids and baskets were rinsed in boiling distilled water. Illumination was supplied from above by Thorn 'white' fluorescent tubes providing an intensity of 4000 lux at the level of the baskets, on a 16:8, light/dark photoperiod. The entire apparatus was placed in a constant temperature room at 15 ± 1^{o}C, the heating action of the lights, however, acted so as to locally raise the temperature around the flasks to 17^{o}-19^{o}C during the light period. A unialgal culture of *C. glomerata* (ref. 505/3, Culture Collection of Algae and Protozoa, Cambridge) served as inoculum. Algal material for inoculation was grown under culture conditions as described in this section. Inoculum for each experiment was 100 ± 50 mg (wet weight) used in experimentation. Each basket was weighed prior to inoculation with a small tuft of *Cladophora* placed in the culture flask for one hour, after which time it was removed and blotted on a 7.0 cm diameter Whatman No. 1 filter. Any drops of medium remaining on the basket were removed with the edge of the same filter and the basket was hung on a light plastic and wire framework positioned on an Oertling single pan balance. Wet weight was thereby determined and the basket replaced in the culture flask. Growth of cultures was followed by making such determinations every 48 ± 2 hours.

At the beginning of each experiment a sample of water was taken from the influent reservoir for full physico-chemical analysis. Where appropriate, water samples were also taken at intervals throughout the course of the experiment for analysis of the physico-chemical factor of interest in that particular experiment. In preliminary experiments (e.g. experiment 3) water samples for physico-chemical analysis were taken from the influent reservoir using a pipette. Water samples taken from three positions in the apparatus (indicated in Fig. 7.2) from three culture flasks, however, showed little difference in physico-chemical 'nature' as indicated by four diverse factors (Table 7.1). Analysis of this data in a 3 x 4 factorial analysis of variance (Table 7.2) showed no significant difference in the concentration of any of the four physico-chemical factors at any of the sampling points ($P>0.20$). For convenience, therefore, water samples were taken for physico-chemical analysis from the small effluent reservoir in the bottom of each culture flask. To do this

Fig. 7.2 Positions of abstraction of water for physico-chemical analysis.

LEGEND

I Influent Reservoir

II Feed Inlet (prior to passing over culture)

III Effluent (small reservoir in culture flask)

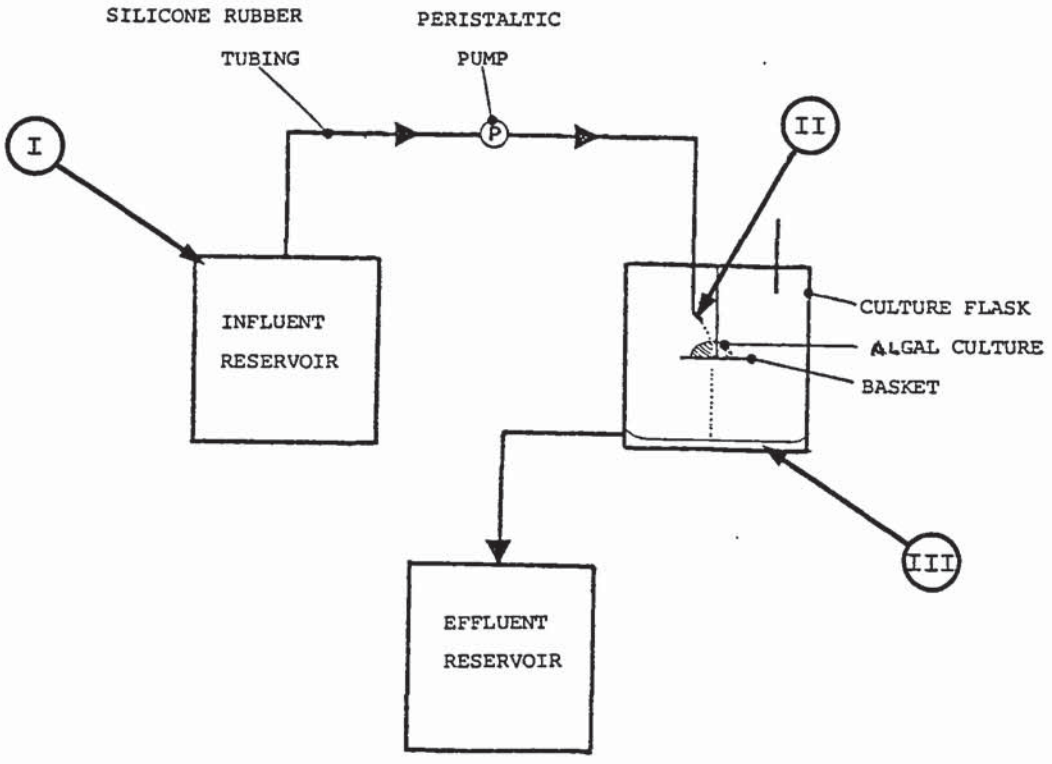


Table 7.1 Physico-chemical data for water samples abstracted from three positions (from three culture flasks).

	Replicates		
	I	II	III
Wet weight of alga (g)	1.063	0.852	0.996

Physico-chemical factor	(units)	Position of abstraction*								
		1			2			3		
		I**	II	III	I	II	III	I	II	III
PO_4-P	(mgl^{-1})	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chloride	(mgl^{-1})	29	31	32	30	30	30	30	30	29
Na^+	(mgl^{-1})	17	17	17	17	16	17	17	17	17
Total Cu	(μgl^{-1})	23	23	23	23	23	23	23	23	23

* = See fig. 7.2

** = Replicates

Table 7.2 Analysis of variance summary table for physico-chemical data from table 7.1.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
A*	2	.501953	.250977	1.00423	($P \geq 0.20$)
B**	3	4431.79	1477.26	5910.98	($P<0.001$)
Interaction	6	1.27734	.212891	.85184	($P>0.20$)
Error	24	5.99805	.249919		
Total	35	4439.57			

* = Position of abstraction

** = Physico-chemical factor

the flask was tilted so that all effluent ran to drain, the reservoir was then allowed to fill, and the flask was once again tilted and the effluent directed into a sample bottle. This procedure enabled physico-chemical sampling to be carried out at regular intervals without any risk of contamination. Where appropriate such sampling was carried out at two day intervals. Water samples were always taken after biological sampling (i.e. wet weighing) since the tilting of the culture flasks inevitably disturbed the flow of nutrients over the basket. Evaluation of concentration of heavy metals, however, required large (100 ml) sample volumes and such samples were taken direct from the influent reservoir with a 50 ml pipette, though the greater probability of contamination resulting from this sampling technique was recognised.

Cumulative mean algal wet weight was plotted against time on linear and ln transformed axes and the period of exponential growth defined. Appropriate data (i.e. that from the period during which growth was exponential) from individual cultures was then ln transformed and entered into a linear regression equation . The slope of the regression equation was then used as an estimate of μ (ln g wet weight day^{-1} units). Comparison of the specific growth rate of various cultures was carried out by analysis of variance incorporating partitioning of the treatment sum of squares using orthogonal polynomial coeffieients (Ridgman, 1975). Resulting F-ratios were compared to standard tables in Fisher and Yates (1963). In cases where lack of significant effect of treatment on specific growth rate was detected ($P>0.05$) incremental growth rate was also calculated by entering appropriate data (i.e. that from the period of incremental growth) into a linear regression equation and using the slope of the equation as an estimate of k (g wet weight day^{-1} units). Analysis was carried out as for values of μ . Resulting response curves were plotted against treatment levels on axes calibrated both in μ and % of maximum μ , or k and % of maximum k.

7.2 INVESTIGATION OF THE EFFECT OF VARIATION IN LIGHT PERIOD, LIGHT INTENSITY AND TEMPERATURE ON *CLADOPHORA* GROWTH.

7.2.1 Experiment 7 - The effect of variation in light period on growth.

7.2.1.1 Introduction and objectives

A review of the relevant literature suggests that little attention has been given to the effect of variation in light period on *Cladophora* growth in either the laboratory or field situation. In laboratory studies a diversity of photoperiods have been used (e.g. Bellis (1968a) 12:12 light/dark, Moore and McLarty (1975) 14:10, Hoffman *et al.* (1974) 16:8, Pitcairn and Hawkes (1973) 18:6, and Whitton (1970a) 24:0) with little experimental justification for their adoption. In field studies Storr and Sweeney (1971) suggested an interaction between temperature and photoperiod to account for the seasonal pattern of growth exhibited by *C. glomerata* populations in the field. Their study, however, involved only two levels of photoperiod (12:12 and 14:10, light/dark). The objective of this study was therefore to investigate the effect of variation in photoperiod on growth of *Cladophora*.

7.2.1.2 Materials and methods

As section 7.1.2. Four photoperiods were chosen for study viz. 8:16, 12:12, 16:8 and 24:0, light/dark. Five replicate culture flasks were used per treatment level.

7.2.1.3 Results and discussion

Chemical analysis of the Langley water used in the experiment is included as table 7.3. *Cladophora* growth data is shown in table 7.4, whilst fig. 7.3 plots mean cumulative algal weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time the period in which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.5) and analysed by analysis of variance to produce table 7.6. Fig. 7.4 summarises the response of *Cladophora* growth to variation in photoperiod.

Table 7.3 Physico-chemical analysis of Langley water.

PARAMETER (mgl^{-1})*	EXPERIMENT								
	7	8	9	10	11	12	14	15	16
pH	8.3	8.4	8.6	8.2	8.2	8.4	8.1	8.1	8.2
Total Alkalinity	65	155	165	125	130	170	120	120	100
Phenolphthalein Alkalinity	0	15	10	0	0	0	0	0	0
Total Hardness	296	338	354	262	270	392	286	286	206
Calcium Hardness	154	220	226	140	144	228	182	182	126
NH_3-N	0.3	0.2	0.1	-	0.4	0.1	0.3	0.3	0.9
NO_3-N	9.3	4.8	9.0	2.4	3.0	-	5.3	5.3	3.4
NO_2-N	0.010	0.025	0.010	0.075	-	0.020	0.01	0.01	0.018
PO_4-P	0.7	0.2	0	0.1	0.1	-	0.1	0.1	0.1
Na^+	18	16	30	15	17	23	15	15	12
K^+	5	5	6	5	5	7	5	5	6
Chloride	23	25	47	28	27	40	25	25	20
Total Cd	0.002	0.002	0.003	0.002	0.001	0.002	0.002	0.002	0.002
Cr	0	0	0	0	0.005	0	0	0	0
Cu	0.038	0.022	0.019	0.017	0.016	0.027	0.017	0.017	0.019
Fe	0.126	0.135	0.099	0.150	0.090	0.055	0.119	0.119	0.123
Ni	0.012	0.013	0.013	0.010	0.008	0.016	0.018	0.018	0.011
Pb	0.017	0.026	0.020	0.015	0.028	0.025	0.017	0.017	0.019
Zn	0.0203	0.0287	0.0231	0.026	0.0154	0.0233	0.192	0.0192	0.0207

* Except pH. Alkalinity and hardness mgl^{-1} as $CaCO_3$

Table 7.4 *Cladophora* growth data for Experiment 7 : growth response to variation in photoperiod.

Treatment (hrs light/ day)	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
8	0.102	0.000	0.016	0.061	0.082	0.068	0.087	0.115	0.139	0.154	0.292
	0.142	0.000	-0.030	0.014	0.031	0.030	0.085	0.175	0.189	0.181	0.311
	0.072	0.000	0.003	0.042	0.102	0.114	0.101	0.135	0.158	0.182	0.254
	0.130	0.000	-0.016	0.020	0.023	0.053	0.065	0.064	0.088	0.116	0.162
	0.122	0.000	0.016	0.001	0.004	0.030	0.042	0.103	0.089	0.090	0.149
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.002	$\bar{x}$ = 0.028	$\bar{x}$ = 0.048	$\bar{x}$ = 0.059	$\bar{x}$ = 0.076	$\bar{x}$ = 0.118	$\bar{x}$ = 0.133	$\bar{x}$ = 0.145	$\bar{x}$ = 0.234
12	0.069	0.000	0.033	0.082	0.098	0.183	0.206	0.245	0.266	0.353	0.403
	0.109	0.000	0.021	0.054	0.057	0.130	0.092	0.219	0.236	0.334	0.263
	0.072	0.000	-0.012	0.030	0.048	0.070	0.093	0.148	0.225	0.309	0.376
	0.089	0.000	-0.001	0.039	0.087	0.108	0.128	0.179	0.213	0.286	0.297
	0.084	0.000	0.008	0.072	0.050	0.092	0.125	0.169	0.186	0.248	0.290
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.010	$\bar{x}$ = 0.055	$\bar{x}$ = 0.068	$\bar{x}$ = 0.117	$\bar{x}$ = 0.129	$\bar{x}$ = 0.192	$\bar{x}$ = 0.225	$\bar{x}$ = 0.306	$\bar{x}$ = 0.326
16	0.109	0.000	0.054	0.096	0.056	0.084	0.133	0.207	0.322	0.332	0.295
	0.125	0.000	0.020	0.043	0.064	0.086	0.150	0.196	0.244	0.353	0.563
	0.076	0.000	0.000	0.028	0.034	0.131	0.198	0.235	0.293	0.412	0.507
	0.108	0.000	0.013	0.041	0.045	0.091	0.133	0.201	0.181	0.204	0.276
	0.116	0.000	-0.020	0.014	0.026	0.054	0.104	0.235	0.358	0.404	0.405
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.013	$\bar{x}$ = 0.044	$\bar{x}$ = 0.045	$\bar{x}$ = 0.089	$\bar{x}$ = 0.144	$\bar{x}$ = 0.215	$\bar{x}$ = 0.280	$\bar{x}$ = 0.341	$\bar{x}$ = 0.409
24	0.169	0.000	0.034	0.089	0.130	0.191	0.329	0.455	0.533	0.690	0.909
	0.111	0.000	0.001	0.072	0.074	0.143	0.139	0.220	0.325	0.395	0.398
	0.097	0.000	0.031	0.072	0.088	0.161	0.247	0.278	0.500	0.624	0.818
	0.098	0.000	-0.013	0.060	0.103	0.118	0.128	0.195	0.222	0.301	0.369
	0.068	0.000	0.058	0.181	0.223	0.245	0.372	0.471	0.486	0.523	0.871
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.022	$\bar{x}$ = 0.095	$\bar{x}$ = 0.124	$\bar{x}$ = 0.172	$\bar{x}$ = 0.243	$\bar{x}$ = 0.324	$\bar{x}$ = 0.413	$\bar{x}$ = 0.507	$\bar{x}$ = 0.673

Fig. 7.3 Growth of *Cladophora* over 18 day experimental period in response to variation in light period.

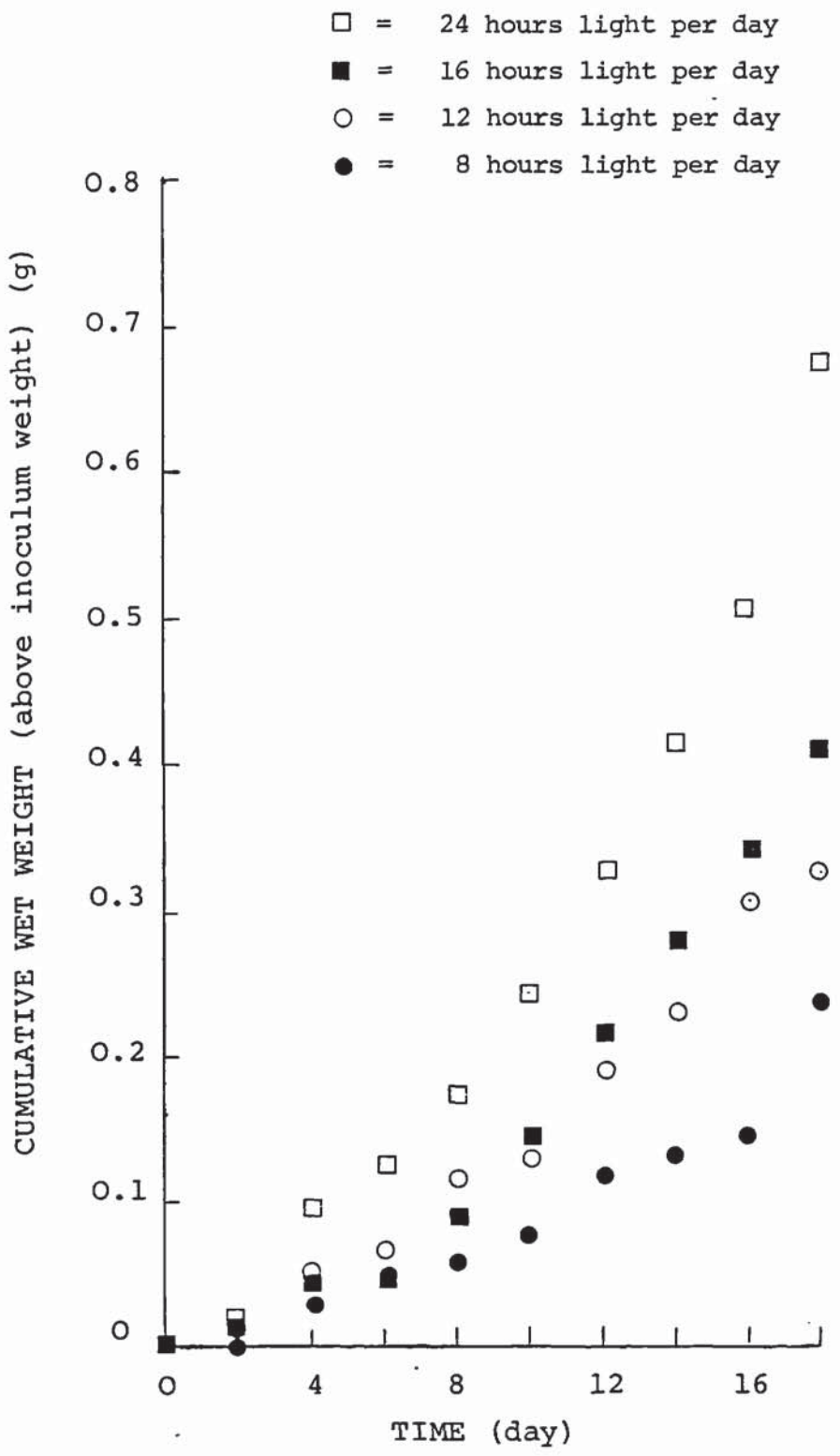


Table 7.5 Calculation of values of μ from growth data of Experiment 7.

Light period (hr/day)	Period over which exponential growth evaluated	Regression of In transformed data		
		Slope (=μ)	Correlation Coefficient (r)	n
8	0 - 18	0.061	0.956	10
	0 - 18	0.072	0.958	10
	0 - 18	0.079	0.952	10
	0 - 18	0.046	0.970	10
	0 - 18	0.054	0.934	10
12	0 - 18	0.101	0.973	10
	0 - 18	0.077	0.965	10
	0 - 18	0.111	0.987	10
	0 - 18	0.088	0.984	10
	0 - 18	0.083	0.981	10
16	0 - 18	0.075	0.949	10
	0 - 18	0.089	0.988	10
	0 - 18	0.124	0.986	10
	0 - 18	0.072	0.982	10
	0 - 18	0.105	0.968	10
24	0 - 18	0.104	0.998	10
	0 - 18	0.092	0.983	10
	0 - 18	0.124	0.996	10
	0 - 18	0.090	0.970	10
	0 - 18	0.123	0.950	10

Table 7.6 Analysis of variance summary table for Experiment 7 - response of μ to variation in light period.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	3	5.6885E-03	1.89618E-03	6.53518	
Linear Eff.	1	4.43017E-03	4.43017E-03	15.2686	(P<0.01)
Quad. Eff.	1	7.78025E-04	7.78025E-04	2.68146	(P<0.20)
Cubic Eff.	1	4.80343E-04	4.80343E-04	1.6555	(P>0.20)
Residual	0	6.51926E-09			
Error	16	4.64240E-03	2.90150E-04		
Total	19	1.03309E-02			

Fig. 7.4 Response of *Cladophora* specific growth rate to variation in photoperiod.

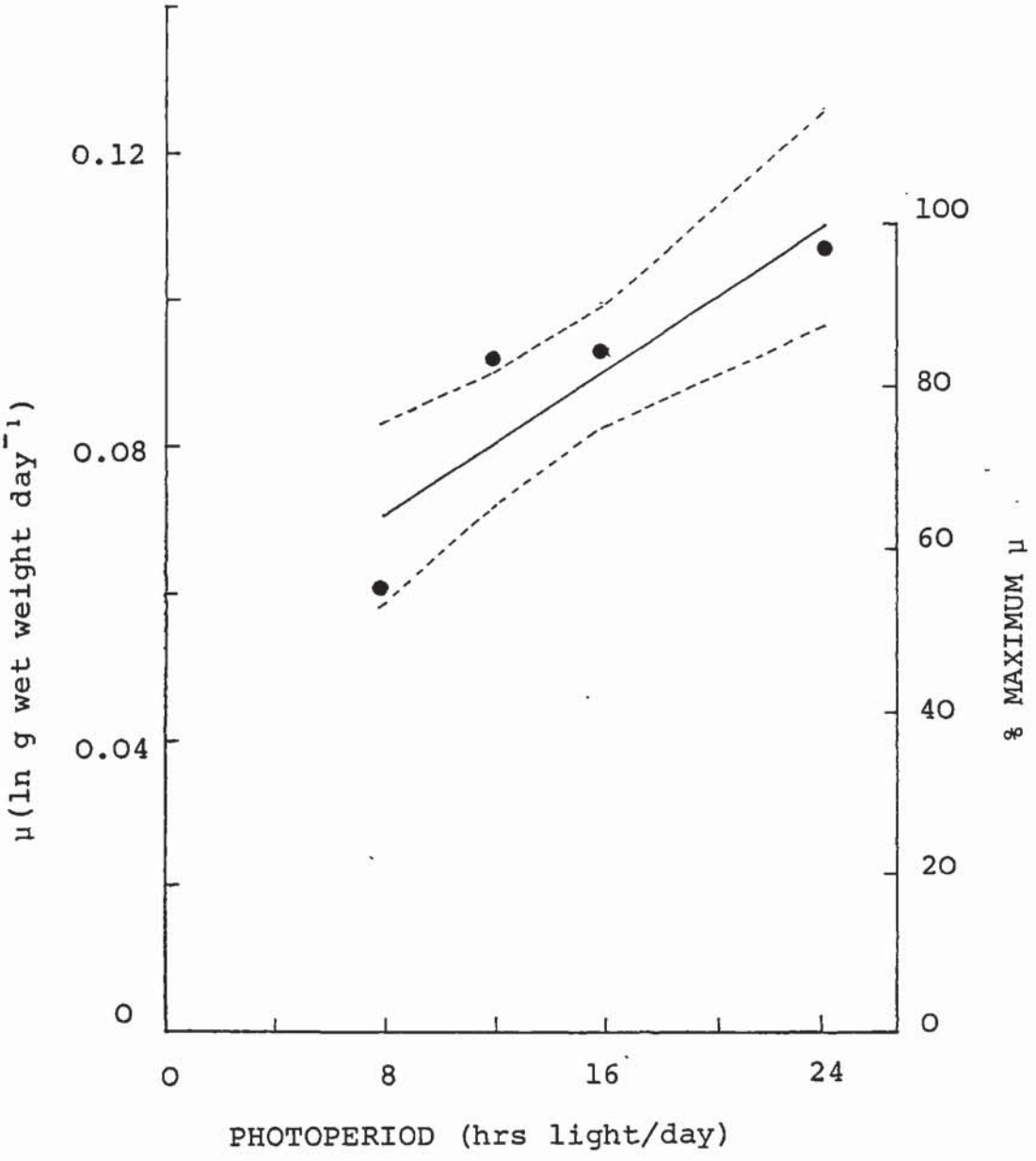
LEGEND

● = Mean (n=5)

——— = Line of best fir

------ = 95% confidence intervals

Significant effects : LINEAR ($P<0.05$)



Results show a significant linear response of *Cladophora* growth rate to increasing duration of light per day ($P<0.01$). No inhibitory effect of continuous lighting is apparent. In the field situation day length is dependant on both season and latitude. At the equator day length is 12 hours throughout the year, whilst at a latitude of 70°N day length may vary from 0 hours throughout December and January to 24 hours throughout June and July. At the latitude 50° (approximately that of the British Isles) day length varies from 8-16 hours (Saunders,1977) ; results suggest that such an increase in the duration of light/day would act so as to increase the growth rate of *Cladophora* by 28% (of the rate with 8 hrs light/day).

The study of Storr and Sweeney (1971) concluded that 'the variation in growth between 12 and 14 hour photoperiod probably represented an exponential increase in growth, with increasing light (and decreasing dark) periods'. Results of this study, however, strongly suggest a linear rather than exponential response of *Cladophora* growth rate to increasing duration of light/day. Modification of the mathematical model of the seasonal pattern of *Cladophora* growth, developed by Storr and Sweeney, would therefore seem expedient.

It was concluded that in further experimental work continuous lighting (24 hr/day) should be provided.

7.2.2 Experiment 8 - The effect of variation in light intensity on growth.

7.2.2.1 Introduction and objectives

Evidence suggests that growth of *C. glomerata* and many other *Cladophora* species is favoured by high light intensity. Jaag (1938) noted that a period in which the sky was cloud covered sufficed to reduce growth of *C. glomerata* in the Upper Rhine. Similarly, Blum (1957) attributed the summer decline in *Cladophora* crop in the Saline River to the reduction in light intensity caused by shading of overhanging vegetation. Whitton (1967) found growth of *Cladophora* to increase upto 7500 lux (the highest level used) in laboratory studies; and whilst Adams and Stone (1973) concluded that photosynthesis was saturated at low intensities both Mantai (1974) and Wood (1975a)

refuted the claim. Indeed McMillan and Verduin (1953) found relatively inefficient utilization of low light intensities. Whitton (1970b), however, comments that not all species of *Cladophora* thrive in well-illuminated situations. Van den Hoek (1963) concluded that *C. basiramosa* is a shade loving species, and *C. aegagropila* is another such species which sometimes flourishes at considerable depth (Whitton, 1970b). The objective of this study was, therefore, to investigate the effect of variation in light intensity on *Cladophora* growth in culture.

7.2.2.2 Materials and methods

As 7.1.2 except that light was provided continuously. Four light intensities were chosen for study viz. 1000, 2000, 4000 and 6000 lux. All light intensities were measured at the level of the baskets using an EEL portable photoelectric photometer (Evans Electroselenium Instruments Ltd., Halstead, Essex). Three replicate culture flasks were used per treatment level.

7.2.2.3 Results and discussion

Chemical analysis of the Langley water used in the experiment is included as table 7.3. *Cladophora* growth data is shown as table 7.7, whilst fig. 7.5 plots mean cumulative algal wet weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time the period over which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.8) and analysed by analysis of variance to produce table 7.9. Fig. 7.6 summarizes the *Cladophora* growth response to variation in light intensity.

Results show a significant linear response of *Cladophora* growth rate to increasing light intensity ($P<0.001$), and are consequently in good agreement with those of Whitton (1967). It must be noted, however that variation in light intensity was obtained by positioning culture flasks at various distances from the light source. Consequently a temperature gradient of 1-2^{o}C accompanies the light intensity gradient, even though the entire apparatus was placed in a constant temperature room and a fan was positioned to ensure circulation of air over

Table 7.7 *Cladophora* growth data for Experiment 8 : growth response to variation in light intensity.

Treatment (lux)	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
1000	0.080	0.000	0.010	0.023	0.027	0.036	0.027	0.054	0.063	0.094	0.080
	0.091	0.000	0.053	0.028	0.043	0.057	0.091	0.101	0.119	0.134	0.166
	0.092	0.000	0.023	0.034	0.032	0.043	0.076	0.057	0.064	0.060	0.089
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.029	$\bar{x}$ = 0.028	$\bar{x}$ = 0.034	$\bar{x}$ = 0.045	$\bar{x}$ = 0.065	$\bar{x}$ = 0.071	$\bar{x}$ = 0.082	$\bar{x}$ = 0.096	$\bar{x}$ = 0.112
2000	0.105	0.000	0.033	0.046	0.033	0.064	0.086	0.122	0.156	0.213	0.246
	0.130	0.000	-0.021	0.004	0.008	0.083	0.093	0.086	0.101	0.195	0.265
	0.093	0.000	0.032	0.028	0.083	0.066	0.152	0.143	0.195	0.232	0.257
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.015	$\bar{x}$ = 0.026	$\bar{x}$ = 0.041	$\bar{x}$ = 0.071	$\bar{x}$ = 0.110	$\bar{x}$ = 0.117	$\bar{x}$ = 0.151	$\bar{x}$ = 0.213	$\bar{x}$ = 0.256
4000	0.063	0.000	0.042	0.033	0.103	0.101	0.102	0.142	0.194	0.262	0.357
	0.072	0.000	0.029	0.051	0.089	0.128	0.217	0.336	0.408	0.563	0.578
	0.094	0.000	0.007	0.059	0.107	0.146	0.223	0.259	0.359	0.484	0.538
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.026	$\bar{x}$ = 0.048	$\bar{x}$ = 0.100	$\bar{x}$ = 0.125	$\bar{x}$ = 0.181	$\bar{x}$ = 0.246	$\bar{x}$ = 0.320	$\bar{x}$ = 0.436	$\bar{x}$ = 0.491
6000	0.129	0.000	0.021	0.087	0.184	0.314	0.409	0.695	0.796	0.989	0.941
	0.106	0.000	0.034	0.044	0.144	0.204	0.318	0.438	0.574	0.716	0.701
	0.103	0.000	0.040	0.051	0.113	0.163	0.236	0.380	0.515	0.646	0.863
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.032	$\bar{x}$ = 0.061	$\bar{x}$ = 0.147	$\bar{x}$ = 0.227	$\bar{x}$ = 0.321	$\bar{x}$ = 0.504	$\bar{x}$ = 0.628	$\bar{x}$ = 0.784	$\bar{x}$ = 0.835

Fig. 7.5 Growth of *Cladophora* over 18 day experimental period in response to variation in light intensity.

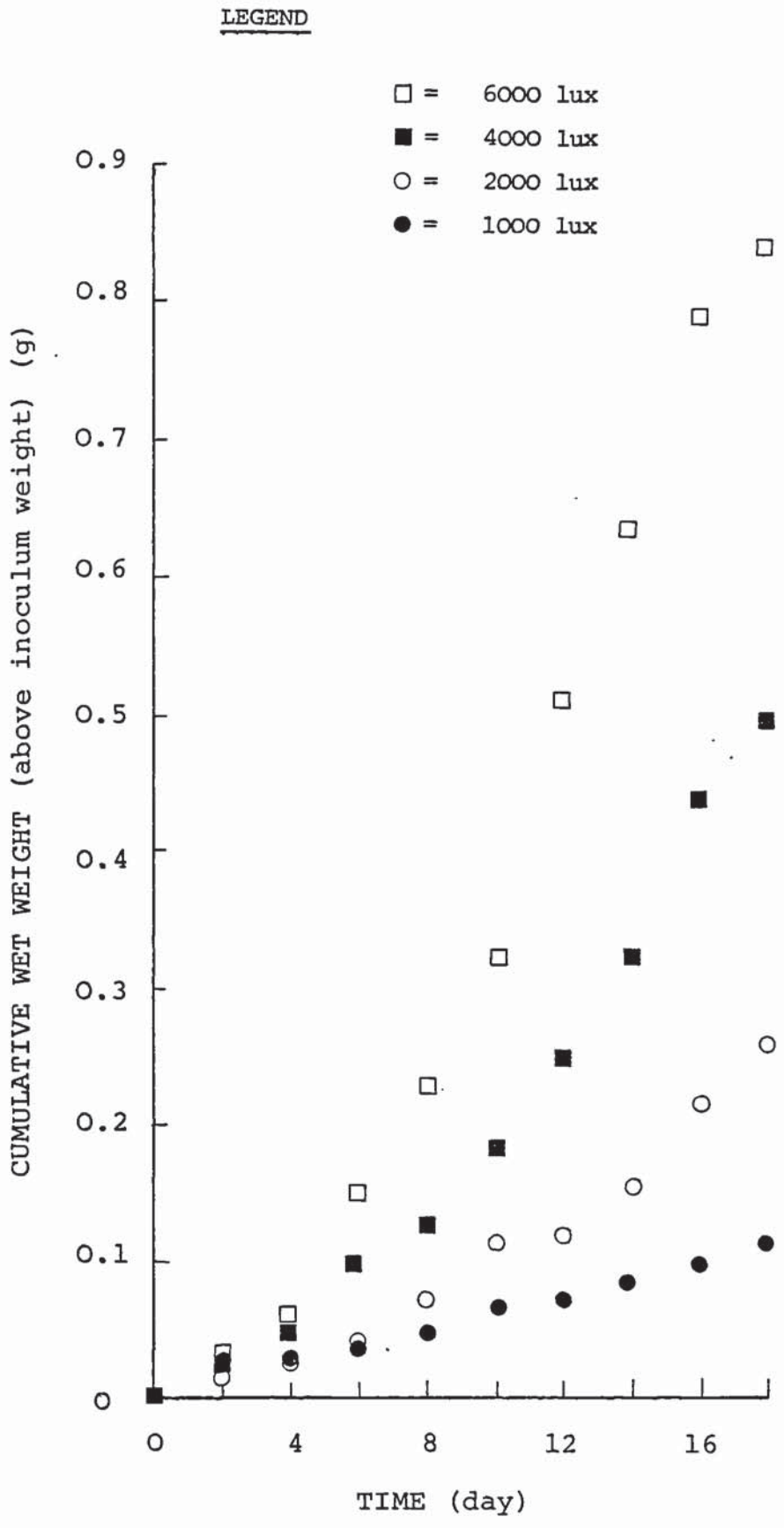


Table 7.8 Calculation of values of μ from growth data of Experiment 8.

Light intensity (lux)	Period over which exponential growth calculated	Regression of ln transformed data		
		Slope (= μ)	Correlation Coefficient (r)	n
1000	0 - 18	0.040	0.964	10
	0 - 18	0.050	0.954	10
	0 - 18	0.030	0.906	10
2000	0 - 18	0.064	0.981	10
	0 - 18	0.066	0.951	10
	0 - 18	0.074	0.977	10
4000	0 - 18	0.093	0.972	10
	0 - 18	0.129	0.994	10
	0 - 18	0.111	0.993	10
6000	0 - 12	0.158	0.996	7
	0 - 12	0.140	0.992	7
	0 - 12	0.123	0.993	7

Table 7.9 Analysis of variance summary table for Experiment 8 - response of μ to variation in light intensity.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	3	.017875	5.95833E-03	31.4285	
Linear Eff.	1	1.76001E-02	1.76001E-02	92.8357	(P< 0.001)
Quad. Eff.	1	2.73948E-04	2.73948E-04	1.445	(P>0.20)
Cubic Eff.	1	9.12661E-07	9.12661E-07	4.81403E-03	(P>0.20)
Residual	0	0			
Error	8	1.51667E-03	1.89584E-04		
Total	11	1.93917E-02			

Fig. 7.6 Response of *Cladophora* specific growth rate to variation in light intensity.

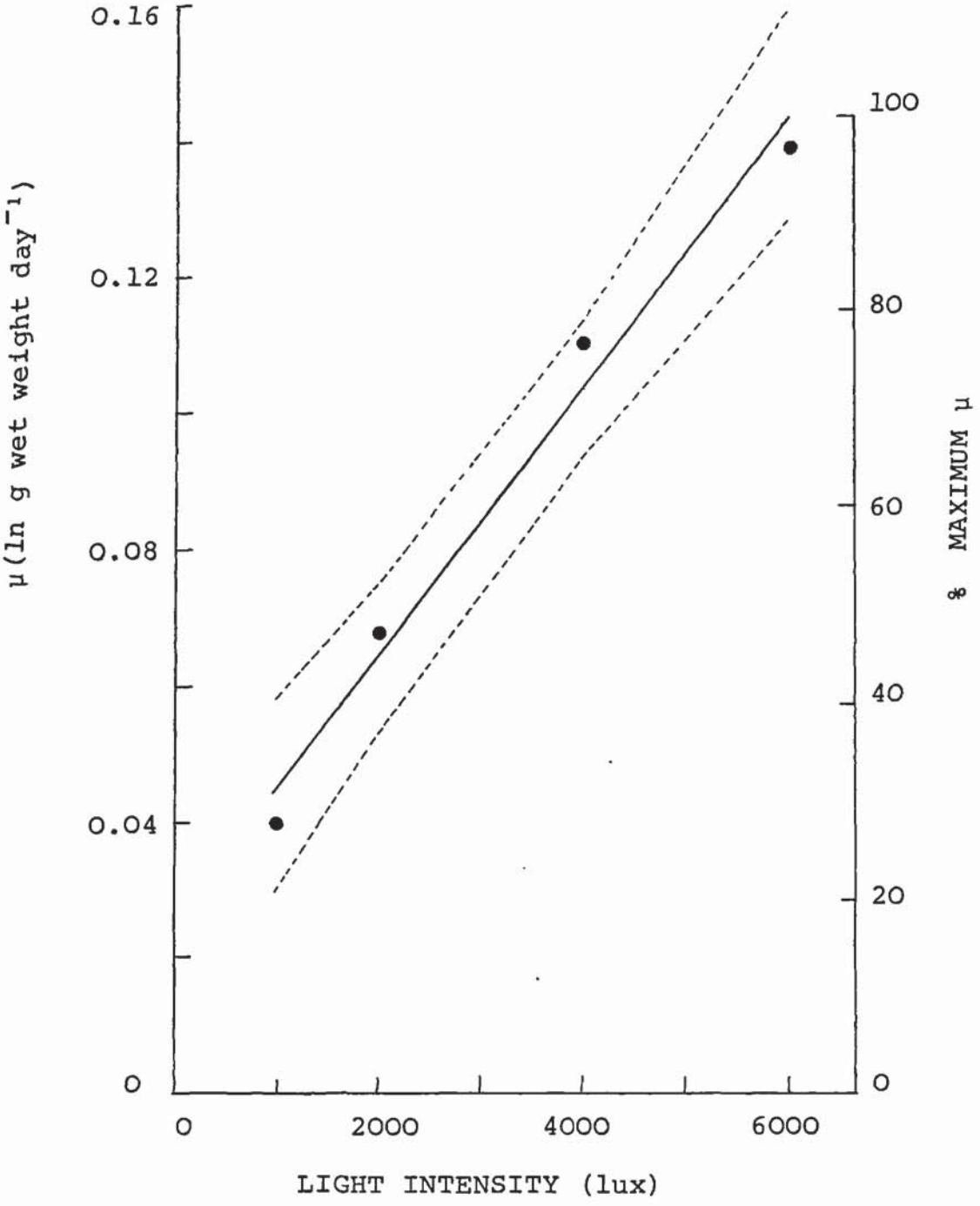
LEGEND

● = Mean (n=3)

——— = Line of best fit

------ = 95% confidence intervals

Significant effects : LINEAR (P<0.001)



the apparatus.

Comparison of experimental results with either results of other experiments or observations from the field situation is unfortunately difficult, owing to the complex nature of 'light' itself . Light is a physiological, not a physical, expression and refers to the spectral sensitivity of the human eye (≈380-760 nm), and since the photosynthetically active part of the solar spectrum comprises 390-710 nm the usage of the term in studies involving photosynthetic processes does not fully coincide with its general usage (Vollenweider, 1974). In this study light intensity (or illuminance) was measured in lux (= lumen m^{-2}) using an EEL portable photoelectric photometer . Vollenweider (1974) considered that such 'barrier layer' instruments are particularly useful in that their spectral sensitivity coincides reasonably well with both the sensitivity range of the human eye and that part of the spectrum which is important in photosynthesis (i.e. photosynthetically active radiation - PAR). McLean (1972), using similar instrumentation noted that the range of intensities recorded for underwater light readings, in spring, at river sites where *Stigeoclonium* had been found varied from 1200-3600 lux. However, *Cladophora* is known to inhabit the riffle zones of less polluted waterways than *Stigeoclonium*; light intensities at such sites may therefore be expected to be nearer to those found in the terrestrial situation. Ginzburg and Ginzburg (1981), in Israel, estimated such intensity to be 1×10^5 lux between the hours of 8 am and 4 pm under a clear sky and 3.5×10^4 lux under an overcast sky. Similarly Tyschen and Fletcher (1971) estimated light intensity to be 1×10^4 lumen ft^{-2} ($\simeq 1 \times 10^5$ lux) during daylight hours. The highest light intensity used in this study (6000 lux) was thus only 6% of the values reported for the intensity of sunlight, on a clear day, in the terrestrial situation. Since, however, light was provided continuously in the experimental situation, comparison of total daily energy input in the experimental and natural system was also worthy of consideration. Monteith (1965) provides estimates of impinging solar radiation as 2650, 4000 and 6000 k cal m^{-2} day^{-1} (for England, Hawaii and Israel respectively), whilst Gates (1962) estimates direct solar radiation as 0-10000 kcal m^{-2} day^{-1} (depending on latitude and season) and Odum (1971) adopts a value of 2-3000 kcal m^{-2} day^{-1} as typical of the input of solar energy to an ecosystem. However, solar radiant energy covers a spectral wavelength range of 300-3000nm

and the proportion of photosynthetically active radiation varies from 43.5% (cloudy day, no sun) to 53% (cloudless day; sun elevation 65°) (Vollenweider, 1974). Using an estimate of photosynthetically active radiation of 50% of total radiation, the input of PAR to an ecosystem may be estimated as 1-2000 kcal m^{-2} day^{-1}. In comparison white fluorescent lights emit 4 x 10^{-6} cal cm^{-2} min^{-1} PAR/lux (Vollenweider, 1974). Thus 6000 lux (continuous lighting) provides approximately 350 kcal m^{-2} day^{-1} i.e 17.5-35% of the energy input to a terrestrial ecosystem. However since the passage of light through water is known to attenuate certain wavelengths of light more than others (see for example Spence, 1981) it is difficult to make further comparison of the field and laboratory situation.

The dependance of the photosynthetic process on light is established, as is that of growth on photosynthesis. Studies of photosynthesis in *Cladophora* have, however, unfortunately been complicated by the tendency of the alga to self-shade when studied by the usual light-and-dark bottle technique. Mantai (1974) found photosynthetic rates three- to fourfold higher than those reported by workers using the light-to-dark bottle when using an oxygen electrode; results suggested this to be a result of self-shading of the alga in bottles, reducing overall photosynthetic rate. Notwithstanding such criticisms Manning *et al.* (1938) found *Cladophora* to exhibit maximum photosynthesis on a bright day at the surface of a lake and whilst Wood (1968) found maximum photosynthetic rate to occur in full sunlight in May and at reduced light intensity in July Mantai (1974) reports that temperatures near the upper limit for growth occurred in July at the site of Wood's study. McMillan and Verduin (1953) suggested that for *Ulothrix* some quality of the surface light rather than intensity was responsible for inhibition of photosynthesis in bottles at the surface of the water - course, and whilst such inhibition was never found for photosynthesis in *Cladophora* it does exemplify the complexity of the situation.

As well as the direct stimulatory effect of increasing light intensity on *Cladophora* photosynthesis it may also be envisaged that the heterogeneous nature of the alga growing in closed continuous culture (an actively growing peripheral area and an inner inactive core - see section 6.3.3) and the heterogeneous nature of the alga in the field situation (as suggested by studies of photosynthetic rate)

both allow for a response of the alga to variation in light intensity in that an increase in light intensity would increase the penetration of light through the algal 'mat'. Overall, algal growth rate would thus increase, as more cells would be capable of photosynthesis and growth. It is thus likely that increasing light intensity would stimulate *Cladophora* growth rate in the field, and in culture, by acting so as to both increase the photosynthetic rate of individual cells and increase the number of cells able to undertake the photosynthetic process.

For further experimental work, it was felt best to provide cultures with 4000 lux (continuous lighting), as used in preliminary studies, since although higher intensities were known to produce better growth the positioning of available light sources to obtain such intensities had the disadvantage of restricting access to culture flasks.

7.2.3 Experiment 9 - The effect of variation in temperature on growth

7.2.3.1 Introduction and objectives

For a given species the specific growth rate (μ) is a function of temperature, light intensity and other environmental variables (Fogg, 1965). Temperature acts both directly, in influencing the rate at which cellular reactions proceed, and indirectly, in affecting the toxicity of a variety of substances found in growth media and natural waters alike (this latter effect is fully reviewed in Cairns *et al.*, 1975). The Q_{10} for μ is usually from 2-4 until unfavourably high temperatures are reached (Fogg, 1965). The optimum temperature for growth of *Cladophora* has been variously reported in laboratory studies : Storr and Sweeney (1971) reported the optimal temperature for growth as 18°C, Whitton (1967) found rapid growth between 15 and 25°C, Bellis (1968a) found the alga to grow with increasing vigour at temperatures between 15 and 30°C and Zuraw (1969) found an optimum at 25°C. It is not known to what extent such differences are attributable to experimental variability, or whether they indicate some degree of ecotypic differentiation in the populations from which isolates were taken. It has been frequently suggested that high summer temperatures result in the reduction of *Cladophora* standing crop in the summer

months in the field situation, and whilst laboratory studies indicate that this would require water temperatures of 20-30°C the study of Wong *et al.* (1978) revealed that the tolerance limit of *C. glomerata* (in the field), at fixed values of the daily mean or daily maximum temperature, changes with daily temperature range. Such a situation could thus explain depression of *Cladophora* growth during the summer months even in temperate climes where mean daily water temperature only reaches 20°c. The objective of this study was therefore to establish the optimum temperature for *Cladophora* growth in culture and to find the range over which growth occurred. With the available equipment no attempt was made to assess the effect of diurnal temperature fluctuations on growth rate.

7.2.3.2 Materials and methods

As section 7.1.2 except that light was provided continuously. Six temperatures were chosen for study viz. 5, 10, 15, 20, 25 and 30°C. For this study the entire apparatus was placed in various temperature control cabinets which maintained the required temperature ±0.5°C. Temperatures were monitored with continuous recording thermometers. Two replicate culture flasks were used per treatment level.

7.2.3.3 Results and discussion

Chemical analysis of the Langley water used in the experiment is included as table 7.3. *Cladophora* growth data is shown as table 7.10, whilst fig. 7.7 plots mean cumulative algal wet weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time the period over which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.11) and analysed by analysis of variance to produce table 7.12. Fig. 7.8 summarizes the *Cladophora* growth response to variation in temperature.

Results show a significant cubic response of *Cladophora* growth rate to increasing light intensity ($P<0.001$). Optimum growth rate occurs at, or near, 20°C; whilst growth at 5 and 30°C was barely detectable. Between 5 and 20°C growth rate increased steadily,

Table 7.10 *Cladophora* growth data for Experiment 9 : growth response to variation in t mperature.

Treatment (°C)	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
5	0.114	0.000	-0.039	-0.017	-0.029	-0.033	-0.016	0.020	0.013	0.018	0.036
	0.121	0.000	-0.014	0.008	-0.003	0.019	0.022	0.069	0.022	0.043	0.060
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.027	$\bar{x}$ = -0.005	$\bar{x}$ = -0.016	$\bar{x}$ = -0.007	$\bar{x}$ = 0.003	$\bar{x}$ = 0.045	$\bar{x}$ = 0.018	$\bar{x}$ = 0.031	$\bar{x}$ = 0.048
10	0.130	0.000	-0.014	-0.013	0.020	0.107	0.234	0.292	0.348	0.409	0.556
	0.108	0.000	0.008	0.016	0.024	0.028	0.078	0.084	0.145	0.155	0.281
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.003	$\bar{x}$ = 0.002	$\bar{x}$ = 0.022	$\bar{x}$ = 0.068	$\bar{x}$ = 0.156	$\bar{x}$ = 0.188	$\bar{x}$ = 0.247	$\bar{x}$ = 0.282	$\bar{x}$ = 0.419
15	0.092	0.000	0.015	0.075	0.162	0.296	0.348	0.506	0.640	0.739	0.775
	0.107	0.000	0.021	0.061	0.118	0.115	0.193	0.255	0.286	0.402	0.557
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.018	$\bar{x}$ = 0.068	$\bar{x}$ = 0.140	$\bar{x}$ = 0.206	$\bar{x}$ = 0.271	$\bar{x}$ = 0.381	$\bar{x}$ = 0.463	$\bar{x}$ = 0.571	$\bar{x}$ = 0.666
20	0.099	0.000	0.033	0.088	0.229	0.401	0.607	0.721	0.940	1.134	1.295
	0.108	0.000	0.003	0.027	0.105	0.257	0.344	0.461	0.524	0.798	1.124
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.018	$\bar{x}$ = 0.058	$\bar{x}$ = 0.167	$\bar{x}$ = 0.329	$\bar{x}$ = 0.476	$\bar{x}$ = 0.591	$\bar{x}$ = 0.732	$\bar{x}$ = 0.966	$\bar{x}$ = 1.210
25	0.110	0.000	0.013	0.071	0.247	0.326	0.478	0.666	0.819	1.002	1.006
	0.106	0.000	0.061	0.070	0.119	0.288	0.331	0.441	0.538	0.559	0.737
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.037	$\bar{x}$ = 0.071	$\bar{x}$ = 0.183	$\bar{x}$ = 0.307	$\bar{x}$ = 0.405	$\bar{x}$ = 0.554	$\bar{x}$ = 0.679	$\bar{x}$ = 0.781	$\bar{x}$ = 0.872
30	0.111	0.000	-0.001	0.012	0.007	0.044	0.094	0.121	0.117	0.132	0.121
	0.098	0.000	0.006	0.026	0.020	0.027	0.014	0.017	0.016	0.024	0.019
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.003	$\bar{x}$ = 0.019	$\bar{x}$ = 0.014	$\bar{x}$ = 0.036	$\bar{x}$ = 0.054	$\bar{x}$ = 0.069	$\bar{x}$ = 0.067	$\bar{x}$ = 0.078	$\bar{x}$ = 0.070

Fig. 7.7 Growth of *Cladophora* over 18 day experimental period in response to variation in temperature.

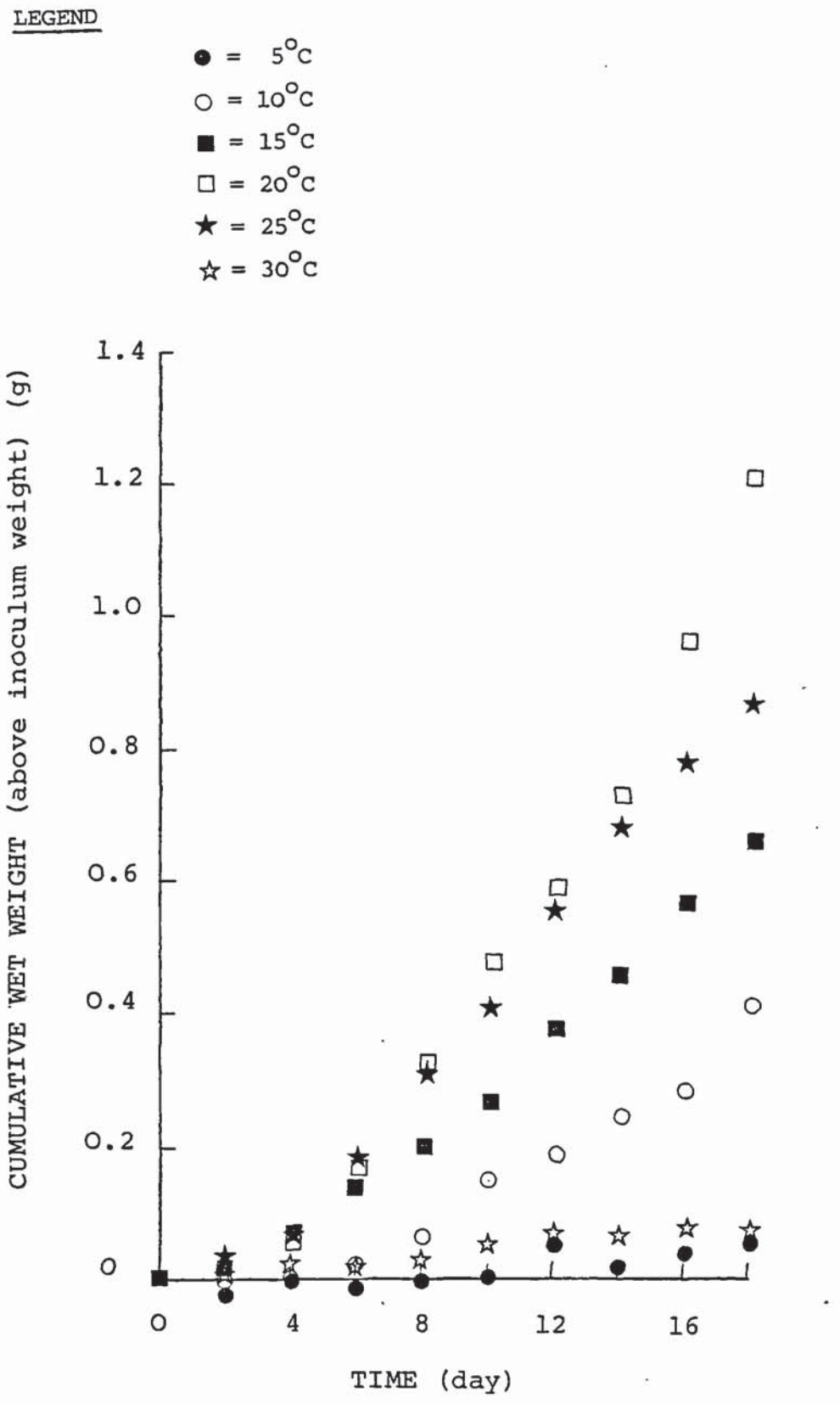


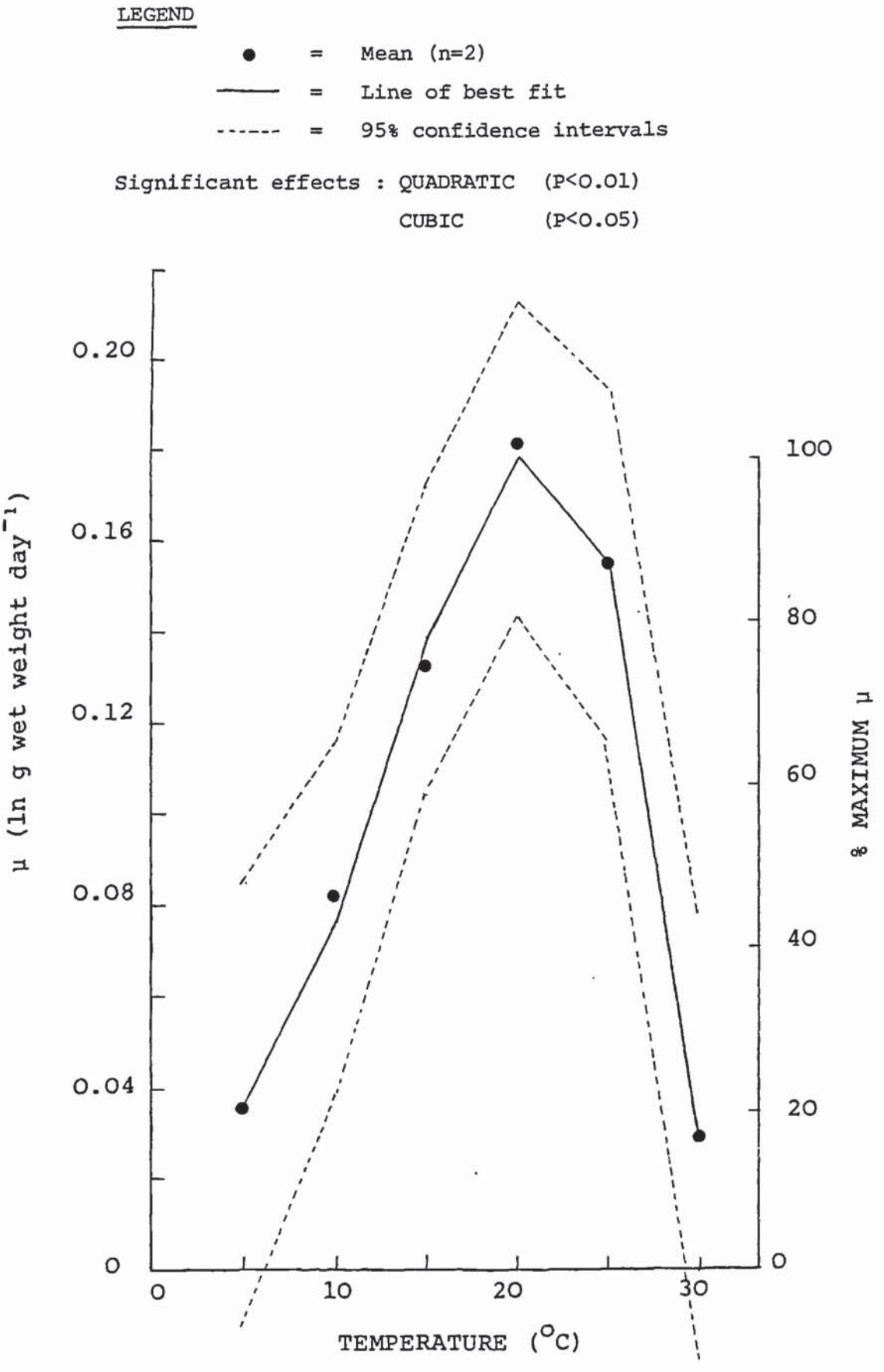
Table 7.11 Calculation of values of μ from growth data of Experiment 9.

Temperature (°C)	Period over which exponential growth evaluated	Regression of In transformed data		
		Slope (= μ)	Correlation Coefficient (r)	n
5	2 - 18	0.042	0.904	9
	2 - 18	0.029	0.847	9
10	10 - 18	0.076	0.990	5
	10 - 18	0.090	0.951	5
15	0 - 12	0.166	0.991	7
	0 - 12	0.101	0.989	7
20	0 - 10	0.205	0.996	6
	0 - 10	0.160	0.968	6
25	0 - 12	0.176	0.986	7
	0 - 12	0.137	0.983	7
30	0 - 18	0.053	0.941	10
	0 - 18	0.006	0.501	10

Table 7.12 *Analysis of variance summary table for Experiment 9 - response of μ to variation in temperature.*

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	5	4.09404E-02	8.18807E-03	9.49797	
Linear Eff.	1	1.63886E-03	1.63886E-03	1.90104	(P>0.20)
Quad. Eff.	1	3.30682E-02	3.30682E-02	38.3583	(P<0.01)
Cubic Eff.	1	6.09264E-03	6.09264E-03	7.06732	(P<0.05)
Residual	2	1.40689E-04			
Error	6	5.17252E-03	8.62087E-04		
Total	11	4.61129E-02			

Fig. 7.8 Response of *Cladophora*: specific growth rate to variation in temperature.



whilst a sharp drop in rate was apparent between 25 and 30°C. At 10°C a noticable lag was found before growth proceeded exponentially.

The isolate used in this study (ref. 505/3, Culture Collection of Algae and Protozoa) had been isolated by George from the River Cam, England, in 1950 and had been maintained at 15-19°C throughout the course of this study. The culture was, therefore, likely to be adapted to temperatures which proved near optimal in this study. The extent to which pre-adaptation to other temperatures would have on the results of similar investigations was not assessed. Results, however, are in good agreement with those already available in the literature, and indicate that mean water temperatures in excess of 20°C would reduce *Cladophora* growth rate from that which is maximal. Combined with the findings of Wong *et al.* (1978) results would thus support the hypothesis that high summer temperatures are (at least in part) responsible for the reduction in *Cladophora* standing crop frequently observed in the field situation.

It was considered that the 17-19°C temperature maintained around the culture flasks by the heating action of the fluorescent lights (room temperature maintained at 15±1°C) and used in preliminary studies was very near to the optimal temperature for *Cladophora* growth ; conditions were therefore not altered in further experimental studies.

7.3 INVESTIGATION OF THE EFFECT OF VARIATION IN CONCENTRATION OF NH_3-N, NO_2-N, NO_3-N AND PO_4-P ON *CLADOPHORA* GROWTH

7.3.1 Experiment 10 - The effect of variation in NH_3-N concentration on growth

7.3.1.1 Introduction and objectives

In higher plants it is generally assumed that the first step in the assimilation of nitrate is its reduction to ammonia via nitrite and hydroxylamine (Wood, 1953). Fogg and Wolfe (1954) concluded that available evidence suggested a similar pathway in blue-green algae. Zobell (1935) concluded that *Nitzschia closterium* and other marine diatoms extracellularly reduce nitrate to nitrite, and Beckwith (1933) demonstrated a similar pathway in *Chlorella*. Zobell (1935), thus considered that since protoplasmic nitrogen is in a reduced state, theoretically nitrite- or ammoniacal nitrogen should be more readily available than nitrate nitrogen; results of Schreiber (1927), Braarud and Foyn (1931) and Bond (1933) supported this hypothesis. McLean (1972), however, quotes Bongers (1956), Fogg (1949), Samejima and Myers (1958) and other authors as suggesting maximum growth rate to be the same in cultures supplied with nitrate- or ammonium nitrogen. However, McLean's results, for the growth rate of *Stigeoclonium* showed nitrate nitrogen to produce significantly better growth than ammoniacal nitrogen at 30 mgN l^{-1} : at 45, 15 and 1.5 mgN l^{-1} no difference was detected. Pratt and Fong (1940) suggested the possibility of such better growth on nitrate salts as a result of the increase in hydrogen ion concentration accompanying absorption of ammoniacal nitrogen, which in poorely buffered media could lead to inhibition of growth. Chu (1942) thus concluded that when nitrogen concentrations were in the optimum range for algal growth then planktonic algae, with few exceptions, grew equally well in media supplied with nitrate- or ammoniacal nitrogen; at lower concentrations of nitrogen, however, growth was generally better with a nitrate source. Little literature concenrning the utilization of NH_3-N by *Cladophora* is apparent. Gerloff and Fitzgerald (1976), however, concluded that *Cladophora* yield per mgN was fairly uniform for NO_2, NO_3 and NH_3.

As well as acting as a potential nitrogen source, ammonia also possesses considerable toxic activity. In aqueous solutions ammonia establishes the equilibrium :

$$NH_3 + nH_2O \rightleftarrows NH_3nH_2O \rightleftarrows NH_4^+ + OH^- + (n-1)\ H_2O$$

The toxicity to aquatic organisms has generally been attributed to the unionized (undissociated) species - NH_3 - (Chipman, 1934; Wuhrmann *et al.*, 1947; Wuhrmann and Woker, 1948)., whilst the ionized (or dissociated) species - NH_4^+ - is considered to be nontoxic or significantly less toxic (Tabata, 1962). Warren (1962) suggested this differential toxicity to be attributable to the relative impermeability of the cell membrane to NH_4^+ but not NH_3. Much of the literature involving ammonia toxicity stems from work on fish, and factors found to affect the overall toxicity include pH and temperature (both of which alter the $NH_3 \rightleftarrows NH_4^+$ equilibrium), dissolved oxygen concentration (Downing and Merkens, 1955) and hardness (Tomasso *et al.*, 1980). Davies (1971) investigated the effects of ammonia on various freshwater invertebrates to find *Hydropsyche angustipennis* and *Rhyacophila dorsalis* most tolerant and *Gammarus pulex* and *Ecdyonurus dispar* least tolerant of the species studied. Watton (1982) reviews the scant literature on ammonia toxicity to freshwater gasteropods and includes results of extensive studies on *Potamopyrgus jenkinsi*. Literature dealing with the tolerance of algal species to ammoniacal nitrogen levels is rather scarce. However, Ludwig (1938) comments that to some algal species some inorganic ammonium salts have occasionally proved to be unavailable or even toxic. Zobell (1935) found 4mM ammoniacal nitrogen to be inhibitory to *Nitzschia closterium* (compared to 25mM for nitrite- and 300mM for nitrate nitrogen); whilst Patrick *et al.* (1968) found the 120 hr TLm (concentration to produce 50% reduction in number of cells produced) to be 420 mgl^{-1} of ammoniacal nitrogen for diatoms - much more than that to produce 50% mortality in either fish or snails. Chu (1942) found reduction in growth of *Pediastrum Boryanum* at concentrations of NH_4NO_3 above 25 ppm; nitrogen (in the form of NH_3) had an inhibitory effect on growth of *Staurastrum paradoxum* at concentrations above 17 ppm; amd growth of *Fragilaria crotonensis* was found to be inhibited at concentrations of $(NH_4)_2SO_4$ in excess of 5 ppm. Gerloff and Fitzgerald (1976) reported *Cladophora* yield to be considerably less at 5 mg NH_3-N l^{-1} than at 2 mg NH_3-N l^{-1}, and considered this to be a result of the lowering of pH which occurred with increasing NH_3-N concentrations.

The objective of this study was limited to an investigation of the toxic effect of NH_3-N on *Cladophora*.

7.3.1.2 Materials and methods

As section 7.1.2. Nominal total ammoniacal nitrogen (TA-N) concentration of 0, 1, 2, 5 and 10 mgl^{-1} were chosen for study. Three replicate culture flasks were used per treatment level. The proportion of total ammoniacal nitrogen present in its undissociated (= toxic) form was calculated from the equation given by Emerson *et al.* (1975) :-

$$f = 1/(10^{pKa-pH} + 1), \text{ where } pKa = 0.9018 + 2729.92/T$$

A temperature of 18°C (291K) and pH of 8.2 were used in calculations to reveal 5.1% of the ammoniacal nitrogen to be in the undissociated form -NH_3. Treatment levels were produced by adding appropriate volumes of a 23.58g l^{-1} $(NH_4)_2SO_4$ (= 5gN l^{-1}) stock solution. No detectable alteration of pH accompanied addition of $(NH_4)_2SO_4$ upto the highest level used. Throughout the duration of the experiment water samples were abstracted for analysis of total ammoniacal nitrogen concentration. Values were processed to produce table 7.13.

Table 7.13 NH_3-N treatment levels for Experiment 10.

Nominal TA-N (mgl^{-1})	Total Ammoniacal Nitrogen				Mean Undissociated Ammoniacal Nitrogen ($\mu g\ l^{-1}$)
	Mean (mgl^{-1})	95% confidence limits ± (mgl^{-1})	Range (mgl^{-1})	n	
0	0.2	0.0	0.1-0.7	30	10
1	1.1	0.1	0.7-1.4	30	56
2	2.2	0.1	1.4-2.6	30	113
5	5.5	0.1	4.9-6.2	30	282
10	10.6	0.2	10.0-11.6	30	544

7.3.1.3 Results and discussion

Physico-chemical analysis of the Langley water used in the experiment is included as table 7.3. *Cladophora* growth data is shown as table 7.14, whilst fig. 7.9 plots mean cumulative algal weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time, the period in which growth was exponential was selected for each treatment level. values of μ were then calculated for each culture (table 7.15) and analysed by analysis of variance to produce table 7.16. Fig. 7.10 summarizes the *Cladophora* growth response to variation in NH_3-N concentration.

Results show the toxic nature of ammonia to *Cladophora*. At mean pH 8.2 and mean temperature 18°C 3.6 mgN l^{-1} serves to reduce *Cladophora* specific growth rate by 50%, and 10.6 mgN l^{-1} reduces growth rate to 18% of that found at 0.2 mgN l^{-1}.

Watton (1982) and Girton (1980) both carried out extensive field sampling throughout Britain. Accompanying physico-chemical data (some supplied courtesy of appropriate Water Authorities) reveals the maximum concentration of total ammoniacal nitrogen recorded at any station to be 22.65 mgN l^{-1}, and the highest mean concentration to be 11.6 mgN l^{-1}. Such high ammonia levels almost certainly came from grossly polluted rivers where *Cladophora* would not be expected to grow; they do, however indicate the range of total ammoniacal nitrogen concentration which may occur in the field situation. Similarly Painter (1971) includes analysis of effluents from three towns in southeast England with total ammoniacal nitrogen concentrations between 21 and 38 mgN l^{-1}. Applying the x8 dilution factor laid down by the Royal Commission on Sewage Disposal (1912), this would produce a total ammoniacal nitrogen concentration of 3-5 mgN l^{-} in the receiving waterway. Concentrations of total ammoniacal nitrogen similar to those used in this experiment thus certainly occur in the field situation. Further, since *Cladophora* is known to favour basic waters, if ammonia toxicity is attributable to the NH_3 species, such conditions favour the formation of this

Table 7.14 *Cladophora* growth data for Experimentt 10 : growth response to variation in NH_3-N concentration.

Treatment (NH_3-N mgl^{-1})	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.2	0.140	0.000	0.023	0.133	0.189	0.363	0.556	0.811	1.117	1.542	1.729
	0.115	0.000	0.020	0.038	0.101	0.194	0.311	0.348	0.506	0.788	0.768
	0.125	0.000	0.010	0.021	0.028	0.075	0.160	0.245	0.341	0.461	0.768
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.018	$\bar{x}$ = 0.064	$\bar{x}$ = 0.106	$\bar{x}$ = 0.211	$\bar{x}$ = 0.342	$\bar{x}$ = 0.468	$\bar{x}$ = 0.655	$\bar{x}$ = 0.930	$\bar{x}$ = 1.088
1.1	0.142	0.000	0.034	0.012	0.021	0.082	0.099	0.208	0.415	0.662	1.085
	0.110	0.000	-0.018	0.013	0.052	0.133	0.265	0.383	0.569	0.771	1.048
	0.150	0.000	-0.006	0.024	0.035	0.108	0.183	0.255	0.341	0.490	0.608
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.003	$\bar{x}$ = 0.016	$\bar{x}$ = 0.036	$\bar{x}$ = 0.108	$\bar{x}$ = 0.182	$\bar{x}$ = 0.282	$\bar{x}$ = 0.442	$\bar{x}$ = 0.641	$\bar{x}$ = 0.914
2.2	0.115	0.000	- 0.006	0.015	0.027	0.025	0.066	0.096	0.131	0.199	0.284
	0.150	0.000	0.002	0.021	0.026	0.069	0.108	0.122	0.150	0.194	0.331
	0.145	0.000	0.011	0.014	0.013	0.045	0.052	0.082	0.139	0.244	0.325
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.002	$\bar{x}$ = 0.017	$\bar{x}$ = 0.022	$\bar{x}$ = 0.046	$\bar{x}$ = 0.075	$\bar{x}$ = 0.100	$\bar{x}$ = 0.140	$\bar{x}$ = 0.212	$\bar{x}$ = 0.313
5.5	0.117	0.000	0.028	0.043	0.045	0.037	0.050	0.073	0.103	0.127	0.154
	0.148	0.000	0.010	0.022	0.038	0.072	0.065	0.096	0.097	0.139	0.166
	0.143	0.000	0.003	0.022	0.020	0.026	0.032	0.071	0.091	0.108	0.165
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.014	$\bar{x}$ = 0.029	$\bar{x}$ = 0.034	$\bar{x}$ = 0.045	$\bar{x}$ = 0.049	$\bar{x}$ = 0.080	$\bar{x}$ = 0.097	$\bar{x}$ = 0.125	$\bar{x}$ = 0.162
10.6	0.092	0.000	0.044	0.071	0.067	0.069	0.069	0.076	0.095	0.088	0.087
	0.145	0.000	-0.033	-0.037	-0.004	-0.012	-0.013	0.005	0.026	0.042	0.057
	0.083	0.000	-0.010	0.003	-0.002	0.012	0.028	0.031	0.011	0.024	0.057
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.000	$\bar{x}$ = 0.012	$\bar{x}$ = 0.020	$\bar{x}$ = 0.023	$\bar{x}$ = 0.028	$\bar{x}$ = 0.037	$\bar{x}$ = 0.044	$\bar{x}$ = 0.051	$\bar{x}$ = 0.067

Fig. 7.9 Growth of *Cladophora* over 18 day experimental period in response to variation in NH_3-N concentration.

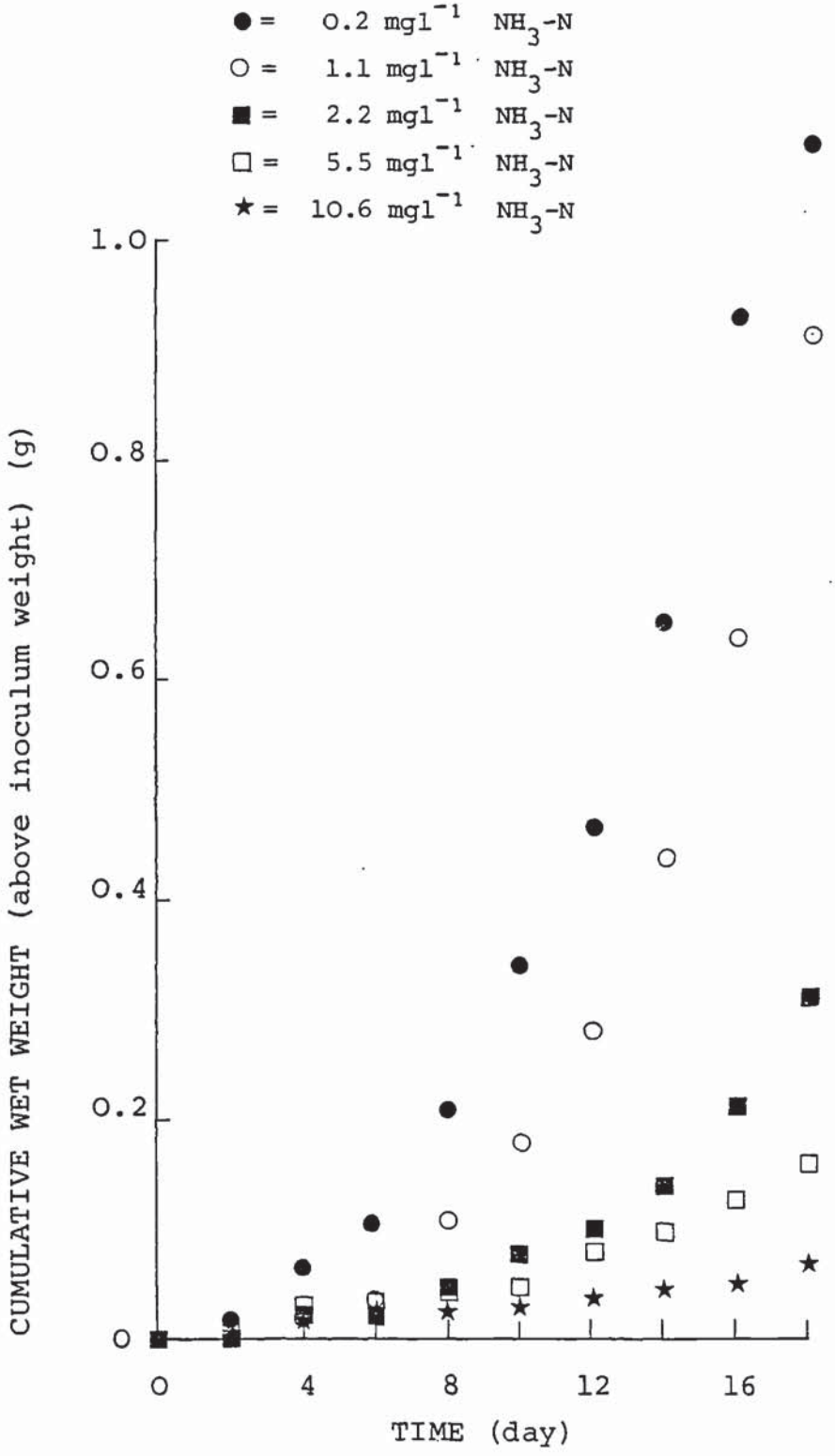


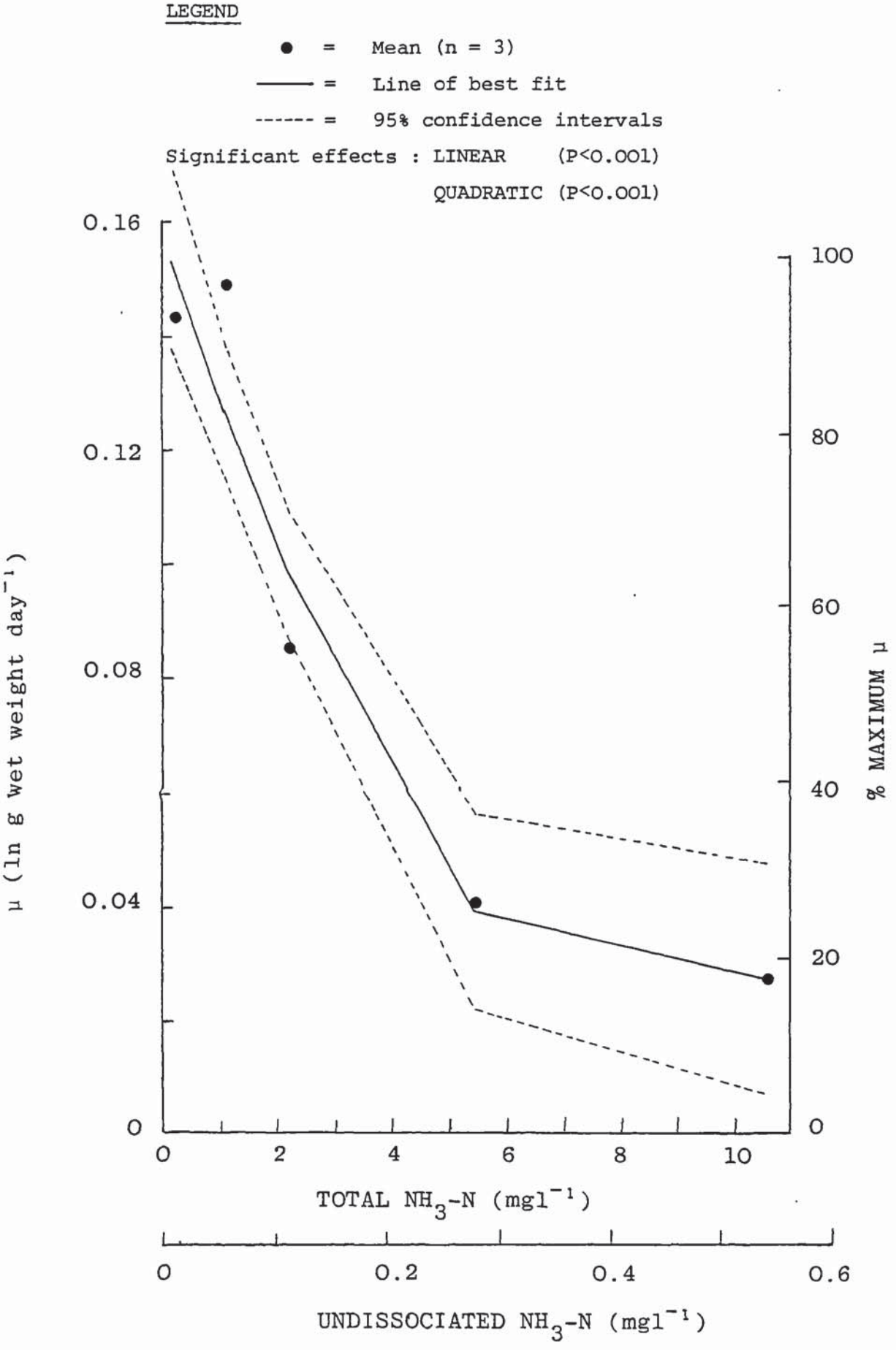
Table 7.15 Calculation of values of μ from growth data of Experiment 10.

NH_3-N (mgl^{-1})	Period over which exponential growth evaluated	Regression of ln transformed data		
		Slope (= μ)	Correlation coefficient (r)	n
	6 - 16	0.160	0.997	6
0.2	6 - 16	0.133	0.989	6
	6 - 16	0.136	0.997	6
	6 - 18	0.169	0.988	7
1.1	6 - 18	0.162	0.995	7
	6 - 18	0.115	0.996	7
	6 - 18	0.090	0.985	7
2.2	6 - 18	0.073	0.974	7
	6 - 18	0.091	0.978	7
	0 - 18	0.040	0.958	10
5.5	0 - 18	0.041	0.989	10
	0 - 18	0.040	0.959	10
	0 - 18	0.027	0.787	10
10.6	0 - 18	0.027	0.815	10
	0 - 18	0.027	0.848	10

Table 7.16 Analysis of variance summary table for Experiment 10 - response of μ to variation in NH_3-N concentration.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	.038121	9.53025E-03	40.247	
Linear Eff.	1	3.03542E-02	3.03542E-02	128.188	(P<0.001)
Quad. Eff.	1	5.44778E-03	5.44778E-03	23.0064	(P<0.001)
Cubic Eff.	1	1.38459E-05	1.38459E-05	5.84723E-02	(P>0.20)
Residual	1	2.30519E-03			
Error	10	2.36794E-03	2.36794E-04		
Total	14	.040489			

Fig. 7.10 Response of *Cladophora* specific growth rate to variation in NH_3-N concentration.



toxic form and therefore may well affect the distribution and growth of *Cladophora* in the field. Ammonia toxicity is also known to be positively correlated with water temperature -at pH 8.5, for example, the proportion of ammoniacal nitrogen present in the toxic (NH_3) form is only 3.8% at 5°C, but rises to 11.2% at 20°C (Emerson *et al.* 1975). Ammonia is thus more likely to produce toxic effects, and consequently be an important factor in affecting the growth of *Cladophora* in the field situation when water temperatures are higher i.e. in the summer months. Indeed, in some situations at least, toxicity of ammonia may help explain the depression in algal standing crop during summer months.

7.3.2 Experiment 11 - The effect of variation in NO_2-N concentration on growth.

7.3.2.1 Introduction and objectives

Nitrite has received increasing attention over the past few years as a potentially important pollutant in aquatic systems. In very low concentrations it is known to be toxic to fishes (Russo and Thurston, 1977) and like ammonia, much of the literature dealing with nitrite toxicity involves work on fish. Nitrite toxicity is reduced by the presence of chloride ions (Russo and Thurston, 1977; Tomasso *et al.*, 1979), calcium (Crawford and Allen, 1977) and increasing pH (Wedemeyer and Yasutake, 1978). In aqueous solutions nitrite establishes the equilibrium :-

$$NO_2^- + H^+ \rightleftarrows HNO_2$$

This equilibrium is pH dependant, Colt and Tchobanoglous (1976) suggested - for fish, at least - that nitrite toxicity may depend upon the concentration of HNO_2 rather than NO_2^- because of the ability of the former to move more readily across biological membranes. Russo *et al.* (1981), however, concluded that NO_2^- contributed significantly to total toxicity and recommended that nitrite criteria to protect freshwater aquatic life be based on total nitrite. Little work has apparently been done on the toxicity of nitrite to algae. Zobell (1935), however, found nitrite toxicity to *Nitzschia closterium* to be between that of ammonium and nitrate - 25 mM NO_2 being evaluated as inhibitory. Fogg (in Fogg and Wolfe, 1954) suggested that 27 mg NO_2-N l^{-1} completely

inhibited growth of *Anabaena cylindrica*, whilst Ludwig (1938) concluded that even at low concentrations KNO_2 seemed to be slightly toxic to *Chlorella* when added to a nitrate medium. Gerloff and Fitzgerald (1976), however, found no toxic effect of nitrite to *Cladophora* growth when up to 5 mg NO_2-N l^{-1} was used as a nitrogen source.

Nitrite may also act as a suitable source of nitrogen. Gerloff *et al.* (1952) found nitrite to be as effective a nitrogen source as nitrate, upto concentrations of 13.6 mgN l^{-1}. Maertens (1914) found nitrite to be suitable for the growth of *Oscillatoria* (but not *Nostoc, Cylindrospermum* or *Calothrix)*, McLean (1972) found nitrite to be the best nitrogen source for *Stigeoclonium tenue* at 30 and 15 mgN l^{-1}, when compared to nitrate- and ammoniacal nitrogen, and Pringsheim (1913) found nitrite to be a suitable nitrogen source for a number of blue-green algal species. Similarly Gerloff and Fitzgerald (1976) after assessing the effects of a variety of nitrogen sources concluded that *Cladophora* yield per mgN was fairly uniform for NO_2, NO_3 and NH_3.

The objective of this investigation was to assess the toxic activity of nitrite on *Cladophora* growth.

7.3.2.2 Materials and methods

As 7.1.2. Nominal NO_2-N concentrations of 0.0, 0.05, 0.10, 0.50 and 1.00 mgl^{-1} were chosen for study. Three replicate culture flasks were used for each treatment level. Analysis of Langley water revealed a Na : K ratio of approximately 3:1; to maintain this ratio treatment levels were produced by adding appropriate volumes of a concentrated stock solution comprising 4.1180 gl^{-1} $NaNO_2$ and 0.9955 g l^{-1} KNO_2 (= 1gN l^{-1}; 1.3721 g Na l^{-1}; 0.4574gKl^{-1}) No detectable alteration of pH accompanied addition of Na/KNO_2 upto the highest level tested. Throughout the duration of the experiment water samples were abstracted for analysis of NO_2-N concentration. Values were processed to produce table 7.17.

Table 7.17 NO_2-N treatment levels for Experiment 11.

Nominal NO_2-N (mgl^{-1})	Mean (mgl^{-1})	95% confidence limits ± (mgl^{-1})	Range (mgl^{-1})	n
0.00	0.077	0.009	0.015-0.100	30
0.05	0.090	0.013	0.045-0.145	30
0.10	0.141	0.012	0.100-0.190	30
0.50	0.574	0.023	0.480-0.680	30
1.00	1.057	0.054	0.825-1.340	30

Work carried out at the University of Aston (Hawkes personal communication) had suggested that storage of samples ready for automated analysis may have led to severe underestimation of NO_2-N concentrations in past studies. At the time of experimentation the best storage method was considered to be freezing (at -5°C), and since medium was nearly (or totally) sterile no conversion of NO_2-N to NO_3-N was expected. NO_3-N concentration was only measured at the beginning of the study, however visual inspection of NO_2-N concentrations revealed no overall reduction of NO_2-N levels throughout the duration of experimentation (as would be expected if nitrification was proceeding in the medium in the influent reservoirs).

7.3.2.3 Results and discussion

Physico-chemical analysis of the Langley water used in the experiment is included as table 7.3. *Cladophora* growth data is shown as table 7.18, whilst Fig. 7.11 plots mean cumulative algal wet weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time the period in which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.19) and analysed by analysis of variance to produce table 7.20. Results show NO_2-N concentration to have no effect on specific growth rate. Plots of untransformed

Table 7.18 *Cladophora* growth data for Experiment 11 : growth response to variation in NO_2-N concentration.

Treatment NO_2-N mgl^{-1}	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.077	0.131	0.000	0.033	0.040	0.134	0.289	0.549	0.750	0.992	1.247	1.302
	0.132	0.000	0.039	0.145	0.354	0.605	0.715	0.884	1.126	1.529	1.733
	0.096	0.000	0.034	0.063	0.087	0.180	0.303	0.434	0.562	0.791	0.860
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.035	$\bar{x}$ = 0.083	$\bar{x}$ = 0.192	$\bar{x}$ = 0.358	$\bar{x}$ = 0.522	$\bar{x}$ = 0.689	$\bar{x}$ = 0.893	$\bar{x}$ = 1.189	$\bar{x}$ = 1.298
0.090	0.105	0.000	0.027	0.115	0.327	0.484	0.684	0.819	1.137	1.588	1.569
	0.090	0.000	0.043	0.080	0.163	0.210	0.309	0.370	0.486	0.742	0.687
	0.093	0.000	0.034	0.111	0.273	0.446	0.593	0.760	0.900	1.404	1.364
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.035	$\bar{x}$ = 0.102	$\bar{x}$ = 0.254	$\bar{x}$ = 0.380	$\bar{x}$ = 0.529	$\bar{x}$ = 0.650	$\bar{x}$ = 0.841	$\bar{x}$ = 1.245	$\bar{x}$ = 1.207
0.141	0.109	0.000	0.044	0.096	0.225	0.453	0.605	0.703	0.997	1.254	1.272
	0.092	0.000	0.041	0.081	0.123	0.283	0.413	0.624	0.710	1.030	1.084
	0.141	0.000	0.051	0.185	0.362	0.540	0.802	0.825	0.943	1.393	1.326
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.045	$\bar{x}$ = 0.121	$\bar{x}$ = 0.237	$\bar{x}$ = 0.425	$\bar{x}$ = 0.607	$\bar{x}$ = 0.717	$\bar{x}$ = 0.883	$\bar{x}$ = 1.226	$\bar{x}$ = 1.227
0.574	0.143	0.000	0.052	0.138	0.451	0.650	0.739	0.861	1.022	1.628	1.381
	0.113	0.000	0.033	0.109	0.186	0.429	0.577	0.649	0.749	1.218	1.125
	0.102	0.000	0.041	0.166	0.454	0.619	0.717	0.808	0.873	1.177	1.141
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.042	$\bar{x}$ = 0.138	$\bar{x}$ = 0.364	$\bar{x}$ = 0.566	$\bar{x}$ = 0.678	$\bar{x}$ = 0.773	$\bar{x}$ = 0.881	$\bar{x}$ = 1.341	$\bar{x}$ = 1.216
1.057	0.081	0.000	0.069	0.171	0.341	0.453	0.529	0.696	0.828	1.053	1.177
	0.125	0.000	0.044	0.109	0.295	0.365	0.410	0.546	0.621	0.949	0.935
	0.109	0.000	0.038	0.067	0.129	0.192	0.311	0.443	0.461	0.525	0.619
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.050	$\bar{x}$ = 0.116	$\bar{x}$ = 0.255	$\bar{x}$ = 0.337	$\bar{x}$ = 0.417	$\bar{x}$ = 0.562	$\bar{x}$ = 0.637	$\bar{x}$ = 0.842	$\bar{x}$ = 0.910

Fig. 7.11 Growth of *Cladophora* over 18 day experimental period in response to variation in NO_2-N concentration.

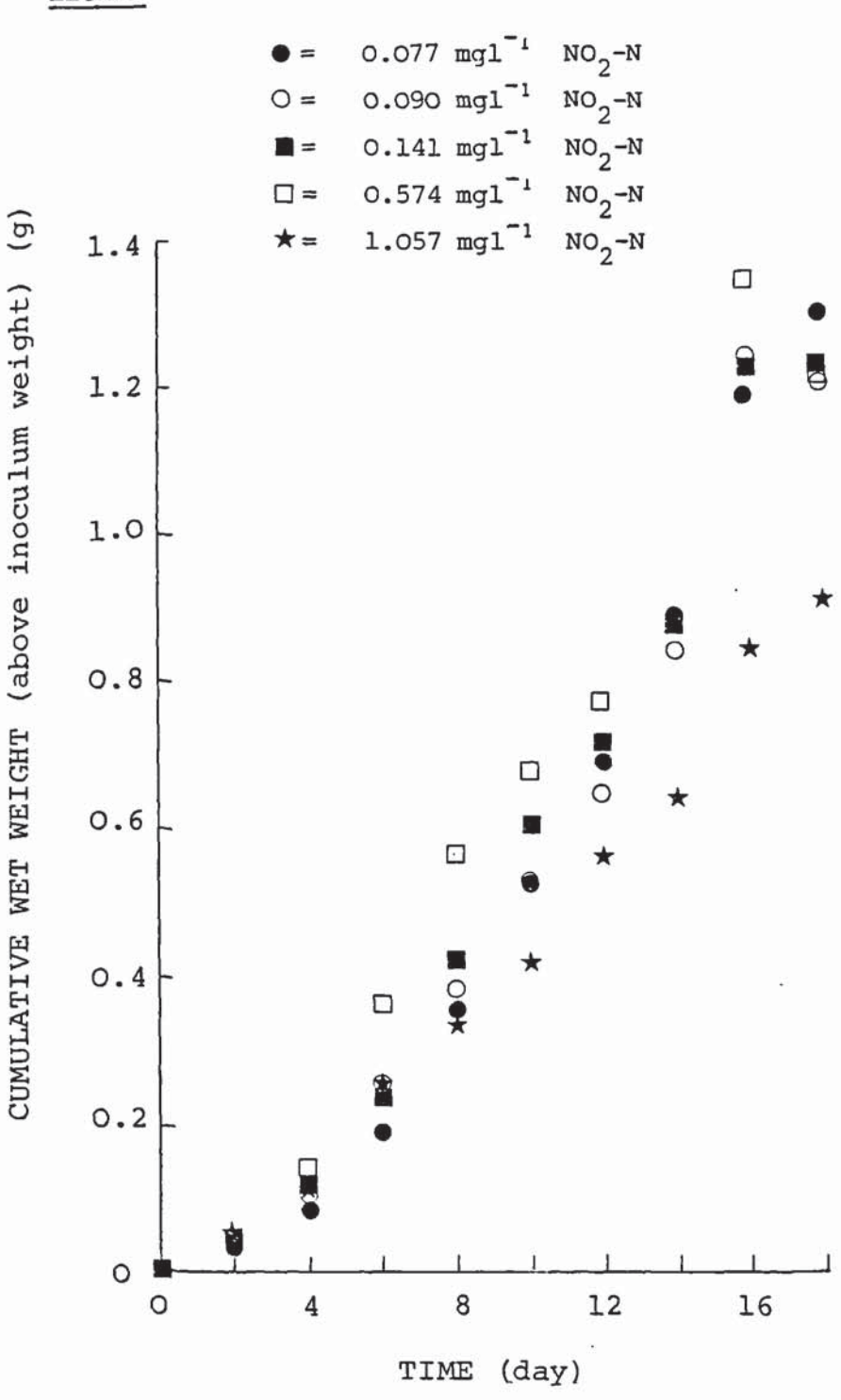


Table 7.19 Calculation of values of μ from growth data of Experiment 11.

NO_2-N (Mgl^{-1})	Period over which exponential growth evaluated	Regression of ln transformed data		
		Slope (=μ)	Correlation coefficient (r)	n
	0 - 8	0.140	0.958	5
0.077	0 - 8	0.224	0.994	5
	0 - 8	0.123	0.986	5
	0 - 8	0.232	0.988	5
0.090	0 - 8	0.153	0.993	5
	0 - 8	0.229	0.996	5
	0 - 10	0.197	0.995	6
0.141	0 - 10	0.169	0.992	6
	0 - 10	0.196	0.996	6
	0 - 8	0.227	0.987	5
0.574	0 - 8	0.193	0.990	5
	0 - 8	0.263	0.989	5
	0 - 6	0.274	0.999	4
1.057	0 - 6	0.198	0.986	4
	0 - 6	0.126	0.995	4

Table 7.20 Analysis of variance summary table for Experiment 11 - response of μ to variation in NO_2-N concentration.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	6.89173E-03	1.72293E-03	.724915	
Linear Eff.	1	1.35601E-03	1.35601E-03	.570535	(P>0.20)
Quad. Eff.	1	3.06221E-03	3.06221E-03	1.28841	(P>0.20)
Cubic Eff.	1	1.30788E-05	1.30788E-05	5.50285E-03	(P>0.20)
Residual	1	2.46043E-03			
Error	10	2.37674E-02	2.37674E-03		
Total	14	3.06591E-02			

Table 7.21 Calculation of yalues of k from growth data of Experiment 11.

NO_2-N (mgl^{-1})	Period over which incremental growth evaluated	Regression of untransformed data		
		Slope (=μ)	Correlation coefficient (r)	n
	12 - 18	0.096	0.971	4
0.077	12 - 18	0.148	0.992	4
	12 - 18	0.075	0.982	4
	12 - 18	0.135	0.942	4
0.090	12 - 18	0.060	0.898	4
	12 - 18	0.116	0.919	4
	12 - 18	0.098	0.949	4
0.141	12 - 18	0.085	0.959	4
	12 - 18	0.098	0.900	4
0.574	12 - 18	0.108	0.807	4
	12 - 18	0.095	0.880	4
	12 - 18	0.065	0.903	4
1.057	12 - 18	0.083	0.993	4
	12 - 18	0.075	0.922	4
	12 - 18	0.030	0.961	4

Table 7.22 Analysis of variance summary table for Experiment 11 - response of k to variation in NO_2-N concentration.

Source of Variation	Degrees of Freedom	Sum of Square	Mean Square	F-Ratio	
Treatment	4	3.62435E-03	9.06087E-04	1.05564	
Linear Eff.	1	3.33624E-03	3.33624E-03	3.88688	(P<0.10)
Quad. Eff.	1	6.05871E-05	6.05871E-05	7.05869E-02	(P>0.20)
Cubic Eff.	1	2.27419E-04	2.27419E-04	.264954	(P>0.20)
Residual	1	1.00583E-07			
Error	10	8.58334E-03	8.58334E-04		
Total	14	1.22077E-02			

and ln transformed mean cumulative wet weight against time indicated that all cultures were growing incrementally from day 12 onwards. Day 12-18 data were entered into a regression equation and slopes (table 7.21) analysed by analysis of variance to produce table 7.22.

Results show that variation in NO_2-N concentration within the range 0.077-1.057 mgN l^{-1} had no significant effect on exponential or incremental growth of *Cladophora* in culture ($P>0.05$).

Painter (1971) found NO_2-N concentrations of 0.2-1.8 in domestic sewage effluents from three towns in southeast England. Applying the x8 dilution factor laid down by the Royal Commission on Sewage Disposal (1912), this would result in concentrations of NO_2-N in the receiving waterway within the range 0.025-0.225 mg N l^{-1} . Results of this study suggest that such concentrations would be expected to have no significant effect on *Cladophora* growth.

7.3.3 Experiment 12 - The effect of variation in NO_3-N and PO_4-P concentration on growth

7.3.3.1 Introduction and objectives

Data appears compatible with the conclusion that *Cladophora* as a genus avoids waters very low in phosphate and nitrate. Further, it is generally assumed that the obvious increases in crops within the last couple of decades are a response to increased levels of nutrients resulting from increased population, detergents and fertilizers (Whitton, 1970b). Neil (1975) thus concluded that priority should be given to studies of the phosphorus and nitrogen requirements of *Cladophora* and, indeed, a number of such investigations are apparent in the literature.

Neil and Owen (1964) describe field experiments in which phosphate and/or combined nitrogen were added to sites in Lake Huron. To ensure availability of 'seed' material, rocks having *Cladophora* on them were moved to the areas under study. Added nitrogen failed to stimulate new growth of the alga, but added phosphorus resulted in the establishment of a new bed of *Cladophora*, as did the addition of nitrogen, phosphorus and potassium in combination. Eichenberger (1967a, 1967b),

using the experimental channels at Zurich, found *C. glomerata* to grow best in the channel receiving 2% effluent (0.07 mg PO_4-P l^{-1}, 0.6 mg NO_3-N l^{-1} and 0.2 mg NH_3-N l^{-1}). Presumably the poorer development of the alga in channels with higher concentrations of nutrients was mainly a result of competition with sewage fungus (Whitton, 1970b). Similarly Bolas and Lund (1974) grew *Cladophora* in 'streams' on the banks of the R. Stour, Kent; supplementing 'streams' with various combinations of nitrate and phosphate. Results showed phosphorus to be a major controlling element, whilst nitrogen was not. Hoffman *et al.* (1974) also concluded that phosphorus was the most probable limiting element controlling growth in the Eau Gallie River, Florida. Pitcairn and Hawkes (1973), after sampling a number of waterways in England, established a significant correlation (r = 0.54) between mean annual *Cladophora* dry weight and mean annual concentration of total inorganic phosphorus, but found no significant correlation (r = 0.22) between biomass and mean annual concentration of NO_3-N. Wong and Clark (1976) established a direct correlation (r = 0.87) between ambient phosphorus concentration in the water and the phosphorus content of the plant tissue in six rivers in southwestern Ontario, but observed no significant correlation between nitrogen content of the tissue and that of the water. Critical concentrations (i.e. the minimum concentration which permits maximum yield) of total phosphorus were 60 µg l^{-1} in stream water and 1.6 mg g^{-1} dry weight plant tissue. Lin (1977) similarly found concentrations of hot-water extractable phosphorus from most *Cladophora* samples to be within the range 0.6-6.8 µg mg l^{-1} dry weight alga which correlated closely to the total dissolved phosphorus in the ambient Lake Michigan water. Gerloff and Fitzgerald (1976) found critical cell concentrations of 11 mgN g^{-1} alga and 0.6 mgP g^{-1} alga. Thus Herbst (1969) quotes Kehr and Poston (pers. comm.) to conclude that water concentrations of 0.3 mg l^{-1} inorganic nitrogen and 0.03 mg l^{-1} phosphorus are generally accepted as the critical concentrations if all other conditions remain

favourable for growth. However, Pitcairn and Hawkes (1973) suggested a much higher critical phosphorus concentration of 1 mgP l^{-1}, whilst Turano (1963) found little alteration of photosynthetic rate over the range 0-0.3 mgP l^{-1}, but did find a linear relationship between the two factors over the range 0.7-5.0 mgP l^{-1} - photosynthetic rate at 5.0 mgP l^{-1} being approximately twice that at 0.7 mgP l^{-1}.

Most authors do, therefore, suggest phosphorus to be the controlling nutritional factor affecting *Cladophora* growth in the field situation and Whitton (1970b) concludes that there is apparently no instance in the literature where lack of combined nitrogen has been indicated as the major factor limiting the development of *Cladophora* populations. Pitcairn and Hawkes (1973), however, found an interaction between NO_3-N and PO_4-P concentration in that the highest level of NO_3-N tested in their laboratory culture work (7.7 mgl^{-1}) enhanced growth at the lowest PO_4-P concentration (0.5 mgl^{-1}), but reduced algal growth at higher PO_4-P concentrations. The results of Zuraw (1969) also suggested that the cation composition of the medium could influence the efficiency with which the alga could make use of nitrate; $NaNO_3$-N being required at 10x the concentrations of $Ca(NO_3)_2$-N or urea-N to stimulate the same growth.

The objective of this study was therefore to assess the effect of NO_3-N and PO_4-P on *Cladophora* growth in continuous culture.

7.3.3.2 Materials and methods

As section 7.1.2. Nominal NO_3-N concentrations of 7, 10 and 15 mgl^{-1} and PO_4-P concentrations of 0.0, 0.5, 1.0, 2.0 and 5.0 mg l^{-1} were chosen for study. The experiment was designed as a 3 x 5 factorial, with two replicate culture flasks per treatment level. Shortage of equipment necessitated the design be blocked. Analysis of Langley water revealed a Na:K ratio of approximately 3:1; to maintain this ratio treatment levels were produced by adding appropriate volumes of a concentrated stock solution comprising 50.7225 gl^{-1} $NaNO_3$ and 11.8268 gl^{-1} KNO_3 (= 10 gN l^{-1}; 13.7206 gNa l^{-1}; 4.5735gK l^{-1}) and a solution comprising 9.5816 g l^{-1} Na_2HPO_4 and 2.3041 g l^{-1} K_2HPO_4 (= 2.5 gP l^{-1} ; 3.1034 gNa l^{-1}; 1.0345 gK l^{-1}). No detectable

alteration of pH accompanied addition of Na/KNO_3 and Na_2/K_2HPO_4 upto the highest levels tested. Throughout the duration of the experiment water samples were abstracted for analysis of NO_3-N and PO_4-P concentration. Values were processed to produce table 7.23.

Table 7.23. NO_3-N and PO_4-P treatment levels for Experiment 12.

Nominal NO_3-N (mgl^{-1})	Mean (mgl^{-1})	95% confidence limits (mgl^{-1})	Range (mgl^{-1})	n
7	7.2	0.1	5.4 - 8.2	100
10	10.3	0.2	8.2 - 11.4	100
15	15.2	0.2	12.8 - 16.4	100

Nominal PO_4-P (mgl^{-1})	Mean (mgl^{-1})	95% confidence limits (mgl^{-1})	Range (mgl^{-1})	n
0.0	0.0	0.0	0.0-0.5	60
0.5	0.5	0.0	0.3-1.2	60
1.0	1.0	0.0	0.8-1.1	60
2.0	1.9	0.0	1.5-2.5	60
5.0	4.9	0.2	4.2-8.7	60

7.3.3.3 Results and discussion

Physico-chemical analysis of the Langley water used in the experiment is included in table 7.3. *Cladophora* growth data is shown as table 7.24, whilst fig 7.12 plots mean cumulative algal weight over the experimental period. From plots of untransformed and ln transformed mean cumulative wet weight against time, the period in which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.25) and analysed

Table 7.24 *Cladophora* growth data for Experiment 12 : growth response to variation in NO_3-N/PO_4-P concentrations.

Treatment		Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
NO_3-N mgl^{-1}	PO_4-P mgl^{-1}		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
7.2	0.0	0.145	0.000	0.002	0.044	0.145	0.233	0.352	0.527	0.639	0.774	0.930
		0.101	0.000	0.001	0.017	0.035	0.086	0.181	0.233	0.323	0.364	0.627
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.002	$\bar{x}$ = 0.031	$\bar{x}$ = 0.090	$\bar{x}$ = 0.160	$\bar{x}$ = 0.267	$\bar{x}$ = 0.380	$\bar{x}$ = 0.481	$\bar{x}$ = 0.569	$\bar{x}$ = 0.779
	0.5	0.108	0.000	0.012	0.059	0.123	0.264	0.345	0.486	0.587	0.708	0.896
		0.145	0.000	-0.004	0.006	0.041	0.111	0.211	0.338	0.478	0.643	0.710
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.004	$\bar{x}$ = 0.033	$\bar{x}$ = 0.082	$\bar{x}$ = 0.188	$\bar{x}$ = 0.278	$\bar{x}$ = 0.412	$\bar{x}$ = 0.533	$\bar{x}$ = 0.676	$\bar{x}$ = 0.803
	1.0	0.120	0.000	0.025	0.056	0.135	0.197	0.315	0.459	0.626	0.802	0.943
		0.142	0.000	0.006	0.004	0.046	0.091	0.121	0.226	0.348	0.470	0.650
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.016	$\bar{x}$ = 0.030	$\bar{x}$ = 0.091	$\bar{x}$ = 0.144	$\bar{x}$ = 0.218	$\bar{x}$ = 0.343	$\bar{x}$ = 0.487	$\bar{x}$ = 0.636	$\bar{x}$ = 0.797
	1.9	0.123	0.000	0.024	0.034	0.079	0.156	0.225	0.365	0.453	0.552	0.668
		0.072	0.000	0.028	0.018	0.062	0.117	0.163	0.235	0.431	0.525	0.692
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.026	$\bar{x}$ = 0.026	$\bar{x}$ = 0.071	$\bar{x}$ = 0.137	$\bar{x}$ = 0.194	$\bar{x}$ = 0.300	$\bar{x}$ = 0.442	$\bar{x}$ = 0.539	$\bar{x}$ = 0.680
	4.9	0.122	0.000	0.008	0.035	0.058	0.073	0.090	0.134	0.157	0.212	0.244
		0.122	0.000	-0.020	0.007	0.043	0.084	0.087	0.143	0.144	0.167	0.084
			$\bar{x}$ = 0.000	$\bar{x}$ = -0.006	$\bar{x}$ = 0.021	$\bar{x}$ = 0.051	$\bar{x}$ = 0.079	$\bar{x}$ = 0.089	$\bar{x}$ = 0.139	$\bar{x}$ = 0.151	$\bar{x}$ = 0.190	$\bar{x}$ = 0.164
10.3	0.0	0.118	0.000	0.011	0.098	0.253	0.461	0.637	0.799	0.873	1.046	1.205
		0.079	0.000	0.047	0.056	0.104	0.159	0.186	0.240	0.306	0.337	0.451
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.029	$\bar{x}$ = 0.077	$\bar{x}$ = 0.179	$\bar{x}$ = 0.310	$\bar{x}$ = 0.412	$\bar{x}$ = 0.520	$\bar{x}$ = 0.590	$\bar{x}$ = 0.692	$\bar{x}$ = 0.828
	0.5	0.137	0.000	0.004	0.018	0.064	0.118	0.163	0.229	0.313	0.478	0.634
		0.095	0.000	-0.007	0.018	0.089	0.121	0.192	0.292	0.408	0.556	0.631
			$\bar{x}$ = 0.000	$\bar{x}$ = -0.002	$\bar{x}$ = 0.018	$\bar{x}$ = 0.077	$\bar{x}$ = 0.120	$\bar{x}$ = 0.178	$\bar{x}$ = 0.261	$\bar{x}$ = 0.361	$\bar{x}$ = 0.517	$\bar{x}$ = 0.633
	1.0	0.138	0.000	0.043	0.051	0.119	0.160	0.389	0.615	0.990	1.194	1.394
		0.086	0.000	0.023	0.023	0.072	0.140	0.197	0.292	0.431	0.542	0.666
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.033	$\bar{x}$ = 0.037	$\bar{x}$ = 0.096	$\bar{x}$ = 0.150	$\bar{x}$ = 0.293	$\bar{x}$ = 0.454	$\bar{x}$ = 0.711	$\bar{x}$ = 0.868	$\bar{x}$ = 1.030
	1.9	0.099	0.000	0.047	0.049	0.102	0.208	0.322	0.452	0.659	0.781	0.980
		0.136	0.000	0.022	-0.001	0.036	0.136	0.317	0.547	0.699	0.997	1.281
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.035	$\bar{x}$ = 0.024	$\bar{x}$ = 0.069	$\bar{x}$ = 0.172	$\bar{x}$ = 0.320	$\bar{x}$ = 0.500	$\bar{x}$ = 0.679	$\bar{x}$ = 0.889	$\bar{x}$ = 1.131
	4.9	0.126	0.000	0.051	0.049	0.070	0.107	0.133	0.155	0.210	0.270	0.287
		0.100	0.000	0.029	0.038	0.051	0.102	0.145	0.192	0.253	0.351	0.397
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.040	$\bar{x}$ = 0.044	$\bar{x}$ = 0.061	$\bar{x}$ = 0.105	$\bar{x}$ = 0.139	$\bar{x}$ = 0.174	$\bar{x}$ = 0.232	$\bar{x}$ = 0.311	$\bar{x}$ = 0.342
15.2	0.0	0.076	0.000	0.066	0.142	0.233	0.416	0.569	0.796	0.927	1.015	1.108
		0.076	0.000	0.028	0.056	0.085	0.166	0.239	0.293	0.389	0.468	0.614
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.047	$\bar{x}$ = 0.099	$\bar{x}$ = 0.159	$\bar{x}$ = 0.291	$\bar{x}$ = 0.404	$\bar{x}$ = 0.545	$\bar{x}$ = 0.658	$\bar{x}$ = 0.742	$\bar{x}$ = 0.861
	0.5	0.111	0.000	-0.004	0.021	0.082	0.178	0.262	0.375	0.459	0.493	0.683
		0.105	0.000	0.019	0.002	0.032	0.111	0.122	0.164	0.291	0.418	0.514
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.008	$\bar{x}$ = 0.012	$\bar{x}$ = 0.057	$\bar{x}$ = 0.145	$\bar{x}$ = 0.192	$\bar{x}$ = 0.270	$\bar{x}$ = 0.375	$\bar{x}$ = 0.456	$\bar{x}$ = 0.599
	1.0	0.099	0.000	0.040	0.057	0.072	0.166	0.233	0.319	0.474	0.617	0.792
		0.098	0.000	-0.012	0.011	0.101	0.143	0.246	0.350	0.608	0.893	1.084
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.014	$\bar{x}$ = 0.034	$\bar{x}$ = 0.087	$\bar{x}$ = 0.155	$\bar{x}$ = 0.240	$\bar{x}$ = 0.335	$\bar{x}$ = 0.541	$\bar{x}$ = 0.755	$\bar{x}$ = 0.938
	1.9	0.150	0.000	0.007	0.013	0.058	0.170	0.222	0.422	0.571	0.730	0.927
		0.078	0.000	0.037	0.010	0.079	0.182	0.219	0.326	0.443	0.576	0.750
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.022	$\bar{x}$ = 0.012	$\bar{x}$ = 0.069	$\bar{x}$ = 0.176	$\bar{x}$ = 0.221	$\bar{x}$ = 0.374	$\bar{x}$ = 0.507	$\bar{x}$ = 0.653	$\bar{x}$ = 0.839
	4.9	0.106	0.000	0.025	0.059	0.065	0.107	0.171	0.216	0.275	0.366	0.372
		0.085	0.000	0.029	0.032	0.021	0.056	0.103	0.156	0.190	0.215	0.234
			$\bar{x}$ = 0.000	$\bar{x}$ = 0.027	$\bar{x}$ = 0.046	$\bar{x}$ = 0.043	$\bar{x}$ = 0.082	$\bar{x}$ = 0.137	$\bar{x}$ = 0.186	$\bar{x}$ = 0.233	$\bar{x}$ = 0.291	$\bar{x}$ = 0.303

Fig. 7.12 Growth of *Cladophora* over 18 day experimental period in response to variation in NO_3-N/PO_4-P concentration.

LEGEND

● = 0.0 mgl^{-1} PO_4-P
○ = 0.5 mgl^{-1} PO_4-P
■ = 1.0 mgl^{-1} PO_4-P
□ = 1.9 mgl^{-1} PO_4-P
★ = 4.9 mgl^{-1} PO_4-P

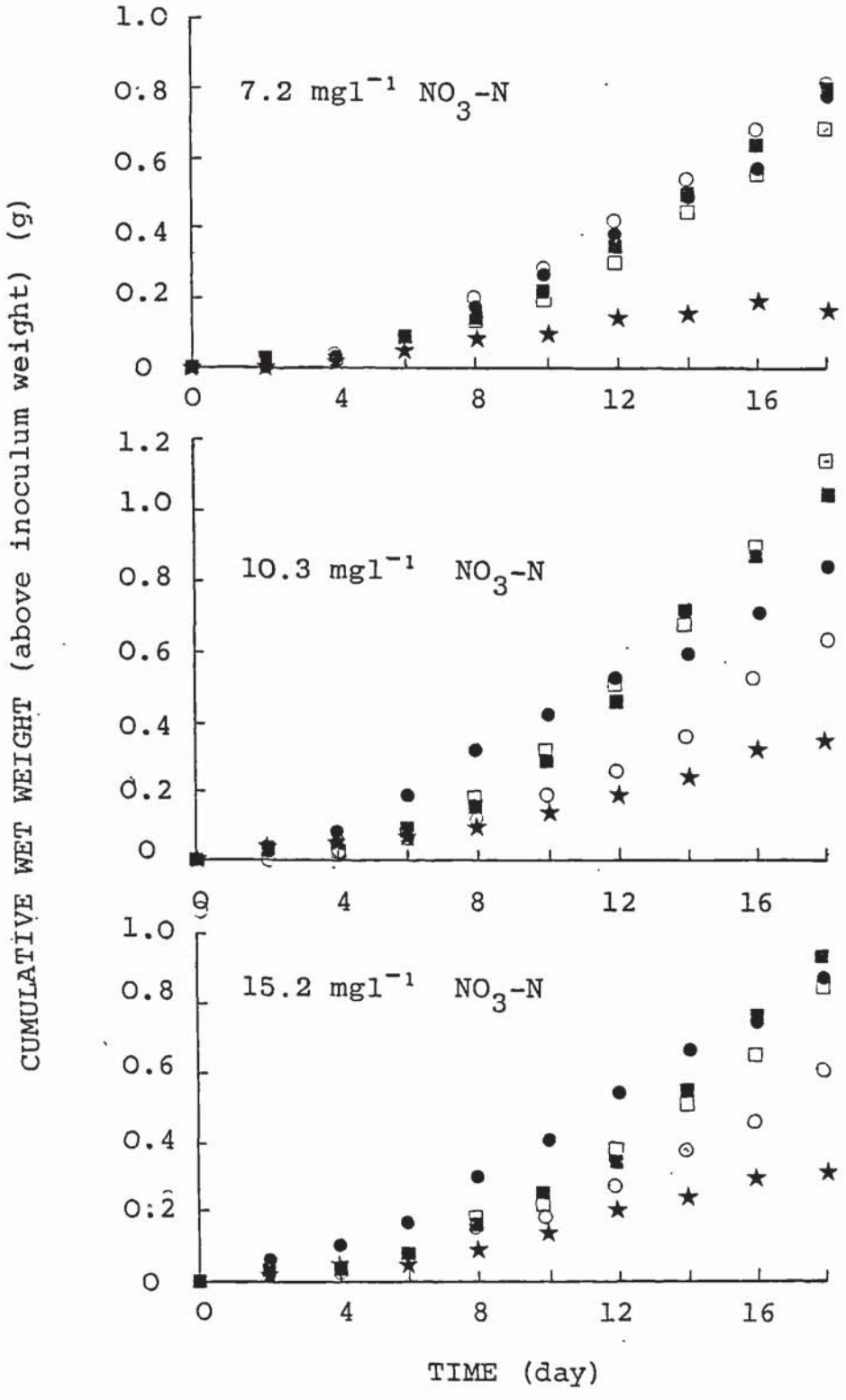


Table 7.25 Calculation of values of μ from growth data of Experiment 12.

NO_3-N	PO_4-P	Period over which incremental growth assessed	Regression of ln transformed data		
			Slope (= μ)	Correlation coefficient	n
7.2	0.0	2 - 18	0.127	0.989	9
		2 - 18	0.124	0.992	9
7.2	0.5	4 - 12	0.161	0.990	5
		4 - 12	0.149	0.997	5
7.2	1.0	4 - 12	0.146	0.998	5
		4 - 12	0.109	0.991	5
7.2	1.9	4 - 12	0.141	0.998	5
		4 - 12	0.151	0.993	5
7.2	4.9	0 - 18	0.062	0.997	10
		0 - 18	0.052	0.869	10
10.3	0.0	0 - 8	0.212	0.982	5
		0 - 8	0.129	0.981	5
10.3	0.5	2 - 18	0.108	0.997	9
		2 - 18	0.136	0.994	9
10.3	1.0	4 - 16	0.174	0.992	7
		4 - 16	0.145	0.997	7
10.3	1.9	4 - 12	0.168	0.997	5
		4 - 12	0.211	0.994	5
10.3	4.9	0 - 18	0.063	0.989	10
		0 - 18	0.091	0.995	10
15.2	0.0	0 - 10	0.211	0.993	6
		0 - 10	0.141	0.996	6
15.2	0.5	4 - 12	0.163	0.995	5
		4 - 12	0.117	0.964	5
15.2	1.0	4 - 18	0.130	0.996	8
		4 - 18	0.168	0.994	8
15.2	1.9	0 - 18	0.123	0.986	10
		0 - 18	0.137	0.984	10
15.2	4.9	0 - 18	0.087	0.994	10
		0 - 18	0.078	0.972	10

using program ANOV 2 (included in appendix 2) to produce table 7.26 (though the 'blocks' sum of squares was calculated manually). Fig 7.13 summarizes the *Cladophora* growth response to variation in NO_3-N and PO_4-P concentration. Line of best fit and confidence intervals were calculated manually for this figure.

Results (table 7.26) show a significant effect of PO_4-P concentration on *Cladophora* specific growth rate ($P<0.05$). Inspection of fig 7.13, however, reveals little effect of PO_4-P over the range 0.0-1.9 mgP l^{-1}, although a slight drop in specific growth rate at concentrations below 0.5 mgP l^{-1} is suggested. The 'nutritional' effect of PO_4-P is thus demonstrated to be small over the range of concentrations considered. Since, however, the precision of PO_4-P determination was only to the nearest 0.05 mg l^{-1} the lowest concentration used (0.0 mg l^{-1}) could actually have been as high as 0.097 mg l^{-1} and since the critical PO_4-P concentration has been estimated as 30-60µg l^{-1} such results do not necessarily seem unreasonable. Inspection of fig 7.13 reveals the toxic effect of PO_4-P at high concentrations : specific growth rate at 4.9 mg l^{-1} being only 48% of that at 1.9 mg l^{-1}. It is considered that it is largely this (toxic) effect of PO_4-P on specific growth rate of *Cladophora* which was detected in the analysis of variance (table 7.26).

Langley water used in experimentation was unfortunately high in NO_3-N and variation in concentration from 7.2-15.2 mgN l^{-1} had no significant effect on growth. Nor was there any significant nitrate/phosphate interaction as postulated by Pitcairn and Hawkes (1973), over the ranges studied.

Painter (1971) found NO_3-N and PO_4-P concentrations within the range 21-28 mg l^{-1} and 6.2-9.6 mg l^{-1} respectively in sewage effluents from three towns in southeast England. Applying the x8 dilution factor laid down by the Royal Commission on Sewage Disposal (1912), this would produce concentrations in the receiving waterways of 3-4 mgNO_3-N l^{-1} and 0.8-1.2 mgPO_4-P l^{-1}. Experimental results would suggest such concentrations of phosphate to be near optimal for growth of *Cladophora*. Watton (1982) found the highest mean PO_4-P concentration at any site under study to be 1.7 mg l^{-1}, whilst Girton (1980) found the highest mean concentration to be 3.3 mg l^{-1} (orthophosphate) in his study.

Table 7.26 Analysis of variance summary table for Experiment 12 - response of μ to variation in NO_3-N and PO_4-P concentrations.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
A*	2	2.35450E-03	1.17725E-03	1.6209	
A linear	1	6.02822E-04	6.02822E-04	.829998	(P>0.20)
A quadratic	1	1.75175E-03	1.75175E-03	2.4119	(P<0.20)
B**	4	2.98144E-02	7.45359E-03	10.2625	
B linear	1	2.43086E-02	2.43086E-02	33.4694	(P<0.001)
B quadratic	1	3.43597E-03	3.43597E-03	4.73083	(P<0.05)
B cubic	1	1.88259E-03	1.88259E-03	2.59205	(P<0.20)
INTERACTION	8	7.38478E-03	9.23097E-04	1.27097	
AL x BL	1	1.39859E-06	1.39859E-06	1.92565E-03	(P>0.20)
AL x BQ	1	2.00734E-03	2.00734E-03	2.76381	(P<0.20)
AL x BC	1	1.60324E-05	1.60324E-05	2.20743E-02	(P>0.20)
AQ x BL	1	2.94564E-05	2.94564E-05	4.05571E-02	(P>0.20)
AQ x BQ	1	8.97033E-04	8.97033E-04	1.23508	(P>0.20)
AQ x BC	1	1.81967E-03	1.81967E-03	2.50542	(P<0.20)
BLOCKS	1	6.34789E-04	6.34789E-04	.874012	(P>0.20)
ERROR	14	1.01681E-02	7.26294E-04		
Total	29	5.03565E-02			

* = NO_3-N concentration

** = PO_4-P concentration

Fig. 7.13 Response of *Cladophora* specific growth rate to variation in NO_3-N/PO_4-P concentration.

LEGEND

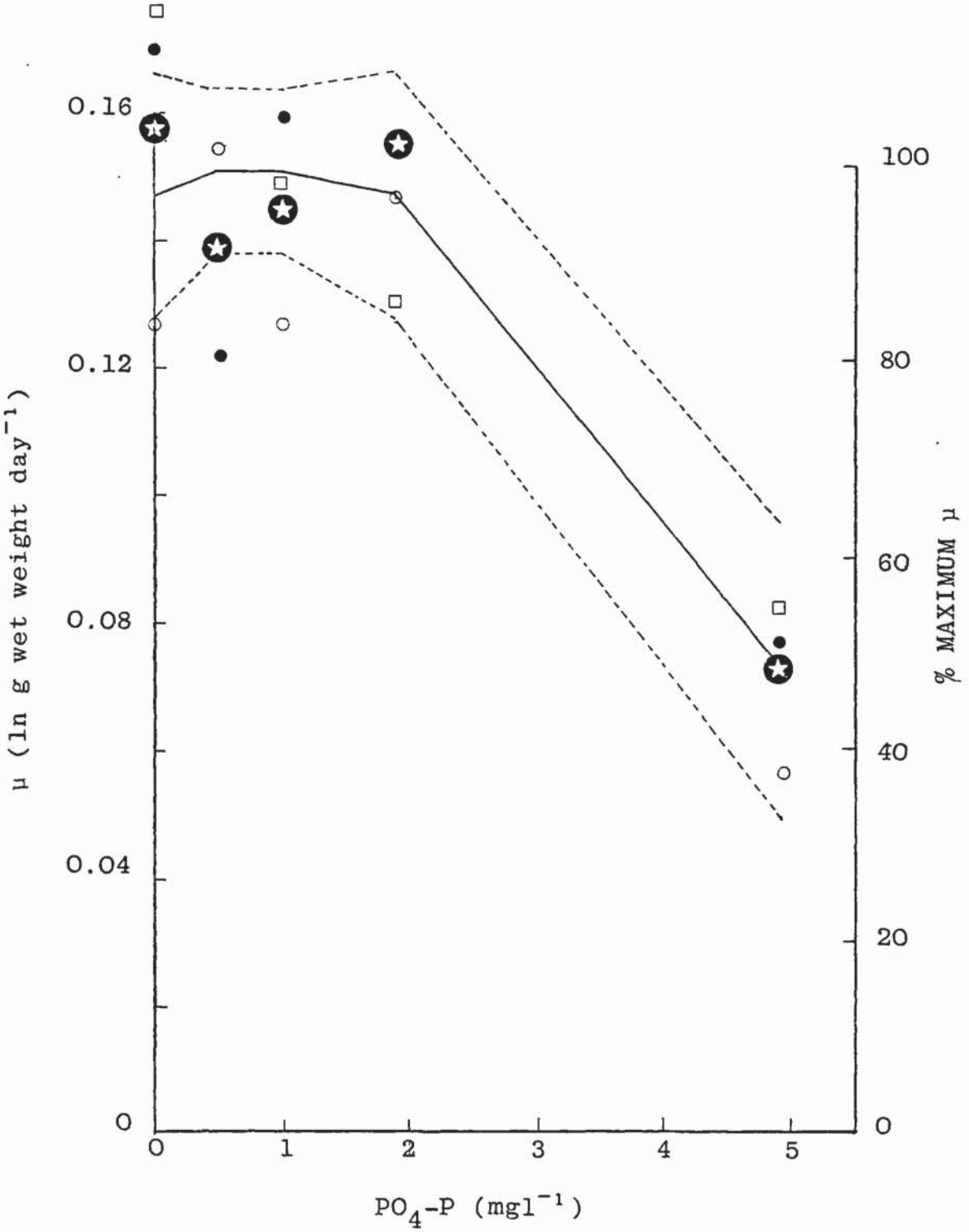
○ = Mean, 7.2 mg l^{-1} NO_3-N (n-2) ● = Mean, 10.3 mg l^{-1} NO_3-N (n=2)

□ = Mean, 15.2 mg l^{-1} NO_3-N (n-2) ✪ = Mean, all levels of NO_3-N (n=6)

——— = Line of best fit ------- = Confidence intervals

Significant effects : LINEAR (P< 0.001)

QUADRATIC (P< 0.05)



It seems unlikely, therefore, that phosphate toxicity is a major factor controlling the growth of *Cladophora* in the field situation.

7.4 INVESTIGATION OF THE EFFECT OF VARIATION IN CONCENTRATION OF HEAVY METALS ON *CLADOPHORA* GROWTH.

7.4.1 Experiment 13 - Evaluation of 'solubility' of heavy metals

7.4.1.1 Introduction and objectives

Stiff (1971) comments that in the aquatic environment copper is found in three physical states, broadly classified as particulate, colloidal and soluble. Soluble matter is defined as that which passes through a 0.45 µm membrane filter and includes copper both as the free ion and as soluble complexes. It is also possible that precipitated species or clays may exist in part, in a finely divided state able to pass through a 0.45 µm filter. Experimental evidence suggests that only soluble forms of copper are available (i.e.toxic) to fish, at least (Stiff 1971), and it seems likely that the toxic form(s) of other metal species also belong to this soluble fraction. It is common practice, therefore, for Water Authorities etc. to analyse filterable metal concentrations in water samples as well as (or instead of) total metal concentrations.

In this series of experiments, Langley water had already been passed through Whatman GF/C filters prior to sterilization (see section 7.1.2). Such filtration is known to retain the majority of particles exceeding 0.5 µm dimater (Melbourne, 1964). It was therefore considered likely that a major proportion of the metals could be filterable through a 0.45 µm membrane filter. Thus the objective of this experiment was to assess the proportion of metals in the 'soluble' state, so as to aid interpretation of further experiments intended to assess the toxicity to *Cladophora* of copper (experiment 14), zinc (experiment 15) and lead (experiment 16).

7.4.1.2 Materials and methods

Langley water was filtered through a Whatman GF/C filter collected in 3 litre vessels, and free-steamed for 40 minutes in an autoclave. Medium was allowed to cool and aliquots of concentrated $CuSO_4$, $ZnSO_4$ and $PbCl_2$ stock solutions added to make up solutions of 0.01 and

Table 7.27 Concentration of metals (in µg) from variously treated Langley water samples.

Cu				Zn				Pb			
Filtered		Not Filtered		Filtered		Not Filtered		Filtered		Not Filtered	
0.01	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.01	0.10	0.10	0.10
28	72	22	84	25.1	85.3	15.5	90.5	23	101	33	108
22	81	25	87	21.7	91.8	30.8	99.2	26	102	26	108
23	79	29	81	37.8	88.8	41.3	93.2	27	103	27	104

Table 7.28 Analysis of variance summary table for Experiment 13 data.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
A*	2	1007.2	503.602	17.2787	(P<0.001)
B**	1	124.687	124.687	4.27806	(P<0.05)
C***	1	38389.9	38389.9	1317.17	(P<0.001)
A x B	2	.71875	.359375	1.23302E-02	(P>0.20)
A x C	2	722.5	361.25	12.3946	(P<0.001)
B x C	1	34.0312	34.0312	1.16762	(P>0.20)
A x B x C	2	7.70312	3.85156	.132148	(P>0.20)
Error	24	699.5	29.1458		
Total	35	40986.2			

* = Cu/Zu/Pb ** = Filtered/Not Filtered *** = 0.01/0.10 mgl^{-1}

and 0.1 mg l^{-1} Cu, Zn and Pb. Media were vigorously agitated and then left to equilibrate for 24 hours at 15°C. Triplicate samples of medium for each combination of treatments were then membrane filtered through 0.45 µm filters and triplicate samples remained unfiltered. 100 ml samples were then digested and analysed by atomic absorption spectrophotometer as outlined in table 6.1.

7.4.1.3 Results and discussion

Results included as table 7.27 were entered into a 3-way analysis of variance to produce an analysis of variance summary table (table 7.28).

Analysis reveals a significant difference in metal concentration between samples which were membrane filtered and those which were not ($P<0.05$). No significant interaction with either, or both, of the other two treatments (i.e. metal species and metal concentration) was found ($P>0.05$). Averaged over all levels of other treatments metal concentrations in samples which had been membrane filtered were 94% of that of samples which had not undergone filtration. Thus filtration removed, on average, only 6% of the metal from 'solution' : within the context of expected experimental variability this was considered negligible. In experiments 14-16, therefore, only total metal concentrations were evaluated.

7.4.2 Experiment 14-16 - Effect of variation in concentration of Cu, Zn and Pb on growth.

7.4.2.1. Introduction and objectives

Since many of the principles of heavy metal toxicity and uptake are common to all, or most, metal species it was considered best to present studies involving metal toxicity to, and uptake by, *Cladophora* as a single study rather than as separate experiments. The heavy metals, copper, zinc and lead were chosen for study.

The literature dealing with metal toxicity to algae is both extensive and complex : of the metal species under study, copper is

perhaps most studied. Whitton (1970c) comments that most reports in the literature indicate *Cladophora* to be exceptionally sensitive to heavy metals, though a few indicate quite the opposite. It seems likely that such contradictions are a result of the toxicity of metals only being attributable to certain species. With respect to copper, Andrew *et al.* (1977) found toxicity to *Daphnia magna* to be clearly related to the concentrations of the ionic species Cu^{2+}, $CuOH^{+}$ and $Cu_2(OH)_2^{2+}$. Pagenkopf *et al.* (1974), considered Cu^{2+} to be the major toxic species to fish, whilst Waiwood and Beamish (1978) found only Cu^{2+} and $CuOH^{+}$ to be significantly related to the swimming performance of *Salmo gairdneri*. Evidence thus suggests that Cu^{2+} is the major toxic species of copper. In waters relatively free of other complexing agents, pH and alkalinity are the major modifying factors governing copper speciation (Spear and Pierce, 1979); high alkalinity and high pH reducing overall toxicity. Water hardness *per se* does not appear to be a significant modifying factor. However, Mierle and Stokes (1976) showed Ca^{2+} to reduce copper accumulation and increase copper tolerance in *Scenedesmus*. In most aquatic systems, however, inorganic and organic complexation, and absorption and precipitation processes are capable of reducing free copper levels even in the presence of high levels of total copper (Sylva 1976), Gachter *et al.* (1973) list further factors capable of reducing copper toxicity including nitrilotriacetic acid (NTA) (Ericksen *et al.* 1970a, 1970b), ethylendiamine tetraacetic acid (EDTA) (Soeder *et al.* 1967, Steeman Nielsen and Kamp-Nielsen 1970), extracellular polypeptides (Fogg amd Westlake, 1955), a protein digest (Steeman Nielsen and Wium Andersen , 1971) and a zooplankton extract (Barber and Ryther, 1969).

Less is known of the toxically active species of zinc or lead. Stiff (1971), however, comments that it seems likely that the toxic forms of metals other than copper are also soluble (i.e. able to pass through a 0.45 µm membrane filter).

Algal species are known to differ greatly in their tolerance to metal toxicity. For copper, for example, inhibitory concentrations have been reported as low as 0.01 mg l^{-1} in *Skeletonema costatum* (Jensen *et al.* 1974) and as high as 2.3 mg l^{-1} in *Chlorella vulgaris*

(Hassall 1963). As regards *Cladophora*, Whitton (1970a) considered inhibitory concentrations to be 0.19 mgCu l^{-1} , 0.14 mgZn l^{-1} and 3 mgPb l^{-1}. Williams and Mount (1965) found *Cladophora* absent from canals receiving 1, 3 or 9 mgZn l^{-1} but abundant in a control canal with no zinc addition. Morgan and Whitton (in Whitton 1970c), however, after surveying various metal polluted streams found *Cladophora* in a stream containing 0.17 mgZn l^{-1}: stream water at this site was noted to be hard (90 mgCa l^{-1} and 10 mgMg l^{-1}). Zinc levels in the sediment exceeded 700 µg g^{-1} leading Whitton to comment that it seems probable that the metal content of sediments has little if any direct effect. *Cladophora* species were noticably absent from the lead polluted streams studied by Carpenter (1924) and Reese (1937) indicating a possible lack of tolerance to lead pollution. Indeed Whitton (1970a) after studying metal toxicity to 37 isolates of freshwater algae found *Cladophora* to be the taxa most intolerant to the toxic effects of lead.

The uptake of heavy metals to produce an internal concentration greater than that in the external environment appears to be widespread in aquatic organisms (Whitton and Say, 1975). General aspects of metal accumulation by aquatic organisms are discussed by Bryan (1976) and Whitton and Say (1975). The apparent uptake of any element by an organism can be generally explained by one or a combination of three main processes : adsorption, ionic exchange and accumulation. Adsorption involves the concentration of solute ions on a solid/solvent interface, and may proceed until all but a trace of the solute ions are left, dependant upon the previous saturation of the solid surface with the element. Ionic exchange necessitates the organism to maintain an exchangeable internal concentration of the element in addition to that which may be concentrated on the solid surfaces. Accumulation resembles adsorption in that it involves active removal of the element from solution, but differs in that the ions may be inaccessibly bound as they are utilized by the cells (Gutknecht, 1961). In discussive sections, therefore, the term ' uptake' is used to denote any uptake reaction between a solid and a dissolved substance.

It has been suggested that *C. glomerata* is able to act as a biological monitor of water quality, concentrating trace metals present in the aqueous environment with a reasonably constant concentration

Table 7.29 Concentration factors quoted for *Cladophora* in the literature.

SOURCE	ELEMENT	CONCENTRATION FACTOR x 10^{-3}	
Keeney *et al.* (1976)	Cu	1.9 and 2.2	$\frac{\mu g\ g^{-1}\ dry\ wt.}{\mu g\ ml^{-1}\ medium}$
	Zn	1.0 and 2.9	
	Pb	16 and 20	
	Cd	18 and 49	
Thorpe (1981)	Cd	1.5 - 5.2	$\frac{\mu g\ g^{-1}\ dry\ wt.}{\mu g\ ml^{-1}\ medium}$
	Cr	3.1 - 11.4	
	Cu	1.2 - 6.2	
	Pb	1.3 - 7.6	
	Ni	0.6 - 2.5	
	Zn	3.1 - 8.9	
Burkett (1975)	$CH_3{}^{203}Hg$	0.065 - 5.597	$\frac{\mu g\ g^{-1}\ dry\ wt.}{\mu g\ ml^{-1}\ medium*}$
Williams (1970)	^{85}Cs	0.388 - 2.216[a]	$\frac{\mu g\ g^{-1}\ dry\ wt.}{\mu g\ ml^{-1}\ medium*}$
	^{137}Sr	0.408 - 1.708[a]	
	^{85}Cs	0.275 - 0.401[b]	
	^{137}Sr	0.142 - 0.191[b]	
Kulikova (1960)	^{91}Y	7.807	
	^{144}Ce	1.571	
	^{90}Sr	0.855	
Sikes (1977)	^{45}Ca	0.416 - 0.998	$\frac{cpm\ g^{-1}\ alga}{cpm\ ml^{-1}\ medium}$
	^{85}Sr	0.374 - 1.585	
Gileva (1964)	^{32}P	0.020 - 5.390	$\frac{moles\ kg^{-1}\ dry\ wt.}{concn.\ in\ solution\ (M)}$
	^{106}Ru	0.610 - 0.630	
	^{45}Ca	0.035 - 2.020	
	^{65}Zn	0.008 - 0.310	
	^{86}Rb	0.110 - 2.100	
	^{90}Sr	0.250 - 0.540	
	^{137}Cs	0.105 - 0.315	
Whitton (1979)	Zn	1.3	$\frac{\mu g\ g^{-1}\ dry\ wt.}{\mu g\ ml^{-1}\ medium}$

a = alga alive b = alga dead

* = measured at begining of experiment

factor (CF) for each element (Keeney *et al.*, 1976); concentration factors (μg g^{-1} alga over μg ml^{-1} medium) ranging from 1.0×10^3 to 49×10^3 for the samples and elements (Cu, Zn, Pb, Cd) studied. Results of Thorpe (1981) support this hypothesis. Gileva (1964) however, concluded that for a given plant the concentration factor for a variety of microelements is constant within a broad range of microconcentrations (if all other conditions are constant). But when the concentration of an element in solution is increased above 10^{-4} -10^{-3} M the concentration factor begins to decrease, although the absolute accumulation still increases (but no longer in direct proportion to the increase in solution concentration), and when the concentration of element in the medium is sufficiently high 'saturation' may occur. Sikes (1977), similarly, found total calcium uptake to be directly proportional to the concentration of the ion in the medium at 2-20 mgCa l^{-1} but to be saturated at high concentrations of 20-100 mgCa l^{-1}. A list of concentration factors quoted for *Cladophora* by various authors is included as table 7.29.

The objectives of this series of three experiments were thus to investigate the toxicity of copper, zinc and lead to, and uptake by, *Cladophora* in closed continuous culture.

7.4.2.2 Materials and methods

As section 7.1.2. Two replicate flasks were used per treatment level in each experiment. Treatment levels were produced by adding appropriate volumes of stock solutions containing 0.3929 g l^{-1} $CuSO_4.5H_2O$ (100 mgCu l^{-1}), 0.4399 g l^{-1} $ZnSO_4$ (100 mgZn l^{-1}), or 0.1342 g l^{-1} $PbCl_2$ (100 mgPb l^{-1}). Since large volumes of lead stock solution were needed to produce the required treatment levels the stock solution was made using autoclaved Langley water as solvent. All solutions were made up 24 hours prior to use so that each introduced metal was able to establish an equilibrium of its various species prior to assessment of toxicity. No detectable alteration of pH accompanied the addition of $CuSO_4.5H_2O$, $ZnSO_4$ or $PbCl_2$ upto the highest level used. Throughout the duration of the experiments water samples were abstracted for analysis of Cu, Zn or Pb concentration. Values were processed to produce tables 7.30-7.32.

Table 7.30 Cu treatment levels for Experiment 14.

Nominal Cu ($\mu g\ l^{-1}$)	Mean ($\mu g\ l^{-1}$)	95% confidence limits ($\mu g\ l^{-1}$)	Median ($\mu g\ l^{-1}$)	Range ($\mu g l^{-1}$)	n
0	21	3	20	9-38	20
20	65	33	43	20-340	20
50	50	5	49	35-73	20
100	79	5	79	57-98	20
200	155	15	15	100-221	20

Table 7.31 Zn treatment levels for Experiment 15.

Nominal Zn ($\mu g\ l^{-1}$)	Mean ($\mu g\ l^{-1}$)	95% confidence intervals ($\mu g\ l^{-1}$)	Median ($\mu g\ l^{-1}$)	Range ($\mu g\ l^{-1}$)	n
0.0	52.2	16.5	40.5	25.3-165	20
20.0	89.8	29.8	65.7	36.6-275	20
50.0	90.6	18.4	76.5	55.6-205.4	20
100.0	96.1	5.5	97.4	61.9-112.6	20
200.0	181.7	5.7	180.9	155.1-204.7	20

Table 7.32 Pb treatment levels for Experiment 16.

Nominal Pb ($\mu g\ l^{-1}$)	Mean ($\mu g\ l^{-1}$)	95% confidence intervals ($\mu g\ l^{-1}$)	Median ($\mu g\ l^{-1}$)	Range ($\mu g\ l^{-1}$)	n
0	20	3	20	12-36	20
200	197	19	185	157-322	20
500	416	24	403	366-606	20
1000	764	30	750	684-892	20
2000	1411	66	1417	1144-1700	20

At the end of each experiment the crop of *Cladophora* was removed from each basket and dried to constant weight at 105°C. They were then transferred to pre-cleaned digestion flasks and digested in atomic absorption grade HNO_3 (about 70%, SG 1.42) and perchloric (about 60%, SG 1.54) acids. After cooling samples were made up to 10 ml with distilled water and aspirated directly into an atomic absorption spectrophotometer.

7.4.2.3 Results and discussion

Physico-chemical analyses of the Langley water used in the experiments are included as table 7.3. Visual inspection of raw data of metal concentrations revealed occasional very high values (especially for zinc) which were considered likely to have arisen from contamination during preparation of the samples for analysis. The total rejection of such spurious values was, however, not considered valid, but to largely avoid the distortions of the distribution which the inclusion of such values made, it was considered better to use the median than the (arithmetic) mean as an estimate of the central tendency of the distributions, and thereby as an estimate of the treatment levels to be used in further calculations. *Cladophora* growth data are shown as tables 7.33-7.35, whilst figs. 7.14-7.16 plot mean cumulative algal wet weight over the experimental period for the three experiments. From plots of untransformed and ln transformed mean cumulative algal wet weight against time the period in which growth was exponential was selected for each treatment level. Values of μ were then calculated for each culture (table 7.36, 7.38 and 7.40) and analysed by analysis of variance to produce table 7.37, 7.39 and 7.41. Figs. 7.17-7.19 summarize the growth response of *Cladophora* to variation in Cu, Zn and Pb respectively.

Metal accumulation data is included as table 7.42-7.44. The response of both metal content (μg metal g^{-1} alga dry weight) and concentration factor (μg metal g^{-1} algal dry weight/μg metal ml^{-1} medium) to variation in concentration of metals in the medium was entered into analysis of variance (tables 7.45-7.50). Figs. 7.20-7.25 summarizing the response of both of these terms to variation in

Table 7.33 *Cladophora* growth data for Experiment 14 : growth response to variation in Cu concentration.

Treatment Cu mgl^{-1}	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.020	0.143	0.000	0.012	0.057	0.144	0.412	0.681	0.919	0.829	1.168	1.154
	0.105	0.000	0.039	0.081	0.196	0.423	0.652	0.701	0.714	0.844	0.958
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.026	$\bar{x}$ = 0.069	$\bar{x}$ = 0.170	$\bar{x}$ = 0.418	$\bar{x}$ = 0.667	$\bar{x}$ = 0.810	$\bar{x}$ = 0.772	$\bar{x}$ = 1.006	$\bar{x}$ = 1.056
0.043	0.111	0.000	0.055	0.060	0.128	0.164	0.147	0.284	0.342	0.488	0.633
	0.121	0.000	0.054	0.040	0.088	0.142	0.207	0.335	0.440	0.541	0.674
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.055	$\bar{x}$ = 0.050	$\bar{x}$ = 0.108	$\bar{x}$ = 0.153	$\bar{x}$ = 0.177	$\bar{x}$ = 0.310	$\bar{x}$ = 0.391	$\bar{x}$ = 0.515	$\bar{x}$ = 0.654
0.049	0.108	0.000	0.053	0.038	0.040	0.034	0.052	0.052	0.054	0.044	0.046
	0.101	0.000	0.045	0.036	0.073	0.053	0.069	0.048	0.059	0.042	0.039
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.049	$\bar{x}$ = 0.037	$\bar{x}$ = 0.057	$\bar{x}$ = 0.044	$\bar{x}$ = 0.061	$\bar{x}$ = 0.050	$\bar{x}$ = 0.057	$\bar{x}$ = 0.043	$\bar{x}$ = 0.043
0.079	0.137	0.000	0.041	0.043	0.013	0.036	0.046	0.039	0.052	0.045	0.013
	0.112	0.000	0.040	0.048	-0.005	-0.020	-0.012	-0.024	-0.024	-0.017	-0.030
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.041	$\bar{x}$ = 0.046	$\bar{x}$ = 0.004	$\bar{x}$ = 0.008	$\bar{x}$ = 0.017	$\bar{x}$ = 0.008	$\bar{x}$ = 0.014	$\bar{x}$ = 0.014	$\bar{x}$ =-0.009
0.154	0.096	0.000	0.027	0.044	0.027	0.021	0.036	0.041	0.021	0.018	0.041
	0.135	0.000	0.019	-0.007	0.000	-0.002	-0.005	-0.009	0.002	-0.004	-0.016
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.023	$\bar{x}$ = 0.019	$\bar{x}$ = 0.014	$\bar{x}$ = 0.010	$\bar{x}$ = 0.016	$\bar{x}$ = 0.016	$\bar{x}$ = 0.012	$\bar{x}$ = 0.007	$\bar{x}$ = 0.013

Table 7.34 *Cladophora* growth data for Experiment 15 : growth response to variation in Zn concentration.

Treatment Zn mgl^{-1}	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.0405	0.115	0.000	0.016	0.034	0.059	0.069	0.193	0.336	0.543	0.657	0.679
	0.085	0.000	0.025	0.023	0.044	0.068	0.175	0.320	0.381	0.436	0.564
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.021	$\bar{x}$ = 0.029	$\bar{x}$ = 0.052	$\bar{x}$ = 0.069	$\bar{x}$ = 0.184	$\bar{x}$ = 0.328	$\bar{x}$ = 0.462	$\bar{x}$ = 0.547	$\bar{x}$ = 0.622
0.0657	0.109	0.000	0.035	0.031	0.057	0.117	0.184	0.320	0.373	0.446	0.483
	0.113	0.000	0.009	0.014	0.066	0.057	0.073	0.157	0.261	0.324	0.361
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.022	$\bar{x}$ = 0.023	$\bar{x}$ = 0.062	$\bar{x}$ = 0.087	$\bar{x}$ = 0.129	$\bar{x}$ = 0.239	$\bar{x}$ = 0.317	$\bar{x}$ = 0.385	$\bar{x}$ = 0.422
0.0765	0.095	0.000	0.021	0.022	0.031	0.097	0.069	0.097	0.115	0.166	0.149
	0.093	0.000	0.039	0.067	0.067	0.071	0.119	0.156	0.202	0.211	0.189
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.030	$\bar{x}$ = 0.045	$\bar{x}$ = 0.049	$\bar{x}$ = 0.084	$\bar{x}$ = 0.094	$\bar{x}$ = 0.127	$\bar{x}$ = 0.159	$\bar{x}$ = 0.189	$\bar{x}$ = 0.169
0.0974	0.109	0.000	0.047	0.040	0.025	0.061	0.045	0.064	0.064	0.085	0.118
	0.097	0.000	0.015	0.024	0.034	0.056	0.038	0.095	0.098	0.115	0.136
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.031	$\bar{x}$ = 0.032	$\bar{x}$ = 0.030	$\bar{x}$ = 0.059	$\bar{x}$ = 0.042	$\bar{x}$ = 0.080	$\bar{x}$ = 0.081	$\bar{x}$ = 0.100	$\bar{x}$ = 0.127
0.1810	0.095	0.000	0.022	0.028	0.031	0.038	-0.005	0.047	0.030	0.029	0.060
	0.104	0.000	0.033	0.040	0.011	0.029	0.035	0.059	0.039	0.044	0.039
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.028	$\bar{x}$ = 0.034	$\bar{x}$ = 0.021	$\bar{x}$ = 0.034	$\bar{x}$ = 0.015	$\bar{x}$ = 0.053	$\bar{x}$ = 0.035	$\bar{x}$ = 0.037	$\bar{x}$ = 0.050

Table 7.35 *Cladophora* growth data for Experiment 16 : growth response to variation in Pb concentration.

Treatment Pb (mgl^{-1})	Inoculum (g)	Cumulative wet weight (above inoculum weight) (g)									
		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14	DAY 16	DAY 18
0.020	0.110	0.000	0.037	0.025	0.055	0.150	0.257	0.431	0.597	0.696	0.732
	0.112	0.000	0.026	0.061	0.067	0.110	0.182	0.308	0.466	0.593	0.698
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.032	$\bar{x}$ = 0.043	$\bar{x}$ = 0.061	$\bar{x}$ = 0.130	$\bar{x}$ = 0.220	$\bar{x}$ = 0.370	$\bar{x}$ = 0.532	$\bar{x}$ = 0.645	$\bar{x}$ = 0.715
0.185	0.126	0.000	0.012	0.069	0.056	0.112	0.257	0.496	0.784	0.895	0.938
	0.096	0.000	-0.011	0.013	0.012	0.026	0.055	0.103	0.162	0.263	0.313
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.001	$\bar{x}$ = 0.041	$\bar{x}$ = 0.034	$\bar{x}$ = 0.069	$\bar{x}$ = 0.156	$\bar{x}$ = 0.300	$\bar{x}$ = 0.473	$\bar{x}$ = 0.579	$\bar{x}$ = 0.626
0.403	0.129	0.000	-0.012	0.003	0.012	0.038	0.100	0.177	0.332	0.443	0.520
	0.116	0.000	0.006	0.018	0.025	0.050	0.082	0.191	0.276	0.344	0.365
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.003	$\bar{x}$ = 0.011	$\bar{x}$ = 0.019	$\bar{x}$ = 0.044	$\bar{x}$ = 0.091	$\bar{x}$ = 0.134	$\bar{x}$ = 0.304	$\bar{x}$ = 0.394	$\bar{x}$ = 0.443
0.750	0.146	0.000	-0.006	0.003	0.000	0.015	0.041	0.072	0.110	0.102	0.172
	0.113	0.000	0.005	0.018	0.018	0.024	0.059	0.087	0.153	0.245	0.288
		$\bar{x}$ = 0.000	$\bar{x}$ = -0.001	$\bar{x}$ = 0.011	$\bar{x}$ = 0.009	$\bar{x}$ = 0.020	$\bar{x}$ = 0.050	$\bar{x}$ = 0.080	$\bar{x}$ = 0.132	$\bar{x}$ = 0.174	$\bar{x}$ = 0.230
1.417	0.106	0.000	0.035	0.042	0.034	0.027	0.045	0.050	0.078	0.093	0.085
	0.106	0.000	-0.007	0.005	0.009	0.020	0.053	0.085	0.111	0.152	0.203
		$\bar{x}$ = 0.000	$\bar{x}$ = 0.014	$\bar{x}$ = 0.024	$\bar{x}$ = 0.022	$\bar{x}$ = 0.024	$\bar{x}$ = 0.049	$\bar{x}$ = 0.068	$\bar{x}$ = 0.095	$\bar{x}$ = 0.123	$\bar{x}$ = 0.144

Fig. 7.14 Growth of *Cladophora* over 18 day experimental period in response to variation in Cu concentration.

LEGEND

● = 0.020 mgl^{-1} Cu
○ = 0.043 mgl^{-1} Cu
■ = 0.049 mgl^{-1} Cu
□ = 0.079 mgl^{-1} Cu
★ = 0.154 mgl^{-1} Cu

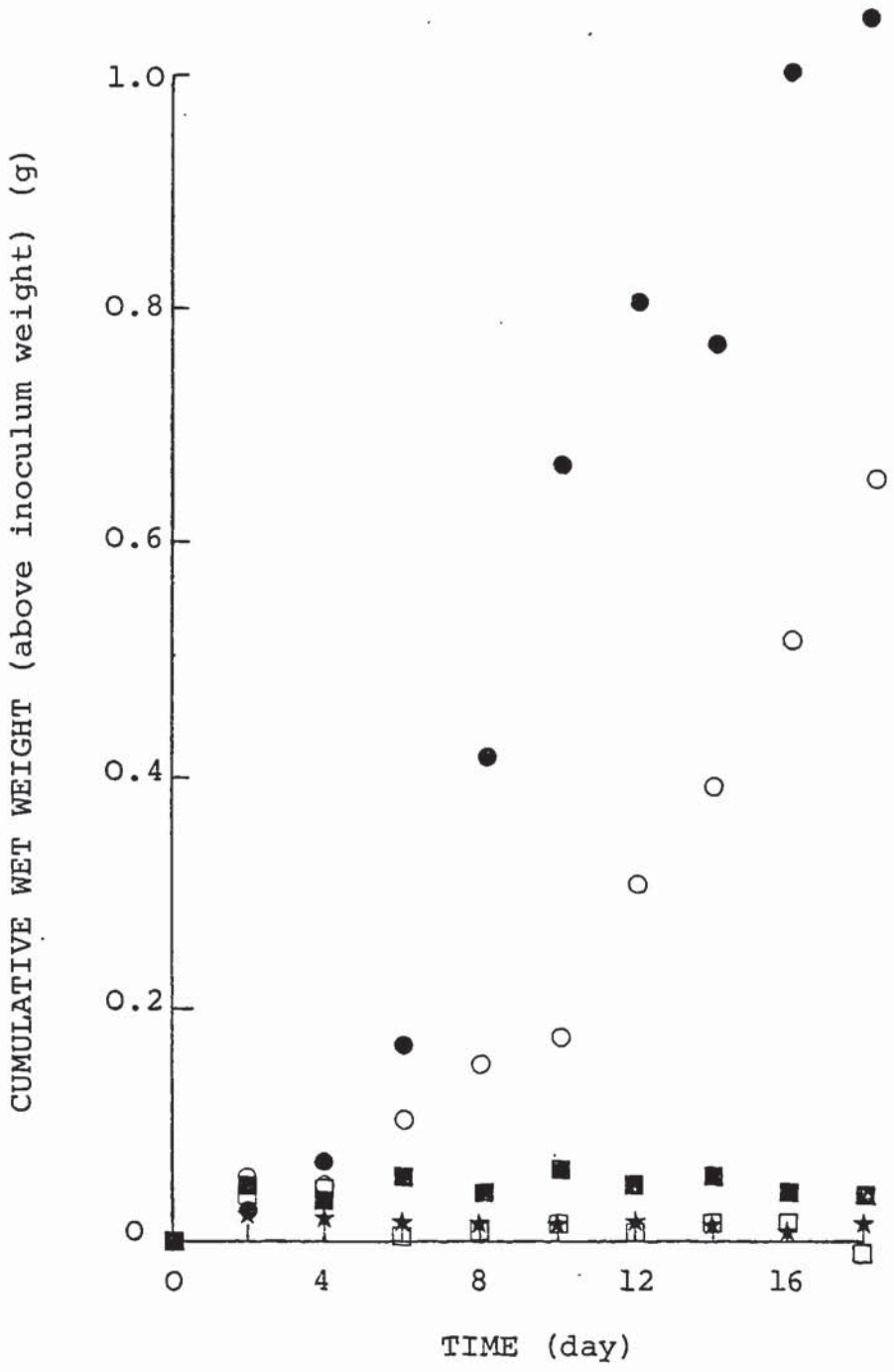


Fig. 7.15 Growth of *Cladophora* over 18 day experimental period in response to variation in Zn concentration.

LEGEND

● = 0.0405 mgl^{-1} Zn
○ = 0.0657 mgl^{-1} Zn
■ = 0.0765 mgl^{-1} Zn
□ = 0.0974 mgl^{-1} Zn
★ = 0.1810 mgl^{-1} Zn

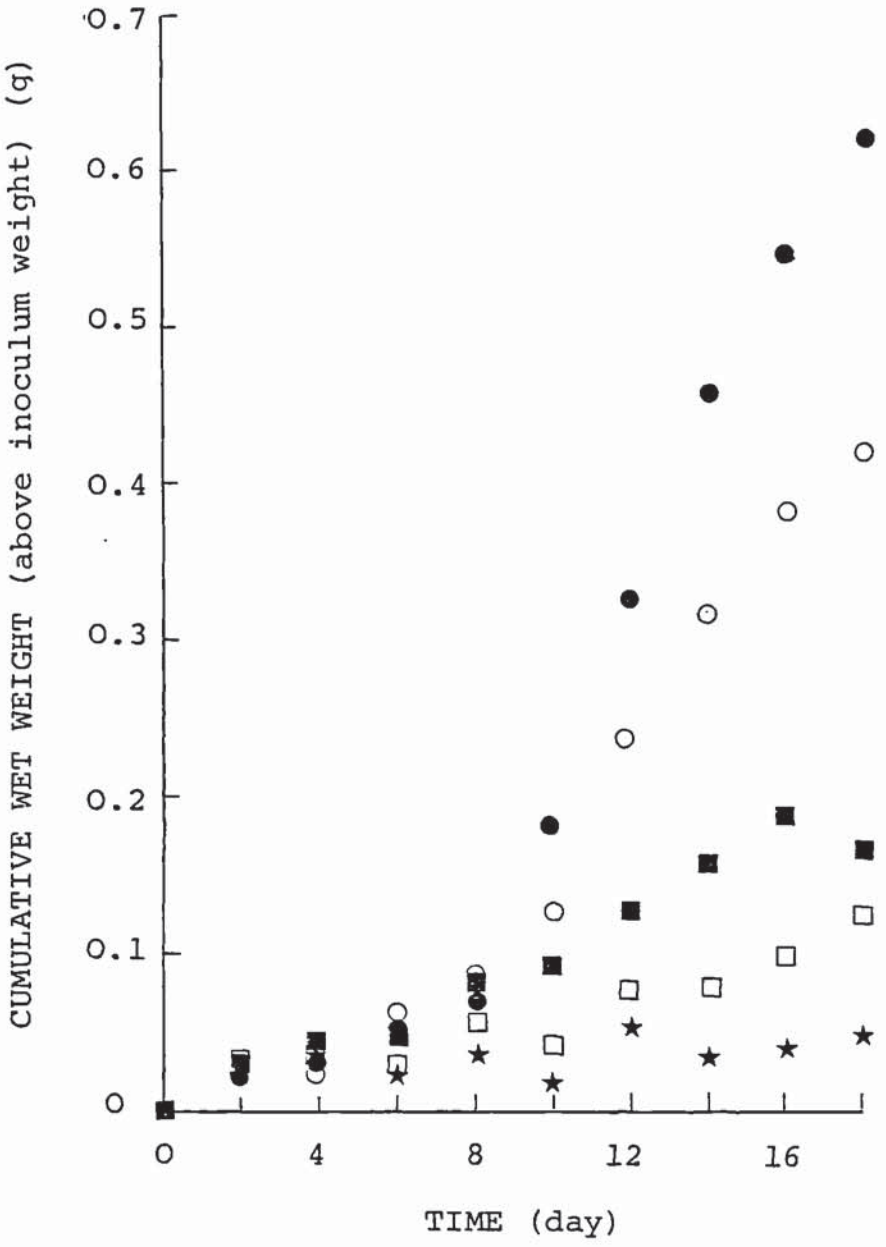


Fig. 7.16 Growth of *Cladophora* over 18 day experimental period in response to variation in Pb concentration.

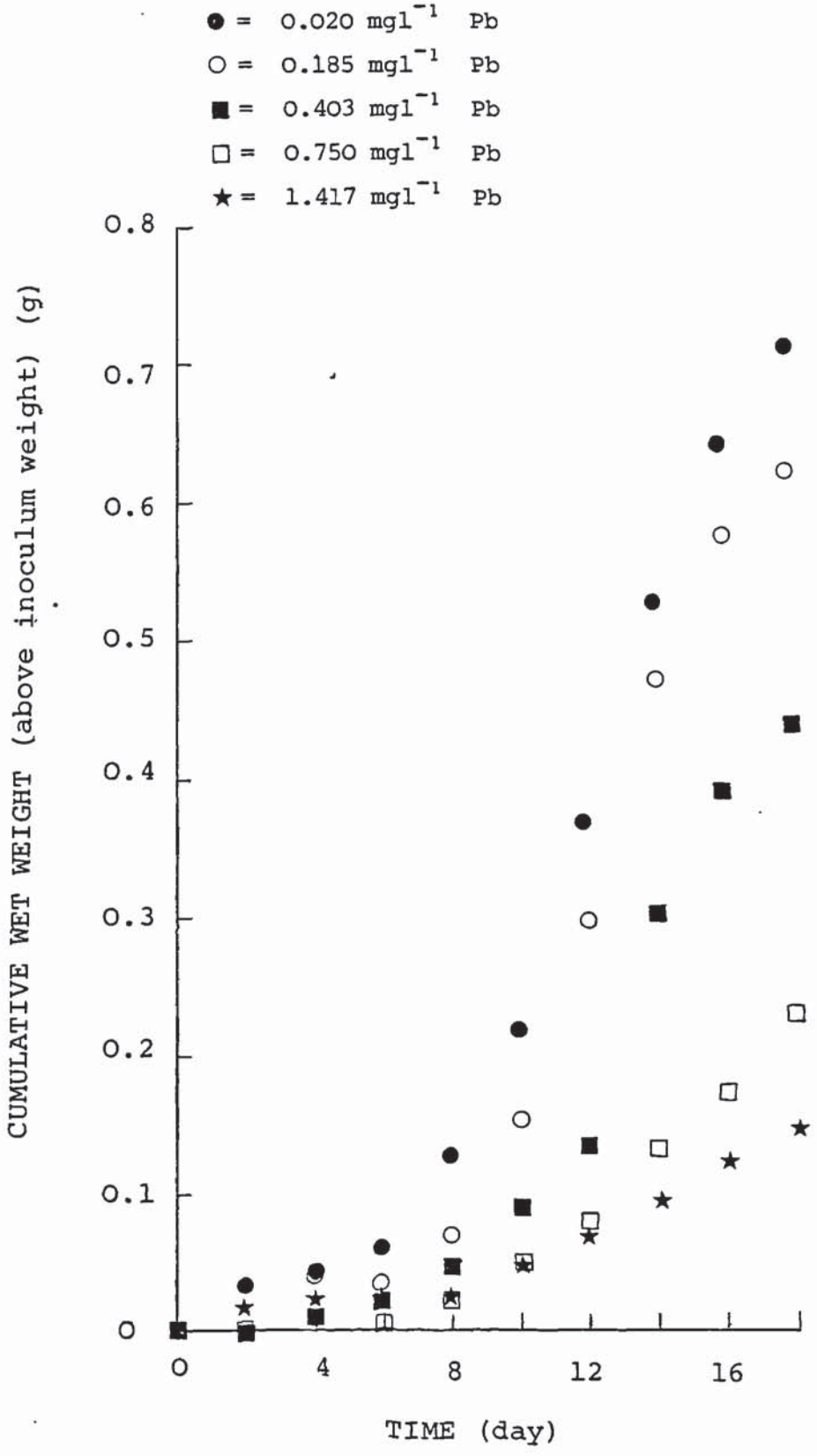


Table 7.36 Calculation of values of μ from growth data of Experiment 13.

Cu (mgl^{-1})	Period over which exponential growth assessed	Regression of ln transformed data		
		Slope (=μ)	Correlation coefficient (r)	n
0.02	2 - 10	0.218	0.988	5
	2 - 10	0.218	0.994	5
0.043	0 - 18	0.098	0.986	10
	0 - 18	0.106	0.991	10
0.049	0 - 18	0.011	0.558	10
	0 - 18	0.010	0.387	10
0.079	0 - 18	0.005	0.296	10
	0 - 18	-0.029	- 0.768	10
0.154	0 - 18	0.007	0.356	10
	0 - 18	-0.007	0.593	10

Table 7.37 Analysis of variance summary table for Experiment 13 - response of μ to variation in Cu concentration.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	7.57876E-02	1.89469E-02	133.713	
Linear Eff.	1	3.45686E-02	3.45686E-02	243.959	(P<0.001)
Quad. Eff.	1	3.47306E-02	3.47306E-02	245.103	(P<0.001)
Cubic Eff.	1	2.04645E-03	2.04645E-03	14.4423	(P<0.05)
Residual	1	4.44199E-03			
Error	5	7.08491E-04	1.41698E-04		
Total	9	7.64961E-02			

Table 7.38 Calculation of values of μ from data of Experiment 14.

Zn (mgl^{-1})	Period over which exponential growth assessed	Regression of ln transformed data		
		Slope (= μ)	Correlation coefficient (r)	n
0.0405	6 - 14	0.178	0.982	5
	6 - 14	0.177	0.982	5
0.0657	4 - 12	0.140	0.993	5
	4 - 12	0.077	0.910	5
0.765	0 - 18	0.055	0.961	10
	0 - 18	0.062	0.960	10
0.0974	0 - 18	0.029	0.874	10
	0 - 18	0.048	0.974	10
0.1810	0 - 18	0.015	0.539	10
	0 - 18	0.014	0.636	10

Table 7.39 Analysis of variance summary table for Experiment 14 - response of μ to variation in Zn concentration.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	.033584	.008396	19.1647	
Linear Eff.	1	2.28945E-02	2.28945E-02	52.259	(P<0.001)
Quad. Eff.	1	.010038	.010038	22.9126	(P<0.01)
Cubic Eff.	1	3.65386E-05	3.65386E-05	8.34029E-02	(P>0.20)
Residual	1	6.14986E-04			
Error	5	2.19049E-03	4.38097E-04		
Total	9	3.57745E-02			

Table 7.40 Calculation of values of μ from data of Experiment 15.

Pb (mgl^{-1})	Period over which exponential growth assessed	Regression of ln transformed data		
		Slope (= μ)	Correlation coefficient (r)	n
0.020	6 - 14	0.182	0.997	5
	6 - 14	0.149	0.996	5
0.185	6 - 14	0.209	0.996	5
	6 - 14	0.112	0.991	5
0.403	6 - 16	0.148	0.995	6
	6 - 16	0.128	0.988	6
0.750	6 - 18	0.063	0.983	7
	6 - 18	0.102	0.987	7
1.417	8 - 18	0.040	0.948	6
	8 - 18	0.087	0.997	6

Table 7.41 Analysis of variance summary table for Experiment 15 - response of μ to variation in Pb concentration.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio
Treatment	4	.017226	4.30651E-03	2.94403
Linear Eff.	*1*	*1.56759E-02*	*1.56759E-02*	10.7164 (P<0.05)
Quad. Eff.	1	7.11106E-04	7.11106E-04	.486129 (P>0.20)
Cubic Eff.	1	8.37671E-04	8.37671E-04	.572651 (P>0.20)
Residual	1	1.36718E-06		
Error	5	7.31397E-03	1.46279E-03	
Total	9	.02454		

Fig. 7.17 Response of *Cladophora* specific growth rate to variation in copper concentration.

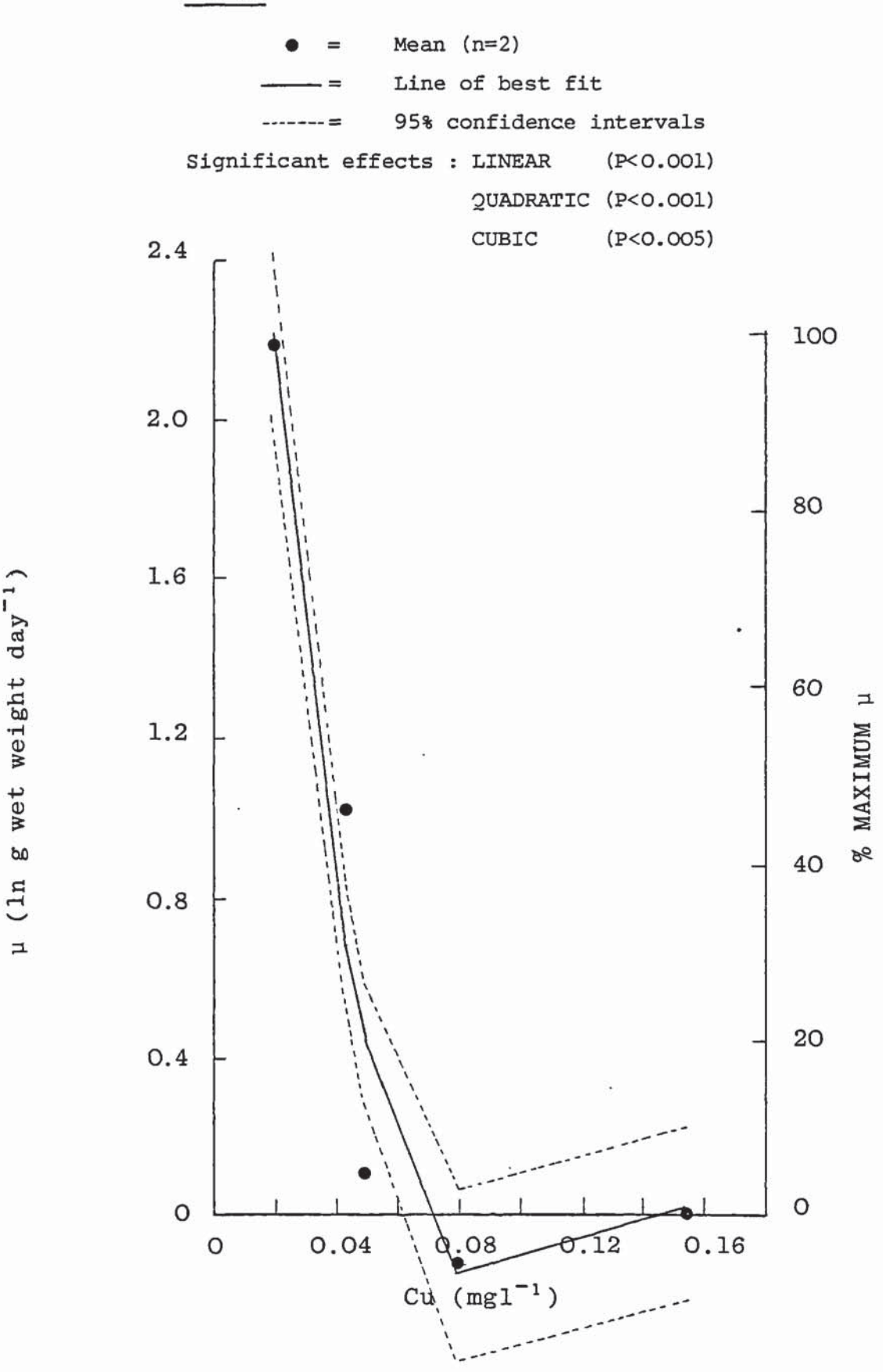


Fig. 7.18 Response of *Cladophora* specific growth rate to variation in zinc concentration.

LEGEND

● = Mean (n=2)

——— = Line of best fit

------ = 95% confidence intervals

Significant effects : LINEAR (P<0.001

QUADRATIC (P<0.01)

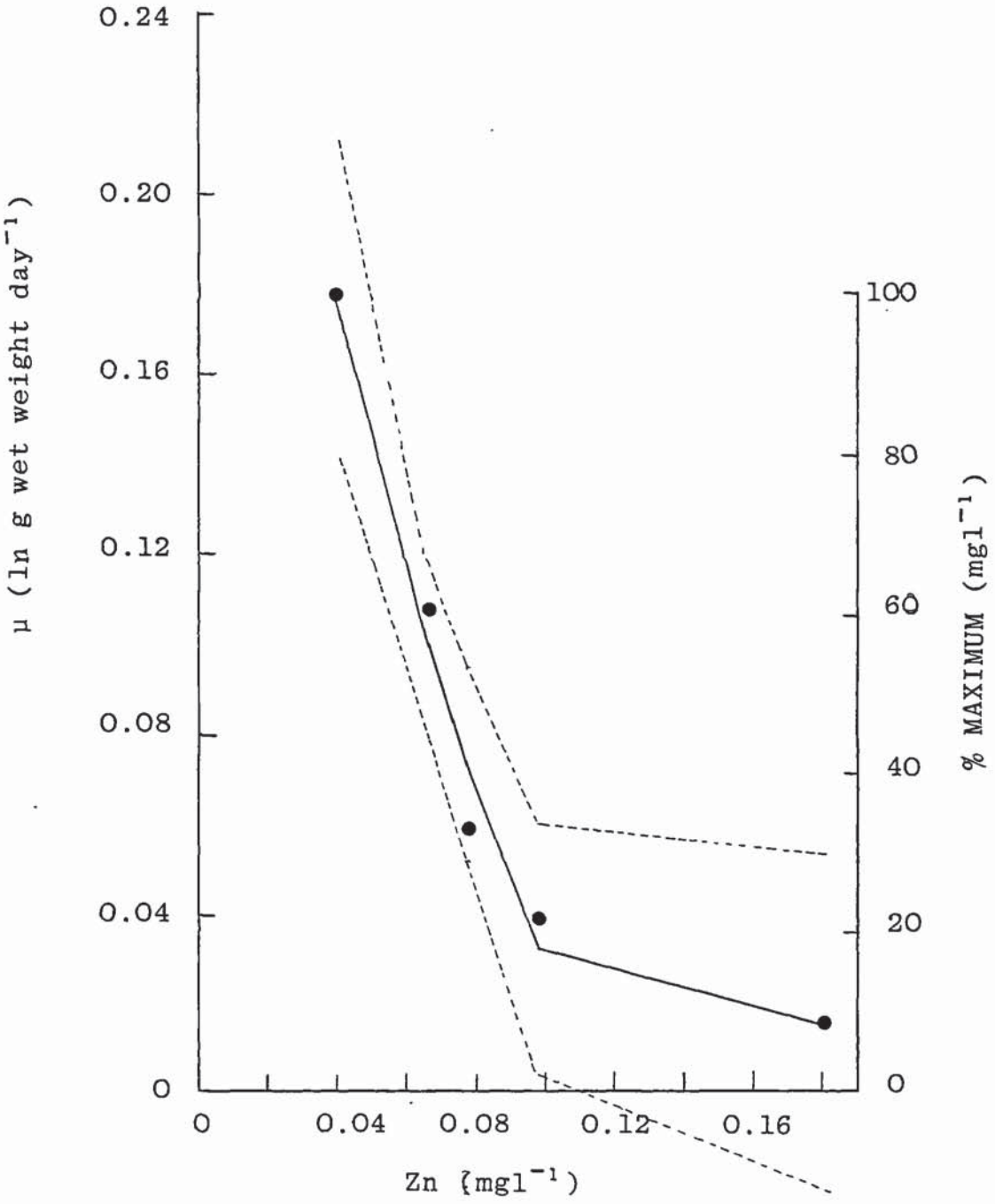


Fig. 7.19 Response of *Cladophora* specific growth rate to variation in lead concentration.

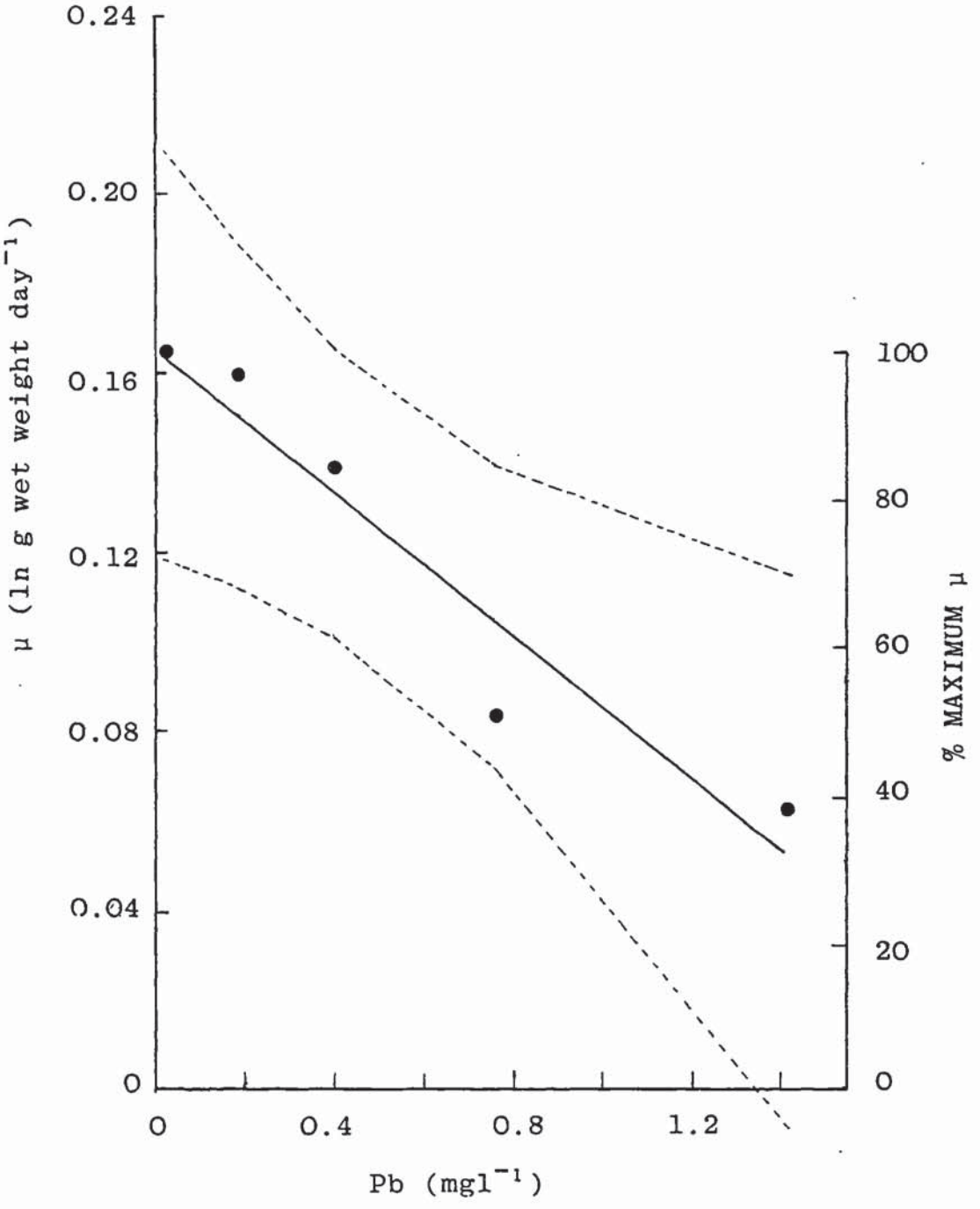
LEGEND

● = Mean (n=2)

——— = Line of best fit

------ = 95% confidence intervals

Significant effects : LINEAR ($P<0.05$)



Tables 7.42 - 7.44 Metal accumulation data.

Nominal Cu (mgl^{-1})	Medium Cu (mgl^{-1})	*Cladophora* dry weight after 18 days (mg)	Total Cu (blank corrected) (µg)	µgCu/ g dry wt.	Concentration factor x 10^{-3}*
0.	0.020	24.4	-0.3	-12	-0.6
		29.9	1.3	43	2.2
0.02	0.043	15.4	0.1	6	0.2
		15.3	0.7	46	1.1
0.05	0.049	3.9	1.2	308	6.3
		3.2	-0.3	-94	-1.9
0.10	0.079	5.2	2.0	385	4.9
		2.6	0.9	346	4.4
0.20	0.154	2.2	5.2	2364	15.3
		2.2	3.2	1455	9.4

Nominal Zn (mgl^{-1})	Medium Zn (mgl^{-1})	*Cladophora* weight after 18 days (mg)	Total Zn (blank corrected) (µg)	µgZn/ g dry wt.	Concentration factor x 10^{-3}*
0.	0.0405	14.1	0.63	45	1.1
		11.7	0.51	44	1.1
0.02	0.0657	8.5	1.02	120	1.8
		7.5	0.35	47	0.7
0.05	0.0765	5.2	0.63	121	1.6
		7.0	0.37	53	0.7
0.10	0.0974	5.5	2.02	367	3.8
		5.2	1.15	221	2.3
0.20	0.1810	2.9	1.18	407	2.2
		3.1	1.83	590	3.3

Nominal Pb (mgl^{-1})	Medium Pb (mgl^{-1})	*Cladophora* dry weight after 18 days (mg)	Total Pb (blank corrected) (µg)	µgPb/ g dry wt.	Concentration factor x 10^{-3}*
0	0.020	22.4	0.3	13	0.7
		19.5	0.4	21	1.0
0.2	0.185	24.2	13.9	574	3.1
		9.0	9.1	1011	5.5
0.5	0.403	14.6	12.2	836	2.1
		13.7	13.4	978	2.4
1.0	0.750	11.1	18.0	1622	2.2
		10.8	21.3	1972	2.6
2.0	1.417	10.9	40.2	3688	2.6
		8.6	44.1	5128	3.6

$$* = \frac{\text{µg metal } g^{-1} \textit{ Cladophora} \text{ dry weight}}{\text{µg metal } ml^{-1} \text{ medium}}$$

Table 7.45 Analysis of variance summary table for Experiment 14 - response of *Cladophora* copper content (µgCu g^{-1} algal dry weight) to variation in copper concentration in the medium.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	5.23495E+06	1.30874E+06	13.166	
Linear Eff.	1	4.88234E+06	4.88234E+06	49.1167	(P<0.001)
Quad. Eff.	1	349921	349921	3.52023	(P<0.20)
Cubic Eff.	1	221.998	221.998	2.23331E-03	(P>0.20)
Residual	1	2464			
Error	5	497015	99403		
Total	9	5.73197E+06			

Table 7.47 Analysis of variance summary table for Experiment 15 - response of *Cladophora* zinc content (µg Zn g^{-1} algal dry weight) to variation in zinc concentration in the medium.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	296897	74224.2	11.4616	
Linear Eff.	1	275999.	275999.	42.6194	(P<0.01)
Quad. Eff.	1	302.823	302.823	4.67615E-02	(P>0.20)
Cubic Eff.	1	17225.7	17225.7	2.65998	(P<0.20)
Residual	1	3369.19			
Error	5	32379.5	6475.9		
Total	9	329276			

Table 7.49 Analysis of variance summary table for Experiment 16 - response of *Cladophora* lead content (µgPb g^{-1} algal dry weight) to variation in lead concentration in the medium.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	2.31213E+07	5.78032E+06	24.0117	
Linear Eff.	1	2.24290E+07	2.24290E+07	93.1711	(P<0.001)
Quad. Eff.	1	416338.	4.6338.	1.72948	(P>0.20)
Cubic Eff.	1	120099.	120099.	.498896	(P>0.20)
Residual	1	155796			
Error	5	1.20365E+06	240730.		
Total	9	2.43249E+07			

Table 7.46 Analysis of variance summary table for Experiment 14 - response of copper concentration factor (x 10^{-3}) to variation in copper concentration in the medium.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	189.526	47.3815	4.27053	
Linear Eff.	1	183.728	183.728	16.5596	(P<0.01)
Quad. Eff.	1	3.15214	3.15214	.284105	(P>0.20)
Cubic Eff.	1	1.30454	1.30454	.117579	(P>0.20)
Residual	1	1.34137			
Error	5	55.4749	11.095		
Total	9	245.001			

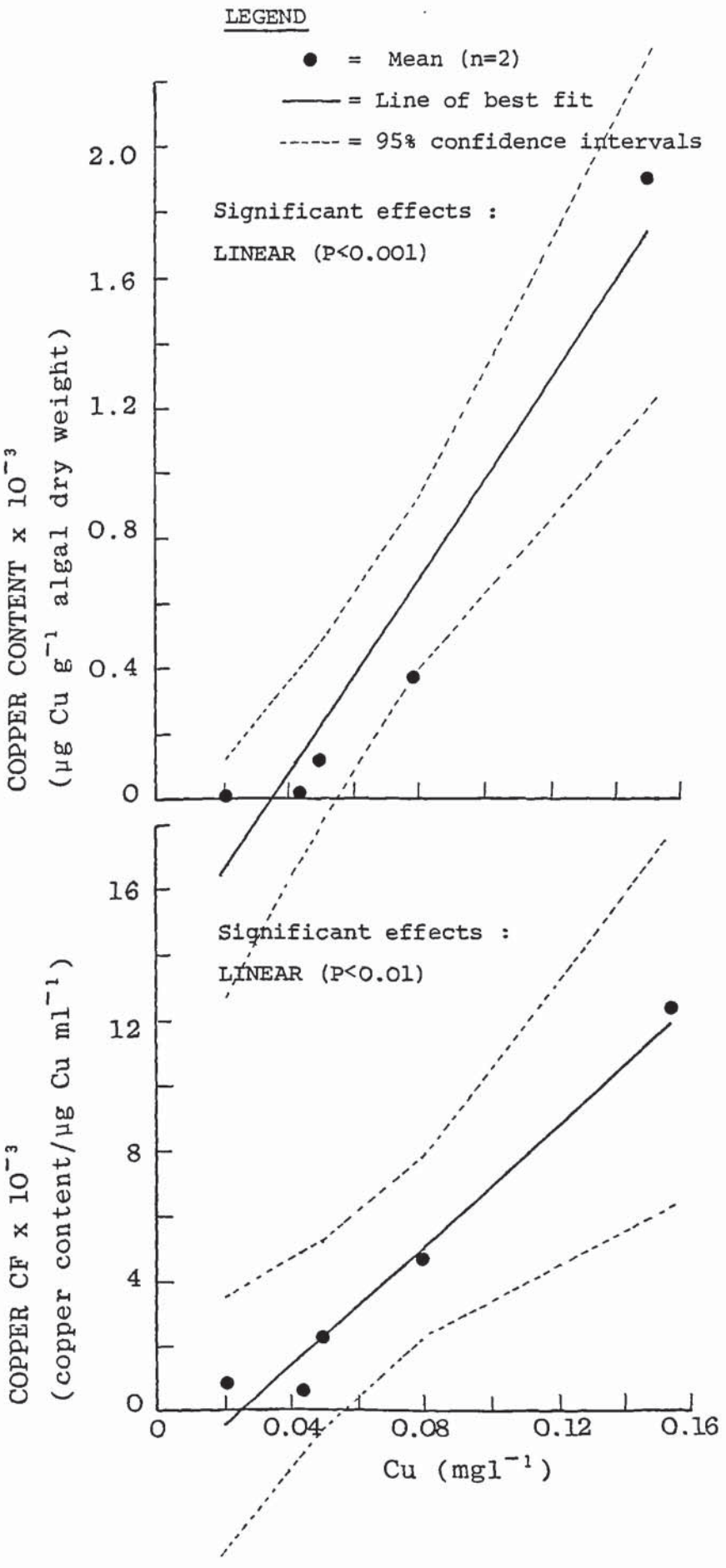
Table 7.48 Analysis of variance summary table for Experiment 15 - response of zinc concentration factor (x 10^{-3}) to variation in zinc concentration in the medium.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	7.324	1.831	3.34125	
Linear Eff.	1	3.99693	3.99693	7.2937	(P<0.05)
Quad. Eff.	1	.799609	.799609	1.45915	(P>0.20)
Cubic Eff.	1	2.13922	2.13922	3.90371	(P<0.20)
Residual	1	.388233			
Error	5	2.73999	.547998		
Total	9	10.064			

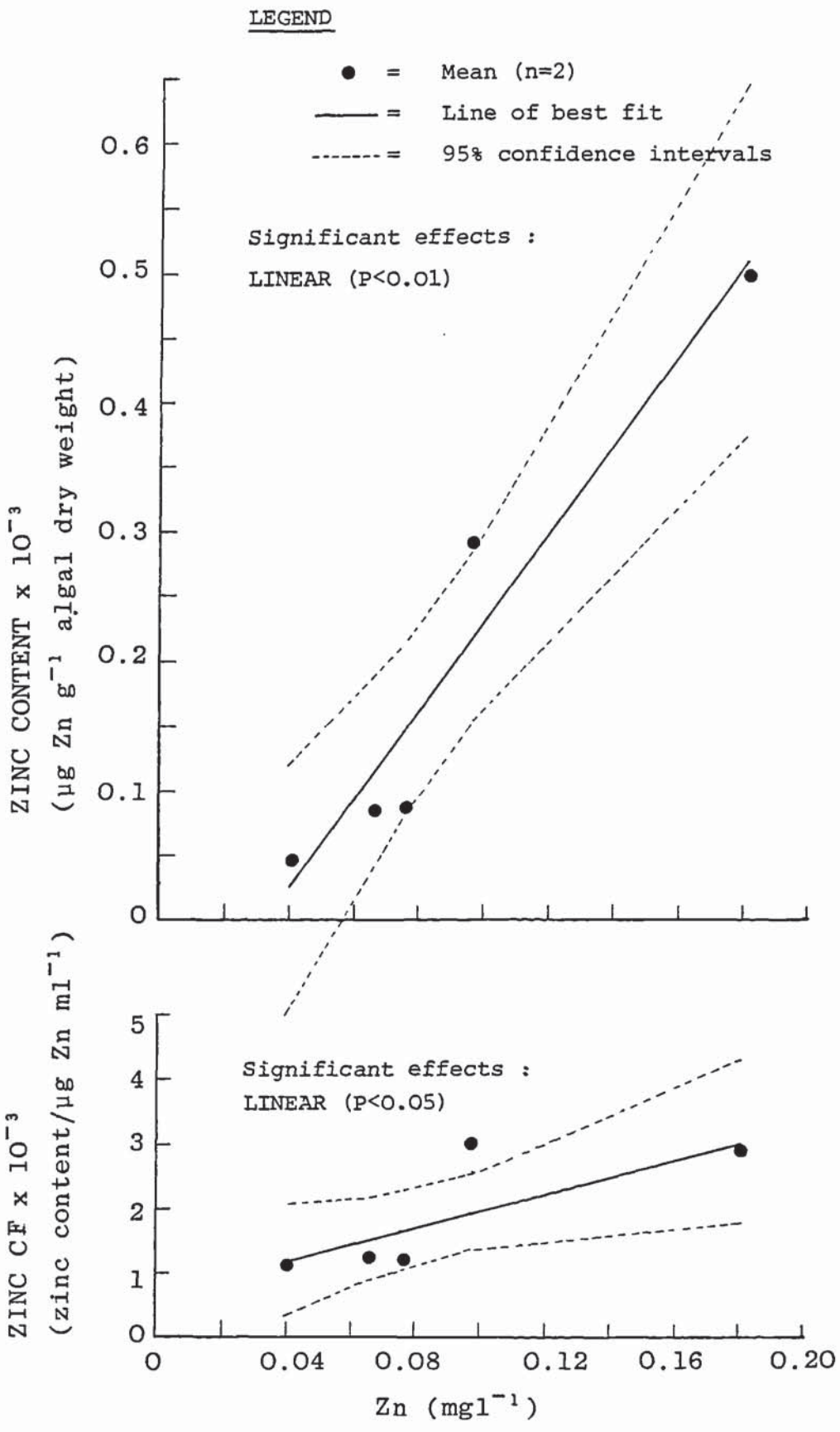
Table 7.50 Analysis of variance summary table for Experiment 16 - response of lead concentration factor (x 10^{-3}) to variation in lead concentration in the medium.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	
Treatment	4	12.726	3.18151	4.48099	
Linear Eff.	1	.922642	.922642	1.29949	(P>0.20)
Quad. Eff.	1	.2554	.2554	.359718	(P>0.20)
Cubic Eff.	1	4.52213	4.52213	6.36919	(P<0.10)
Residual	1	7.02586			
Error	5	3.55	.710001		
Total	9	16.276			

Figs. 7.20 and 7.21 Response of *Cladophora* copper content and concentration factor (CF) to variation in copper concentration.



Figs. 7.22 and 7.23. Response of *Cladophora* zinc content and concentration factor (CF) to variation in zinc concentration.



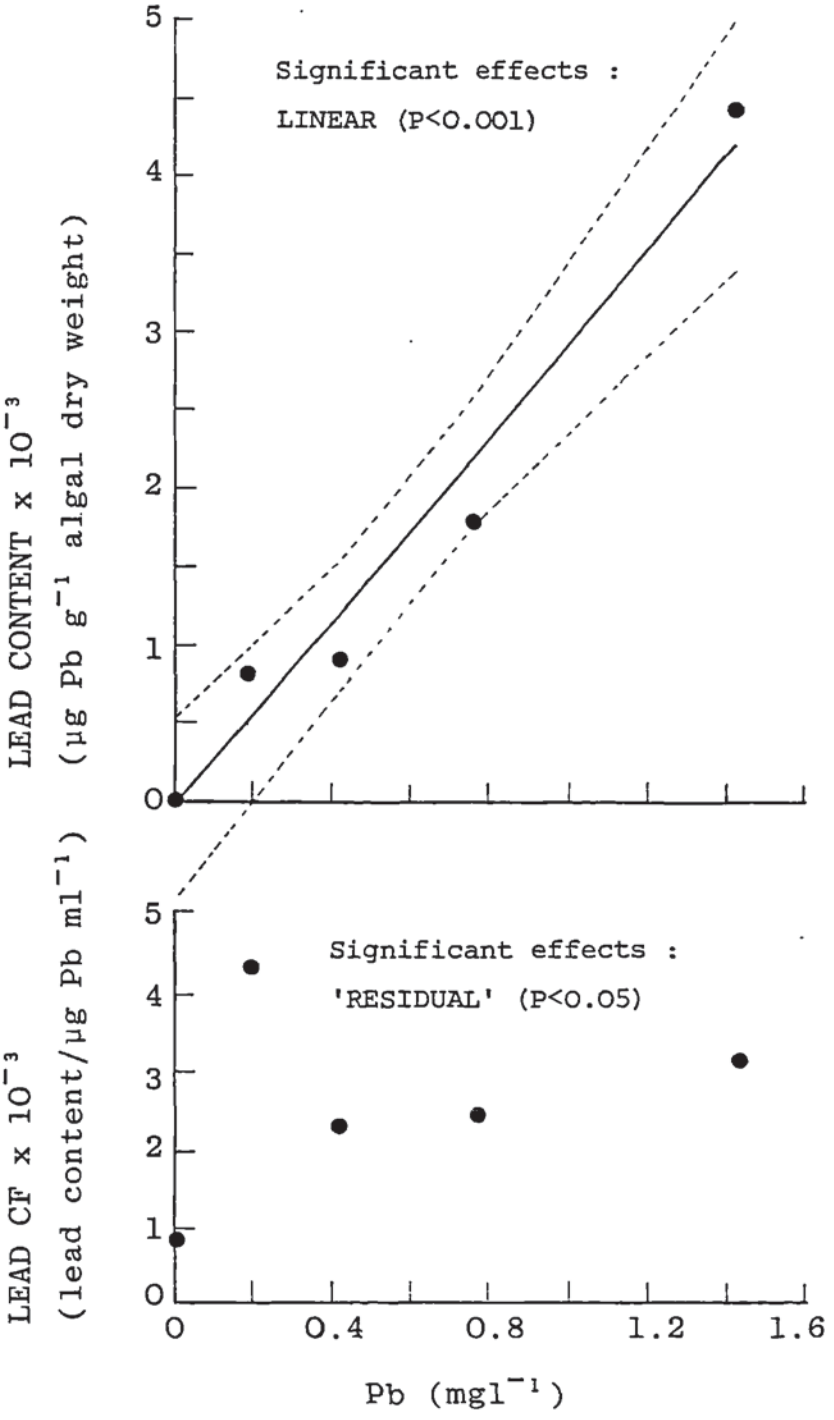
Figs. 7.24 and 7.25 Response of *Cladophora* lead content and concentration factor (CF) to variation in lead concentration.

LEGEND

● = Mean (n=2)

——— = Line of best fit

------ = 95% confidence intervals



metal concentration in the medium.

Inspection of figs. 7.17-7.19 reveal that of the three metals under consideration, copper is the most toxic (0.036 mg l^{-1} reducing growth rate by 50%), zinc is next toxic (0.070 mg l^{-1} reducing growth rate by 50%), and lead the least toxic (1.03 mg l^{-1} reducing growth by 50%). Such results are in reasonably good agreement with those of Whitton (1970a) who evaluated the 'tolerance index concentration' for various *C. glomerata* isolates to be 0.19 mgCu l^{-1} , 0.14 mgZn l^{-1} and 3 mgPb l^{-1}. In this study, however, inhibition of growth rate is apparent at lower concentrations than those quoted by Whitton; this may possibly be explained by the likely loss of metal from solution during batch culture experiments such as those carried out by Whitton. This study also found copper to be more toxic than zinc whilst Whitton claimed the reverse. Out of nine other genera of Chlorophyta studied by Whitton only in certain isolates of *Oedogonium* was a similar result found (i.e. zinc more toxic than copper), whereas to most isolates copper was appreciably more toxic than zinc.

It is noticable that in comparison to the total metal concentrations in the Langley water only small additions of either copper or zinc are required to reduce growth rate. This suggests that much of the copper or zinc already present in the water is in a non-toxic or significantly less toxic form even though results of experiment 13 showed approximately 96% of total metals to be 'soluble'. Similarly, in a river studied by Morgan and Whitton (in Whitton 1970c) *Cladophora* was found at a river site where dissolved zinc levels were 0.17 mg l^{-1} - much of this zinc must have been in relatively non-toxic (though soluble) forms. Extrapolation of laboratory results to the field situation is thus difficult. However, Watton (1982) and Girton (1980) after extensive sampling of rivers throughout England found maximum values of total metals to be 0.08 mgCu l^{-1}, 0.28 mgZn l^{-1} and 0.28 mgPb l^{-1}. Assuming that the proportion of toxic species is approximately the same for each metal, results would thus suggest that copper and zinc are more likely to affect the growth of *Cladophora* in the field situation than is lead.

Results of the uptake study all show a significant linear relationship between metal content of the alga and metal concentration in the medium (figs. 7.20, 7.22, 7.24). For copper and zinc a significant linear relationship is also found between the concentration factor and the metal concentration in the medium (figs. 7.21, 7.23). For lead the situation is rather more complex (fig. 7.25); at the $P = 0.05$ level neither linear, quadratic, nor cubic effects are significant. However, after removal of the variability attributable to such effects the residual variability is significant ($P<0.05$) and since this residual has only one degree of freedom it is, in fact, the quartic effect (i.e the response curve comprises three turning points). Such a relationship is exceedingly complex and unlikely to result from a single biological effect, though the interaction of two or more effects could certainly produce such a curve. This relationship will be further discussed in due course.

As mentioned previously Keeney *et al.* (1976) and Thorpe (1981) both considered metal concentration factors to be fairly stable in *Cladophora*. However, both Sikes (1977) and Gileva (1964) found concentration factors to fall as the concentration of element in solution rose. Findings of Bryan (1969) and Gutknecht (1965) suggest such a relationship to hold true for a variety of algal taxa. Restricting comments to copper and zinc for the moment, results are somewhat surprising in that concentration factors for the two metals increase as their concentration in the medium increases. It is considered likely that this effect is a result of the toxic activity of the metals themselves. Such a hypothesis is not in direct agreement with results of other workers ; Williams (1970) found preserved *Cladophora* took up less ^{85}Sr and ^{137}Cs than the live alga, and Burkett (1975) concluded that live *Cladophora* sorbed more methylmercury than the dead alga at equal exposure concentrations (though the different shaped response curves for dead and live alga made such comparison rather subjective). However, Gutknecht (1961) found zinc uptake by killed *Ulva* to be considerably more rapid and extensive than by living material. Killing was effected by immersing the alga in either 3% formalin or steam and Gutknecht considered that either of these two techniques might, by denaturing cell proteins, produce an abnormally large surface and thereby enhance uptake by the dead

tissue. Application of phenylurethane (a photosynthesis inhibitor) and cyanide (a respiratory inhibitor), however, also increased zinc uptake although such treatment was not considered to have any direct effects on the surface proteins. Gutknecht (1963) also found similar results with species of *Porphyra* and *Laminaria* and concluded that the extensive ^{65}Zn uptake by killed algae was a result of the considerable increase in intracellular pH which, in turn, causes a higher degree of dissociation of internal weak multibasic acids and consequently an increase in the number of available sites for cation adsorption (Lundegardh, 1960), and since the stability of zinc complexes with various ligands is high (Goldberg, 1957) it seems likely that in killed cells ^{65}Zn may replace other cations already bound to previously inaccessible sites. Results with copper and zinc suggest a similar process may occur in *Cladophora* enabling dead cells to take up more metal than live cells. That concentration factors increase more for cells exposed to copper than zinc (i.e. the more toxic species) is evidence in support of this hypothesis.

Results show lead to be much less toxic than either copper or zinc, and even up to the highest concentration tested the lower toxicity of the metal likely causes little cell death. The response of concentration factor to variation in lead concentration is therefore not linear. Results for the upper four treatment levels are readily interpretable : as lead concentration in the medium increases, the lead concentration factor first falls (as suggested by Gileva, 1964; Sikes, 1977; Bryan, 1969; and Gutknecht, 1965) and only rises very slowly as a result of the low toxicity of lead. The lower treatment level, however, does not apparently conform to this trend and may indicate that the metal in the natural Langley water (i.e. that without any metal compound supplement) is in a form relatively unavailable to the alga. This hypothesis has already been suggested in connection with the toxicity of copper and zinc.

7.5 CONCLUSIONS

1. In closed continuous culture the specific growth rate of a unialgal culture of *C. glomerata* increased as 'daylength' increased. No inhibitory effect of continuous lighting was detected.

2. Specific growth rate increased with increasing light intensity up to 6000 lux - the highest intensity tested.

3. Optimum specific growth rate occurred at, or near, 20°C; whilst growth at 5 and 30°C was barely detectable.

4. Ammonia was shown to be highly toxic, 3.6 mg l^{-1} total ammoniacal nitrogen (= 0.185 mg l^{-1} undissociated ammoniacal nitrogen) reduced specific growth rate to 50% of that which occurred with natural Langley water containing 0.2 mg l^{-1} total ammoniacal nitrogen (=0.010 mg l^{-1} undissociated ammoniacal nitrogen).

5. NO_2-N had no significant effect on specific or incremental growth rate (μ or k) up to approximately 1 mg l^{-1} ($P>0.05$).

6. NO_3-N had no significant effect on specific growth rate between 7.2 and 15.2 mg l^{-1}. High PO_4-P concentration was shown to be toxic : specific growth rate at 4.9 mgP l^{-1} was only 48% of that at 1.9 mgP l^{-1} . Results indicated the critical PO_4-P concentration for *Cladophora* to be below 0.1 mgP l^{-1} . No significant nitrate/phosphate interaction was detected over the levels of treatment studied ($P>0.05$).

7. 0.036 mgCu l^{-1}, 0.070 mgZn l^{-1} and 1.03 mgPb l^{-1} were found to reduce *Cladophora* specific growth rate to 50% of that occurring with natural Langley water.

8. Concentration factors for copper and zinc increased as the concentration of metal in solution increased; this was considered attributable to physiological changes occurring within the cells at, or near to, death. For the less toxic lead, if the first treatment level is neglected, the concentration factor initially falls before rising as a result of cell damage/death.

9. Toxicity curves for copper and zinc suggest that metals already in the Langley water may be non-toxic, or significantly less toxic than metals added as $CuSO_4 . 5H_2O$ or $ZnSO_4$; whilst the uptake curve for lead suggests that lead already in solution is less available for uptake than lead added as $PbCl_2$.

8. GENERAL DISCUSSION AND SYNTHESIS

In retrospect, the objectives of the research program were remarkably ambitious. Each of the three major areas of work - field investigation, laboratory batch culture and laboratory continuous culture - is potentially capable of providing a three-year study in itself. The study of all three, in the time available thus necessitated some neglect or superficial study of some factors of great interest and importance. It was, however, considered that the inclusion of these three broadly related area of study provided a more balanced perspective of factors affecting the growth of *Cladophora*. The purpose of this chapter is therefore to integrate findings from each of the major sections of the study so that general conclusions may be formulated in the next chaper.

Cladophora is generally considered notoriously difficult to grow in culture; results of batch culture work support this view. However, it was found that regular replacement of medium (membrane filtered Langley water) at three-day intervals resulted in a three-fold increase in the algal crop produced after 20 days. The pH of the medium was shown to drift from pH 8.2 ± 0.1 to 9.2-10.2 over a similar experimental period, and it was considered that elevated pH limited growth in batch culture without regular medium replacement. Further experiments showed that *Cladophora* growth virtually ceased after 5-10 days if medium was not replaced. Growth of *Cladophora* in continuous culture, in which nutrients were continuously supplied and waste products continuously removed, avoided such problems. Growth, in such culture, proceeds through lag, logarithmic and linear phases. Consideration of growth of *Cladophora* in the lotic situation indicates that growth would generally proceed through similar phases, though self-shading would complicate matters in that the alga could not be considered homogeneous. Unfortunately, little is known of the kinetics of growth of the alga in the field situation. Chudyba (1965) measured the length of *Cladophora* streamers as a part of his study of the Skawa River, Poland. but the infrequency of his measurements makes interpretation difficult. Results of Bellis and McLarty (1967), however, showed filament length to grow at a constant rate of 1.5 cm day^{-1} during the May pulse in vegetative growth.

This linear growth of individual filaments is not, however, incompatible with exponential growth of the entire plant (see for example Katz *et al.* 1972 and Smith, 1924). It was realized that the heterogeneous nature of the alga grown in continuous culture made estimations of the specific growth rate artificially low (although comparison of such values was considered valid). Whitton (1967) and Zuraw (1969), however, estimated the specific growth rate to produce a doubling time in the order of 1-2 days. It seems obvious that such a growth rate could only be maintained, in the field situation, for a short period of time before some physical or chemical factor becomes limiting. As mentioned previously self-shading is one important factor which will certainly limit growth of the alga in the field situation. Both reduction of total intensity and attenuation of spectral composition are likely to affect growth rate. Westlake (1964) discusses some of the complex changes in light upon its passage through weed beds. The effects on *Cladophora* growth are difficult to evaluate except to suggest that generally growth will only proceed at rates approaching the specific growth rate in the upper cells of the algal 'mat'. With our present knowledge of the growth of *Cladophora* in the natural situation it is therefore difficult to apply any index of growth in culture to the field situation with total satisfaction and until more information becomes available consideration of specific growth rate seems to be more satisfactory than any other index. In continuous culture studies both specific growth rate (μ) and incremental growth rate (k) were chosen as indices of growth for comparison. However, in cases of low specific growth rate, the time taken to grow to a size where incremental growth begins, makes estimation of k impracticable in any but long-term experiments and in such cases the ecological value of such estimates is somewhat dubious. Conceivably, however, a treatment may have little effect on specific growth rate but significantly affect incremental growth rate. In experimentation described in this study no such case was found.

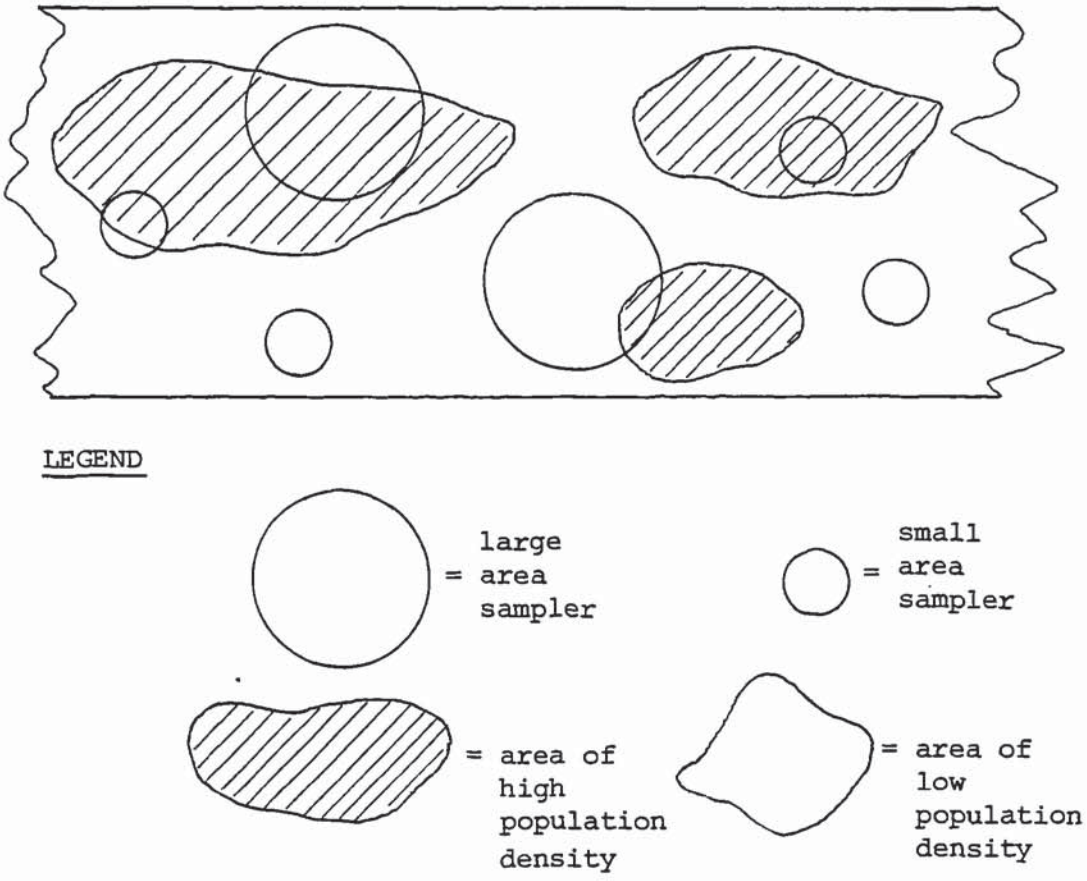
The study of the growth of *Cladophora* in batch culture and continuous culture has been used in this, and other, studies to indicate the growth response of the alga to various factors in the field situation. Various important considerations have, however, been somewhat neglected. No effort has been made to investigate the effects of various physico-

chemical factors on sporulation and spore germination ; no effort has been made to assess whether different stages in the vegetative growth cycle of the alga respond differently to various physico-chemical factors; no effort has been made to investigate the effect of sporulation on the subsequent growth of the parent plant; no effort has been made to assess the effect of fragmentation; and no effort has been made to investigate colonization of the alga. All of these considerations are undoubtedly of importance in determining the overall growth and distribution of *Cladophora* in the aquatic environment yet were necessarily beyond the scope of the present laboratory culture work. All such effects were, however, integrated in determining the distribution of *Cladophora* in the experimental streams studied at Checkley and, since the community comprised a naturally developed flora and fauna, competitive and, other interactive effects were also taken into consideration. The experimental streams thus provided an excellent facility for the study of algae, and it was unfortunate that only a very broad initial survey of the distribution of *Cladophora* was made prior to their closure in September, 1980. The information gained from this study does, however, enable some of the results of laboratory studies to be compared to a carefully controlled field situation and this will be discussed later in the chapter. But first, this seems an appropriate point to briefly discuss some of the problems associated with sampling of *Cladophora* populations in the field, in the light of work presented and experience gained by this study.

Sampling of *Cladophora*, and indeed most macro-algal taxa, in the field is difficult. Quantitative sampling requires the removal of a standardized area of benthos and this is usually done with the aid of Surber or cylinder samplers. The use of such samplers is not without criticism however. Firstly the assumption is made that the substrate surface area is the same within the area delimited by the sampler. This assumption is likely to hold true in the Checkley study were the substratum was standardized, but in field surveys where a diversity of field sites are sampled this assumption may not hold, and this potential source of sampling error should not be overlooked. The second criticism is that quadrat samplers only delimit the plant material within their boundaries and, with macro-algal taxa such as *Cladophora*, algal streamers may be attached to the area in question but extend beyond the quadrat area. Obviously the technique can be adapted so that all of the flora attached to the area in question

is removed, but this would be more time consuming and practically difficult in deep and/or fast flowing waters. The situation may, however, be rationalized by considering a randomly placed quadrat in which macro-algal material attached within the delimited area extends downstream. Similarly, however, macro-algal material attached above the quadrat would extend downstream and enter the delimited area, and if a random quadrat is considered it is not difficult to estimate that the losses and gains of plant material should approximately balance. The two methods of obtaining a sample from a quadrat should thus yield similar results. Other criticisms of quadrat-type sampling have been made by Blum (1957). One of Blum's suggestions was that adequate sampling is difficult or impossible without employing relatively large area quadrats. Small - 0.004 m^2 - samples were, however, preferred in this study since samples had to be analysed for composition following cropping. Undoubtedly the use of a 0.004 m^2 quadrat enabled a larger number of samples to be taken and thus a wider range of habitats to be sampled than if only a few larger samples had been taken. It was realized, however, that the adoption of small area samples had resulted in some of the problems in data analysis, since small area samples are likely to respond more dramatically, to a contagious contribution than larger area samplers. One may envisage, for example, the situation (fig. 8.1) in which a large area sample is likely to include both an area of high population density and an area of low density, whereas a small area sample is more likely to include only one of the two areas. In the time available, however, it is estimated that for instance only two or three 0.05 m^2 cylinder samples could have been processed in the time taken to process nine 0.004 m^2 samples. But certainly in studies where only an estimate of total macro-algal or macro-floral biomass is required larger samples would be recommended to alleviate problems arising as a result of the contagious distribution of the flora : the 0.05 m^2 cylinder sampler used by Thorpe (1981) may well be near the ideal size for such usage. In retrospect the use of the 0.004 m^2 sampler for both pool and riffle sites was unwise. At pool sites diversity was low but biomass was high and it was difficult to drive the small sampler through the dense plant growths. A further problem arises in that since rooted vascular hydrophytes (e.g. *Potamogeton*) predominate in the muddy bottomed pool sites the biomass collected was dependant

Fig. 8.1 Plan view of bethos to show effect of sample area.



on the depth to which the sampler was sunk since the root system of such plants grew to considerable depths. At riffle sites, however, total biomass was lower but comprised a greater diversity of taxa. Rather than use such a small area sampler, therefore, it may have been better to use a larger area (e.g. 0.05 m^2) sampler and use a subsampler for determining composition. Thorpe and Williams (1980) summarize statistical techniques which may be used to evaluate the errors attributable to each stage of sampling and subsampling. Alternatively, and perhaps most useful, would be a stratified sampling regime. Kershaw (1963) comments that if any area on inspection appears to be heterogeneous, it is pointless to sample at random, and that in such circumstances the recognized method is to sub-divide the area into a convenient number of equal sized areas (strata) and to take random samples within each sub-division. Thus, as regards sampling riverain macroalgae, it would seem appropriate to sample the high density 'clumps' and the low density 'patches' separately. Snedecor and Cochrane (1967) discuss the mathematics involved in carrying out such a sampling methodology. Undoubtedly such a sampling regime demands a greater

degree of statistical interpretation and understanding of the user; the advantage of such a technique, however, is that differences between the strata means in the population do not contribute to the sampling error of the estimate of the population mean. By forming strata so that a heterogeneous population is divided into parts, each of which is fairly homogeneous, we may therefore expect a gain in precision over simple random sampling (Snedecor and Cochrane, 1967). Stratified sampling has not been reported in the literature in association with *Cladophora* field studies.

Returning now to the discussion of factors affecting *Cladophora* growth in the labortaory and field, it is apparent that both light intensity and duration have important effects. In the laboratory, increasing light intensity, up to 6000 lux, was shown to significantly increase specific growth rate. However, as a result of qualitative differences in the spectra concerned it is difficult to make direct comparisons of laboratory and field results except to point out that most authors have observed no inhibition of photosynthesis or growth at high levels of irradiance either in the laboratory (Whitton, 1967) or field (e.g. Wood, 1968; McMillan and Verduin, 1953). Similarly, laboratory studies have shown that increasing light period significantly increases specific growth rate. At a latitude of 50° (i.e. approximately that of Britain) specific growth rate with 16 hours light/day (summer) would be expected to be 28% greater than that with 8 hours light/day (winter). Though differences expected over the period of active algal growth (i.e. March to October) would be somewhat less. Field studies reveal that the significance of correlation of mean transformed *Cladophora* biomass with both monthly mean total radiation and maximum radiation was very similar even though 'total radiation' integrated both light intensity and period, whereas 'maximum radiation' was only an index of intensity. Such results suggest that light period may be a rather unimportant environmental variable in influencing *Cladophora* growth rate in the field situation.

Ammonia has already been suggested to be an important factor in influencing the growth of *Cladophora* in the field (section 7.3.1). In the laboratory 3.6 m l^{-1} total ammoniacal nitrogen (TA-N) served to reduce growth rate to 50% of that with 0.2 mg l^{-1} TA-N. In the

Checkley streams, however, mean monthly TA-N concentrations were 0.2-0.6 mgN l^{-1} in A(O), 0.4-1.7 mgN l^{-1} in B(25) and 0.4-2.4 mgN l^{-1} in C(50), with maximum concentrations of 1.2, 3.6 and 4.7 mgN l^{-1} found in A(O), B(25) and C(50) respectively. If TA-N were the toxic agent such levels would certainly be expected to noticably reduce the growth rate of *Cladophora* in streams B(25) and C(50). However, in studies involving ammonia toxicity to fish and invertebrates, it has generally been assumed that only ammonia in its undissociated form NH_3 - is toxic, and the proportion of this toxic form is increased by increasing pH and temperature. Calculation reveals that 3.6 mg l^{-1} TA-N, in the laboratory study, contains 5.1%, 0.185 mg l^{-1} undissociated ammoniacal nitrogen (UDA-N). Using monthly mean temperature, pH and TA-N concentration data from the Checkley study, and calculations as prescribed by Emerson *et al.* (1975) table 8.1 was constructed.

Table 8.1 Mean monthly TA-N and UDA-N concentrations in the Checkley streams.

Month	Total ammoniacal nitrogen (mg l^{-1})			Undissociated ammoniacal nitrogen (µg l^{-1})		
	A(O)	B(25)	C(50)	A(O)	B(25)	C(50)
J	-	-	-	-	-	-
F	0.4	1.6	1.5	3	8	3
M	0.6	1.7	2.4	5	9	5
A	-	-	-	-	-	-
M	-	-	-	-	-	-
J	0.4	0.7	1.0	7	7	6
J	0.2	0.4	0.4	5	5	3
A	0.3	0.4	0.5	7	5	3
S	0.2	0.4	0.4	4	5	2

Calculations reveal UDA-N concentrations in the Checkley streams to be well below that which reduced growth rate by 50% in the laboratory. In fact the highest concentration of 9 μg l^{-1} UDA-N (occurring in stream B(25) in March) is actually below that in the natural Langley water in the laboratory study (0.2 mg l^{-1} TA-N; 10 μg l^{-1} UDA-N). It is therefore suggested that levels of ammonia found in the Checkley streams would have little or no toxic effect if the toxic species were UDA-N, whereas if TA-N were all toxic *Cladophora* growth rate in streams B(25) and C(50) may well be expected to be reduced. Since it is clear that *Cladophora* growth rate (as indicated by standing crop) is significantly higher in streams B(25) and C(50) than in stream A(0) it therefore seems probable that UDA-N is the form of ammonia toxic to the alga. If this supposition is correct ammonium toxicity would therefore be favoured by high pH and high temperature and under conditions of more gross pollution such toxicity may well explain, in part at least, the drop in *Cladophora* standing crops during the summer months.

Laboratory studies showed that NO_2-N concentration, over the range 0.077-1.057 mg l^{-1} had no significant effect on *Cladophora* specific growth rate ($P>0.05$). It is unlikely that NO_2-N concentrations occur in the field situation much in excess of the range considered, and that NO_2-N concentration is an important factor influencing the growth of tha alga. However, high nitrite concentrations may often indicate the presence of a toxic agent, or agents, inhibiting the activity of the organisms responsible for the second stage of nitrification (i.e. the oxidation of nitrite to nitrate) and such agents may also be capable of toxic activity towards *Cladophora*. In the field situation, therefore, sites with high NO_2-N concentration may well have poor *Cladophora* growth, not attributable to the NO_2-N concentration itself.

NO_3-N between 7.2 and 15.2 mg l^{-1} was found to have no significant effect on *Cladophora* growth in culture, as was the nitrate/phosphate interaction ($P>0.05$). High PO_4-P concentrations were shown to be toxic, such that specific growth rate at 4.9 mgP l^{-1} was only 48% of that at 1.9 mgP l^{-1} . Available literature, however, suggests

that PO_4-P concentrations in excess of 2 mg l^{-1} rarely occur at sites other than those which are grossly polluted. Laboratory studies showed *Cladophora* specific growth rate to still be near optimal at 0.0 mgP l^{-1}. However, since the precision of PO_4-P estimation was only to the nearest 0.05 mg l^{-1} this lowest treatment level could actually have been as high as 0.097 mg l^{-1} . Such a value is well above the critical concentrations estimated by Herbst (1969) - 0.03 mgP l^{-1} - or Wong and Clark (1973) - 0.06 mgP l^{-1} . If then, the critical PO_4-P concentration, in water, is so low the question must be asked, why is *Cladophora* growth so poor in Checkley stream A(0) when the mean monthly PO_4-P concentration is between 0.5-0.7 mg l^{-1}? Admittedly the PO_4-P concentration in streams B(25) and C(50) is higher (mean monthly PO_4-P , 1.9-3.1 mg l^{-1} in stream B(25), 3.0-3.8 mg l^{-1} in stream C(50)) but PO_4-P concentration in all streams is higher than the estimates of critical concentration from laboratory culture work. The suggestion of Pitcairn and Hawkes (1973) that an interaction exists between nitrate and phosphate in their ability to stimulate *Cladophora* growth, complicates matters further in that they found the highest level of NO_3-N (7.7 mg l^{-1}) to enhance growth at the lowest PO_4-P level (0.5 mg l^{-1}), but to reduce growth at higher PO_4-P levels. In this study 7.7 mg NO_3-N l^{-1} was within the mean ranges of streams B(25) and C(50), and in these streams PO_4-P concentrations were high, yet *Cladophora* growth (as indicated by standing crop) was significantly higher in these two streams than in A(0). The situation is thus obviously highly complex and will be further discussed later in the chapter.

Laboratory studies showed that 0.032 mgCu l^{-1} , 0.070 mgZn l^{-1} and 1.03 mgPb l^{-1} reduced growth rate to 50% of that which occurred in unsupplemented Langley water. The effects of complexation, etc. in reducing heavy metal toxicity have already been discussed, but results from Checkley further illustrate this phenomenon. Mean monthly concentrations of both total copper and zinc in streams B(25) and C(50) would be expected to have highly toxic effects on *Cladophora* growth if all metal was in the toxic form.

Regarding the uptake of metals by *Cladophora*, it has already been suggested that much of the variation in concentration factors, in culture, occurred as a result of cell damage/death. Consideration of the field situation, however, indicates that since similar concentra-

tions of metals are likely to be less toxic in the field than in the laboratory, and since *Cladophora* is only likely to be found at sites where metal concentrations are non-toxic, then concentration factors may be expected to be more stable than suggested by the results of this study, thereby enabling *Cladophora* to be used as an effective biological monitor as suggested by Keeney *et al.* (1976).

A review of the literature reveals that continuous-flow culture techniques have rarely been used for the culture of filamentous algae. Work of Prance (1973), Prance and Benson-Evans (1973), Harvey and Patrick (1967) and Sikes (1976, 1978) all, however, suggest that the growth and metabolism of filamentous algae in continuous-flow culture differs from that in batch culture. Undoubtedly much of this difference may simply be attributable to the lack of nutrient depletion which inevitably occurs in batch culture, and this study has hopefully highlighted the differences in growth kinetics which occur in batch and closed continuous culture processes. One further factor capable of producing differences between the two culture systems is that of flow. The effect of flow (or current) on the metabolism of filamentous algae has been investigated by Whitford (1960) and Whitford and Schumacher (1961, 1964) who found that all species of Chlorophyceae studied showed higher rates of respiration and ^{32}P uptake in a current. Lotic isolates were found to demonstrate a greater response to current and Whitford and Schumacher (1964) suggested this to indicate a higher metabolic rate of lotic isolates as compared to lentic isolates. Results thus generally supported the hypothesis proposed by Whitford (1960) that a current produces a steep diffusion gradient thereby increasing the exchange of materials between an attached current-inhabiting species and the water. Most of the continuous-flow culture techniques, however, involve little real 'flow' of medium. Harvey and Partrick (1967) adopted a 15 ml hr^{-1} feed rate to a 125 ml sidearm filter flask, Prance and Benson-Evans (1973) maintained a 50 ml hr^{-1} feed rate to a 50 ml culture channel and Sikes (1978) used a 250 ml hr^{-1} feed rate and a 3 l culture. Assuming no mixing, retention times in these systems would therefore be 8.3 hours (Harvey and Patrick, 1967), 1 hour (Prance and Benson-Evans, 1973) and 12 hours (Sikes, 1978). Flow would therefore be only slight. In the technique described in this system, however, the dripping of medium over the alga

results in feed rate being a direct estimate of flow rate. Therefore, without using very high feed rates (and concomitantly large volumes of medium) the technique described in this work allows investigation of the effects of flow rate on algal growth ; work already done along these lines (see section 6.7) suggests flow rate to be an important factor affecting *Cladophora* growth. Further, consideration of the kinetics of growth in closed continuous culture suggest that incremental growth succeeds exponential growth, and if such a phase of growth also occurs in the field situation, as seems likely, then flow rate would be expected to be directly proportional to growth rate if nutrient concentrations remained unaltered. The relationship between river flow and nutrient concentration is, however, a complex one that will vary according to the precise conditions at a particular site and time. Water flow may also be an important factor affecting the seasonal growth of *Cladophora*. If it is accepted that *Cladophora* favours sites where water flow is great, such as riffles, we may then consider the growth of the alga in spring/early summer to continue until the growths seriously affect the amenity value of the river and even (as noted by Pitcairn and Hawkes, 1973) affect drainage by impeding the flow. It is likely therefore that the alga would itself make conditions less suitable for its further growth. In dense growths only the cells at the periphery of the algal 'mat' may receive a flow rate fast enough to allow rapid diffusion of nutrients and waste products so as to allow further growth, whilst cells in the middle, or at the bottom, of such dense growths may be in an environment of slow flow and reduced light intensity. The subsequent death and detachment of filaments of such cells may well explain the reduction in standing crops of *Cladophora* and other macro-algae in the summer months, as noted in this and other studies.

Returning now, to the problem of why *Cladophora* growth is so poor in Checkley channel A(O) where mean monthly PO_4-P concentration is between 0.2 and 0.6 mg l^{-1}, when studies suggest the critical PO_4-P concentration, in water, to be within the range 30-60 µg l^{-1} ; two main hypotheses may be suggested. The first is simply that PO_4-P was not the limiting factor in the Checkley riffles. No evidence is available, however, to suggest an alternative limiting factor, though the literature indicates that various vitamins are necessary for

optimum growth (e.g. thiamine; Moore and McLarty, 1974). The alternative hypothesis makes the assumption that PO_4-P is the limiting factor but suggests that discrepancies between laboratory and field findings occur as a result of differences in the ambient conditions of the two situations. In this context flow itself may be worth considering. If the flow rate (relative to the algal biomass) was greater in laboratory studies than in the field situation differences in diffusion gradients may necessitate higher water PO_4-P concentrations in the field situation (than in the laboratory) so as to obtain the critical PO_4-P concentration in the cells. Even so this hypothesis does not fully explain the finding of Wong and Clark (1976) who correlated 'community metabolism' with ambient PO_4-P concentration *in situ* and still obtained a critical concentration of 60 µg PO_4-P l^{-1}. Results presented in this study are unfortunately unable to prove or disprove either of these two main hypothyses. Whichever hypothesis is correct results of the field study at Checkley are in good agreement with those of Pitcairn and Hawkes (1973) who suggested that rivers with a concentration of less than 1.0 mgP l^{-1} usually only contain modest growths of *Cladophora* . An understanding of the discrepancies of field and laboratory estimates of critical nutrient concentrations may therefore be crucial in the understanding of the ecology of the alga.

To conclude, the author acknowledges the suggestion of Westlake (1981) that perhaps the most noteworthy aspect of the biology of *Cladophora* is its unpredictability and the consequent widespread disagreements about many features of its behaviour. It is, however, hoped that results presented in this report, and attempts to rationalize findings with those of other authors, has helped establish some degree of cosmos, rather than simply add to the chaos. Moreover, it is hoped that some of the techniques developed may prove useful in further studies of the biology of *Cladophora* and thereby lead to a fuller understanding of the factors affecting the growth of the alga in relation to pollution.

9. GENERAL CONCLUSIONS

1. Field studies using the simulated streams at Checkley Applied Hydrobiology Research Station, Checkley, Staffordshire revealed a 'clean water' riffle community dominated by *Vaucheria* to be replaced, upon effluent addition, by a 'polluted water' community comprising *Cladophora*, *Oedogonium*, *Elodea* and *Amblystegium*.

2. *Cladophora* biomass exhibited an overall significant increase upon effluent addition. No significant overall trend in the comparison of the streams receiving 25% and 50% effluent was detected.

3. Factors responsible for the spatial differences in *Cladophora* biomass remain unclear, although the literature suggests phosphorus to be intimately involved. Mean PO_4-P concentrations over the course of the study were 0.6 mgP l^{-1} for stream A(0), 2.5 mgP l^{-1} for stream B(25), and 3.5 mgP l^{-1} for stream C(50).

4. Maximum mean monthly temperatures ($<15^{o}C$) were considered too low to account for the reduction in *Cladophora* biomass in the summer months, even taking into account the effects of diurnal temperature fluctuation.

5. In batch culture investigations, regular replacement of growth medium served to significantly increase yield of *Cladophora* over the 20 day experimental duration ($P<0.001$).

6. Over a similar experimental duration the pH of the medium used was found to drift from 8.0 ± 0.1 to 9.2-10.2. It is suggested that this drift in pH results in limitation of growth in batch culture.

7. Batch culture techniques were, however, used successfully to assess the acute toxicity of various herbicides to *Cladophora*. Results demonstrated that of the herbicides cleared for use in, or near, waterways under the Pesticides Safety Precaution Schemes only diquat (as Reglone 40) and terbutryne (as Clarosan) possess significant algicidal activity towards *Cladophora*.

8. Results of preliminary experiments indicated that the continuous culture technique devised for fungi by Hawkes (1965) and developed by Williams (1971) allowed good growth of *Cladophora* under conditions of continuous nutrient supply and waste removal. Using an appropriate wet weight determination, significantly correlated with dry weight ($P<0.001$), growth of the alga could be followed throughout the course of the experimental duration.

9. Appropriate indices of growth were chosen so as to allow comparison of the growth of variously treated cultures - these were an estimate of μ (the specific growth rate) and an estimate of k (the incremental growth rate).

10. In closed continuous culture, *Cladophora* specific growth rate increased linearly with increasing duration of light per day up to 24 hours.

11. *Cladophora* specific growth rate increased linearly with increasing light intensity up to 6000 lux - the highest intensity tested.

12. Optimum specific growth rate occurred at, or near, $20^{\circ}C$; whilst at 5 and $30^{\circ}C$ growth was barely detectable.

13. Laboratory studies showed ammonia to be highly toxic to *Cladophora* : 3.6 mg l^{-1} total ammoniacal nitrogen (TA-N) (= 185 µg l^{-1} undissociated ammoniacal nitrogen (UDA-N)) was shown to reduce growth rate to 50% of that at 0.2 mg l^{-1} TA-N (= 10µg l^{-1} UDA-N). Consideration of the *Cladophora* growth response in the Checkley streams supports the hypothesis that UDA-N is the form of ammonia toxic to *Cladophora* .

14. NO_2-N, over the range 0.077-1.057 mg l^{-1}, had no significant effect on *Cladophora* growth rate in laboratory continuous culture ($P>0.05$).

15. NO_3-N, over the range 7.2-15.2 mg l^{-1}, has no significant effect on *Cladophora* growth rate in laboratory continuous culture ($P>0.05$). Neither was any NO_3-N / PO_4-P interaction found to be significant ($P>0.05$).

16. High PO_4-P concentrations were shown to be toxic such that *Cladophora* specific growth rate at 4.9 mgP l^{-1} was only 48% of that at 1.9 mgP l^{-1}. Results indicated that the critical PO_4-P concentration (i.e. the lowest concentration to allow maximum growth rate) to be <0.1 mgP l^{-1}.

17. 0.036 mgCu l^{-1}, 0.070 mgZn l^{-1} and 1.03 mgPb l^{-1} were found to reduce *Cladophora* specific growth rate to 50% of that occurring in unsupplemented natural water.

18. Toxicity curves for copper and zinc suggest that metals already present in the unsupplemented natural water may be non-toxic, or significantly less toxic, than metals added as $CuSO_4.5H_2O$ or $ZnSO_4$. Results from the Checkley streams similarly indicate that a large proportion of total or filterable metal in the natural water is in a form, or forms, relatively non-toxic to the alga.

19. In laboratory studies, concentration factors for copper and zinc increased as the concentration of the metal in solution increased; this is considered attributable to physiological changes occurring within the cells at, or near, death. In the case of lead, ignoring the first treatment level, the concentration factor first falls before rising.

20. The discrepancy in the concentration factor at the lowest lead treatment level is interpreted as indicative of the lower availability of lead already in solution in the natural water than that added as $PbCl_2$. This suggestion is in agreement with the lower toxicity of metals already in natural waters (in comparison to those added) as suggested in conclusion 14.

APPENDICES

Appendix 1a — Checkley physico-chemical data for period January-September 1980.

1b — Raw biologival data for Checkley streams, January-September 1980.

2 — BASIC computer programs

3 — Analysis of variance (AOV) summary tables for biomass data of taxa present in the Checkley streams.

APPENDIX 1a

Checkley physico-chemical data for period January - September 1980.

LEGEND

$\bar{x}$	=	means value
95%CL±	=	95% confidence interval
Max	=	monthly maximum value
Min	=	monthly minimum value
n	=	number of values

Physico-chemical parameter	Month	STREAM														
		A(0)					B(25)					C(50)				
		$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n
Temperature (^{o}C)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	5.0	0.3	5.9	4.3	12	6.2	0.4	7.0	5.4	12	6.1	0.5	7.8	5.3	12
	M	4.3	0.3	5.7	3.0	30	5.2	0.3	7.0	3.9	30	5.3	0.3	6.7	3.8	30
	A	9.6	0.8	12.2	7.2	20	10.1	0.8	12.7	7.6	20	10.9	0.9	13.6	8.2	20
	M	7.4	0.5	10.1	5.5	29	8.3	0.5	10.7	6.5	29	8.6	0.5	11.0	6.4	29
	J	10.9	0.4	11.5	10.0	7	11.7	0.3	12.2	11.2	7	13.7	1.0	16.4	10.5	17
	J	11.8	0.6	14.5	10.0	19	12.9	0.6	16.0	11.5	20	13.2	0.6	16.0	10.8	21
	A	13.4	0.6	15.0	10.5	16	14.2	0.6	15.8	11.5	16	14.6	0.6	15.8	11.5	16
	S	11.1	1.4	12.5	10.0	5	11.9	1.5	13.0	10.5	5	12.5	1.9	14.0	11.0	5
Suspended solids (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	29	210	45	12	2	12	45	15	8	2	13	10	22	8	4
	M	14	7	50	4	18	16	6	49	1	18	17	6	4	3	17
	A	-	-	-	-	0	16	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	24	6	52	2	19	23	5	51	7	20	26	6	54	3	18
	J	6	3	33	0	25	9	4	53	0	28	9	3	30	0	26
	A	27	15	175	0	27	17	9	107	0	25	22	10	95	0	25
	S	10	8	98	0	25	7	5	15	1	7	8	6	14	1	7
Dissolved oxygen (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	10.4	0.2	11.0	9.8	12	11.8	0.5	12.1	9.7	12	7.6	0.5	8.6	6.1	12
	M	11.0	0.4	12.5	8.7	30	10.3	0.4	12.0	7.8	30	8.2	0.4	10.5	6.4	30
	A	12.9	0.8	16.0	10.0	20	11.3	0.8	14.6	8.7	20	9.7	0.6	12.1	7.6	20
	M	11.3	0.3	12.7	9.6	29	10.1	0.3	12.5	9.0	29	8.7	0.5	10.5	5 5	29
	J	11.1	0.7	14.0	8.6	18	10.5	0.7	13.8	8.0	18	9.0	0.6	10.6	6.4	18
	J	10.9	0.6	13.2	8.4	21	10.2	0.5	14.0	7.8	27	9.5	0.5	11.7	7.0	27
	A	11.8	0.5	14.1	9.2	29	11.0	0.5	12.9	8.7	30	9.5	0.6	12.8	7.0	29
	S	10.9	1.8	13.1	8.6	6	9.2	0.9	10.6	7.6	9	8.1	1.0	9.5	5.7	9
Chloride (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	50	10	75	38	8	68	11	80	42	8	80	20	116	45	8
	M	46	6	47	43	3	82	5	84	80	3	88	25	90	86	2
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	S	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
Conductivity (μS)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	423	65	500	270	8	559	66	650	370	9	649	100	820	400	9
	M	418	22	500	310	22	550	36	690	400	22	612	31	738	470	22
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	370	381	400	340	2	550	1016	630	470	2	575	762	635	515	2
	J	502	15	565	460	16	581	22	640	510	16	635	35	715	505	15
	J	513	16	560	408	18	618	26	680	465	18	655	30	730	485	18
	A	511	19	555	410	16	589	23	635	460	16	666	32	730	495	16
	S	536	32	580	470	8	619	23	640	595	5	702	44	760	665	5
pH	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	7.85	0.08	7.98	7.6	12	7.56	0.11	7.8	7.3	12	7.2	0.09	7.4	6.9	12
	M	7.85	0.06	7.97	7.55	26	7.62	0.07	7.8	7.2	30	7.23	0.05	7.6	7.05	30
	A	8.18	0.06	8.3	7.8	20	8.04	0.10	8.2	7.5	20	7.57	0.10	7.8	7.2	20
	M	7.85	0.05	8.1	7.68	29	7.59	0.07	7.85	7.2	29	7.36	0.07	7.75	7.05	29
	J	7.93	0.04	8.05	7.7	20	7.7	0.04	7.85	7.45	20	7.42	0.06	7.6	7.1	20
	J	8.12	0.04	8.4	7.85	31	7.76	0.05	8.05	7.5	30	7.45	0.08	7.8	7.0	30
	A	8.02	0.04	8.15	7.8	31	7.72	0.04	7.95	7.5	31	7.29	0.07	7.6	6.9	31
	S	7.95	0.06	8.2	7.5	28	7.73	0.08	7.85	7.5	9	7.27	0.13	7.45	7.0	9

Physico-chemical parameter	Month	STREAM														
		A(0)					B(25)					C(50)				
		$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n
Total Alkalinity ($\mu s\ mgl^{-1}$ $CaCO_3$)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	117	19	150	95	8	140	23	180	90	8	153	21	190	120	9
	M	127	7	150	90	23	149	10	180	97	22	155	10	190	108	21
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	139	6	160	105	19	141	5	155	115	20	143	7	163	112	18
	J	156	5	170	125	24	158	5	170	118	20	158	5	178	121	26
	A	141	8	170	100	26	151	6	165	115	24	150	7	168	110	25
	S	145	5	165	125	23	145	4	150	137	7	149	7	156	134	7
Total Hardness as mgl^{-1} $CaCO_3$	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	171	70	270	64	8	180	87	300	60	8	213	92	390	58	8
	M	226	9	260	174	23	249	13	296	180	22	257	14	308	191	21
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	241	6	270	208	20	240	6	260	212	20	244	9	296	220	18
	J	249	8	270	200	28	257	6	276	214	28	261	5	290	218	27
	A	231	8	260	190	27	238	7	262	196	25	242	7	268	196	28
	S	241	7	282	214	23	252	10	262	236	7	254	10	268	236	7
NH_3-N (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	0.4	0.5	1.2	0	6	1.6	0.9	3.6	0.56	7	1.5	0.7	2.65	0.82	6
	M	0.6	0.2	0.9	0.3	7	1.7	0.7	2.95	0.92	7	2.4	0.9	4.7	1.3	7
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	0.4	0.1	1.2	0.2	18	0.7	0.1	1.2	0.4	18	1.0	0.2	1.6	0.4	16
	J	0.2	0.0	0.3	0.1	23	0.4	0.1	0.8	0.2	27	0.4	0.1	0.9	0.2	25
	A	0.3	0.1	0.8	0.1	27	0.4	0.1	0.8	0.2	24	0.5	0.1	1.0	0.2	23
	S	0.2	0.1	0.3	0.1	7	0.4	0.2	0.7	0.1	7	0.4	0.2	0.8	0.1	7
NO_3-N (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	2.85	1.99	3.7	0	5	6.00	0.89	6.8	4.43	5	6.84	0.91	7.44	5.61	5
	M	3.65	0.51	4.45	3.05	7	5.54	1.25	7.05	4.03	7	6.54	1.09	8.07	5.06	7
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	3.95	0.22	4.8	3.0	18	7.62	0.44	9.55	6.13	19	8.84	1.04	11.6	3.6	18
	J	3.8	0.25	6.4	3.2	24	8.54	0.42	13.0	6.6	29	10.1	0.76	18.6	8.0	27
	A	3.61	0.14	4.6	3.0	27	6.81	0.30	8.4	5.4	24	9.33	0.41	11.2	7.0	23
	S	3.86	0.18	4.2	3.6	7	6.34	0.56	7.2	5.6	7	10.54	3.91	20.0	8.0	7
NO_2-N (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	0	0	0	0	4	0	0	0	0	4	0	0	0	0	4
	M	.002	.003	.005	0	6	.007	.011	.008	.007	2	.003	.006	.010	0	5
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	.012	.004	.030	.005	18	.026	.010	.090	0	19	.062	.039	.325	0	19
	J	.003	.006	.015	0	6	.003	.003	.005	0	6	.002	.003	.005	0	5
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	S	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0

Physico-chemical parameter	Month	STREAM														
		A(0)					B(25)					C(50)				
		$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n
PO_4-P (mgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	0.5	0.2	1.1	0	17	2.2	0.4	3.2	1.1	18	3.0	0.5	4.7	0.6	15
	J	0.7	0.3	2.3	0	22	3.1	0.3	4.0	0	27	3.8	0.2	4.9	2.8	24
	A	0.5	0.1	1.2	0	27	2.2	0.2	3.6	1.2	25	3.4	0.3	4.5	2.2	24
	S	0.6	0.3	1.0	0	7	1.9	0.3	2.2	1.4	7	3.5	0.6	4.5	2.7	7
Total Cd (μgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	3	0.6	4	2	10	7	1.1	9	5	9	9	1.6	14	6	12
	M	1.4	0.2	2	1	22	56	0.7	8.8	2.6	21	6.7	0.7	10	4	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	5.9	5.8	54	0.9	19	4.7	0.6	8	3.1	20	6.7	1.1	13	4.2	17
	J	1.6	0.2	2.5	1	24	3.5	0.3	5.5	2.5	27	4.0	0.3	6	3	26
	A	1.6	0.3	3.8	0.5	30	3.2	0.3	6	2	28	4.1	0.3	6.2	3	27
	S	1.3	0.2	2.6	0.7	23	2.0	0.4	2.5	1.6	5	3.1	0.7	4	2.5	5
Total Cr (μgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	3.3	0.8	5	2	10	25.7	12.7	52	8	9	36.1	20.8	111	14	12
	M	3.3	0.5	5	1	22	15.6	3.8	37	5	21	28.3	8.4	76	7	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	2.1	0.3	3	1	19	14.9	5.0	46	6	19	23.1	10.6	85	10	17
	J	2.5	0.3	4	1	24	11.3	3.2	32	4	27	14.2	4.4	45	4	26
	A	3.4	0.3	5	1.5	31	6.1	0.7	13	4	30	9.6	2.5	39	4.5	28
	S	2.0	0.2	3.3	1.2	22	3.2	0.6	3.8	3	4	6	1.3	7	5	4
Total Cu (μgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	6.2	1.4	9	4	9	17.2	3.8	25	10	9	23.3	3.4	32	16	12
	M	4.1	1.1	11	1	22	13.8	2.6	30	5	21	17.9	2.4	33	12	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	7.1	1.7	16	3	19	15.9	1.9	28	11	19	24.7	3.3	36	15	17
	J	2.8	0.9	8.5	0	26	11.2	0.9	16	6	27	13.6	1.2	20	9	26
	A	5.7	2.2	2	0	31	10.3	1.3	18.5	4	29	15.3	1.7	29	24	28
	S	3.9	1.3	13.5	0.5	23	11	2.2	14	10	5	15.4	4.0	20	12	5
Total Pb (μgl^{-1})	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	13	5	27	5	10	37	12	57	20	9	58	12	100	35	11
	M	25	3	35	15	22	55	11	130	30	21	73	13	148	41	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	23	3	38	13	18	45	5	68	31	19	84	17	140	30	17
	J	17	1	22	12	26	33	3	55	20	27	37	5	68	21	26
	A	22	4	58	6	31	35	4	58	20	29	51	6	100	22	28
	S	16	2	35	11	23	23	7	29	16	5	35	12	45	21	5

Physico-chemical paramter	Month	STREAM														
		A(0)					B(25)					C(50)				
		$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n	$\bar{x}$	95% CL ±	Max	Min	n
Total Ni ($\mu g l^{-1}$)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	10	1	13	7	10	12	2	15	8	9	15	2	19	10	11
	M	10	1	16	5	22	13	1	20	9	21	14	1	20	10	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	11	2	20	6	19	12	1	20	7	20	15	1	22	11	17
	J	9	1	20	5	25	11	1	17	7	26	11	1	17	5	25
	A	18	4	48	3	31	20	6	7.5	8	29	20	6	80	8	28
	S	15	5	71	7	23	9	5	14	5	5	11	3	15	10	5
Total Zn ($\mu g l^{-1}$)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	36	7	48	22	10	107	16	130	88	9	158	15	190	130	12
	M	30	5	58	15	22	100	10	162	66	21	130	9	182	100	20
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	31	8	75	5	19	77	12	158	51	20	131	15	190	82	17
	J	5	4	52	0	26	47	4	70	30	27	62	8	130	32	26
	A	24	16	198	0	31	58	13	176	24	29	99	14	193	44	28
	S	8	7	78	0	23	38	5	40	31	5	78	11	92	69	5
Total Radiation (arbitrary units)	J	-	-	-	-	0										
	F	30	13	60	5	12										
	M	61	16	186	8	29										
	A	178	30	246	57	18										
	M	120	18	211	49	30										
	J	178	39	348	33	20										
	J	120	21	234	35	31										
	A	108	18	200	12	31										
	S	114	19	241	30	29										
Maximum radiation (arbitrary units)	J	-	-	-	-	0										
	F	6	2	11	1	12										
	M	10	2	16	2	29										
	A	14	1	17	7	19										
	M	13	1	17	7	30										
	J	21	4	35	4	20										
	J	14	2	22	5	31										
	A	14	1	19	3	31										
	S	17	2	34	6	30										
Free CO_2 ($mg l^{-1}$)	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	F	4	6	4	3	2	7	10	8	6	2	20	19	21	18	2
	M	4	0	4	4	2	9	13	10	8	2	31	13	40	21	2
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	M	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	J	4	0	4	4	6	5	1	6	4	6	10	3	19	5	9
	J	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	A	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
	S	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0

APPENDIX 1b

Raw biological data for Checkley streams,
January - September 1980.

Stream A

Sample taken

29/JAN/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.2541	0.1613		P									100					P								40			
1ii	0.4979	0.3035											100													76			
1iii	0.8342	0.5046											100													126			
2i	0.9492	0.6847											100													171			
2ii	0.7259	0.4387		P									100					P								110			
2iii	0.7597	0.4890											100													122			
3i	1.0038	0.5633											100													141			
3ii	0.7059	0.4308											100													108			
3iii	0.3448	0.1452											100													36			
LOWER RIFFLE																													
1i																													
1ii																													
1iii																													
2i																													
2ii																													
2iii																													
3i																													
3ii				P														P											
3iii													P													P			
UPPER POOL																													
1i	0.0683	0.0115	30	70									P				1	2								P			
1ii	0.4323	0.2538	P	P									100				P	P								63			
1iii	0.2136	0.0668	40	60													7	10											
2i	0.2703	0.0668	20	80													3	13											
2ii	0.1558	0.0809	100														20												
2iii	0.3197	0.0826	30	70													6	14											
3i	0.5963	0.3467											100													87			
3ii			P	P													P	P											
3iii	0.1748	0.0595											100													15			
UPPER RIFFLE																													
1i			P														P												
1ii			P										P				P									P			
1iii																													
2i	0.0557	0.0234	100														6												
2ii																													
2iii			P														P												
3i																													
3ii			P														P												
3iii																													

Stream A

Sample taken

26/FEB/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL A			Cladophora	Vaucheria	Stigeoclonium	Oedogonium	Microspora	Spirogyra	Batrachospermum	Rhodochorton	Amblystegium	Elodea	Potamogeton	Callitriche	Ranunculus	Other Macrophytes	Cladophora	Vaucheria	Stigeoclonium	Oedogonium	Microspora	Spirogyra	Batrachospermum	Rhodochorton	Amblystegium	Elodea	Potamogeton	Callitriche	Ranunculus	Other Macrophytes
LOWER POOL																														
1i	1.1857	0.5312											100														133			
1ii	1.1030	0.4936											100														123			
1iii	0.6174	0.3002											100														75			
2i	0.1447	0.0808									P		P			100									P		P			20
2ii	1.0091	0.4913		10									90					12									111			
2iii	0.5433	0.2167		100									P					54									P			
3i	0.3070	0.1838											100														46			
3ii	0.3663	0.1666											100														42			
3iii	0.2416	0.1531											100														38			
LOWER RIFFLE																														
1i				P														P												
1ii				P												P		P												P
1iii				P														P												
2i																														
2ii				P							P					P		P							P					P
2iii				P														P												
3i			P														P												
3ii																														
3iii											P					P									P					P
UPPER POOL																														
1i	0.5284	0.4025											100														101			
1ii	1.7620	1.3112											100														328			
1iii	1.0831	0.6502											100														163			
2i	0.8619	0.5624											100														141			
2ii	1.0854	0.6048											100														151			
2iii	0.8796	0.3750											100														94			
3i													P														P			
3ii													P														P			
3iii			P														P													
UPPER RIFFLE																														
1i				P														P												
1ii	0.0169	0.0019		100														P												
1iii	0.0221	0.0028		100									P			P		1									P			P
2i	0.0572	0.0071	P	100													P	2												
2ii																P														P
2iii											P														P					
3i				P														P												
3ii	0.0503	0.0063		100														2												
3iii																														

Stream A

Sample taken

25/MAR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblyst-egium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	1.9115	0.9809											100													245			
1ii	2.0293	1.2427											100													311			
1iii	1.1708	0.6935		P									100					P								173			
2i	1.8571	0.7826											100													196			
2ii	1.5524	0.7403											100													185			
2iii	1.6865	1.0614											100													265			
3i	0.9217	0.6797														100													170
3ii	0.2143	0.0798														100													20
3iii	1.1855	0.7012									P		100			P								P		175			P
LOWER RIFFLE																													
1i											P													P					
1ii																P													P
1iii																													
2i				P														P											
2ii				P														P											
2iii				P														P											
3i			P	P													P	P											
3ii			P	P													P	P											
3iii																													
UPPER POOL																													
1i	0.2892	0.1759			P								100						P							44			
1ii	0.8544	0.4877											100													122			
1iii	1.0834	0.5418											95			5										129			7
2i	1.2373	0.6783											100													170			
2ii	0.8578	0.3722											100													93			
2iii	1.0573	0.5255											100													131			
3i	0.6795	0.4140											95			5										98			5
3ii	0.6124	0.3736											100													93			
3iii	0.2384	0.1767														100													44
UPPER RIFFLE																													
1i			P								P					P		P						P					P
1ii			P													P		P											P
1iii			P								P					P		P						P					P
2i	0.1797	0.0284	100													P		7											P
2ii																													
2iii			P															P											
3i	0.0361	0.0066	100													P		2											P
3ii	0.0273	0.0051	100															1											
3iii			P															P											

Stream A

Sample taken

22/APR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)													
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	1.8154	0.8120											100														203			
1ii	2.3608	1.2105											100														303			
1iii	0.4831	0.2639											90			10											59			7
2i	1.0817	0.5900											100														148			
2ii	1.5351	0.6928											100														173			
2iii	0.4199	0.1658											100														41			
3i	0.3867	0.2482											100														62			
3ii	0.4128	0.2591											90			10											58			6
3iii	0.6808	0.2932		5									95					4									70			
LOWER RIFFLE																														
1i																P														P
1ii				P												P		P												P
1iii			P	P												P	P	P												P
2i				P												P		P												P
2ii				P												P		P												P
2iii																P														P
3i																P														P
3ii				P														P												
3iii																P														P
UPPER POOL																														
1i	0.5793	0.3710											100														93			
1ii	0.2096	0.1617											90			10											36			4
1iii	0.2327	0.1333											100														33			
2i	0.6352	0.3636											100														91			
2ii	0.8921	0.6765											100														169			
2iii	0.4567	0.3491											100														87			
3i	1.5899	1.1023											100														276			
3ii	1.0360	0.7870											100														197			
3iii	0.6903	0.4840											100														121			
UPPER RIFFLE																														
1i	0.4132	0.0424		100														11												
1ii	0.6758	0.4193	5	75									20				5	79									21			
1iii	0.5331	0.3272		20									80					16									65			
2i	0.0680	0.0518		100														13												
2ii			P	P													P	P												
2iii				P									P					P									P			
3i				P														P												
3ii													P														P			
3iii			P	P									P				P	P									P			

Stream A

Sample taken

26/MAY/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	1.0665	0.6825											100														171			
1ii	0.9591	0.6232											100														156			
1iii	1.5198	0.7696											100														192			
2i	1.0902	0.6682											100														167			
2ii	0.8817	0.6525											100														163			
2iii	0.8614	0.5606											100														140			
3i	0.6083	0.3542											100														89			
3ii	0.0036	0.0031	P						100								P						1							
3iii			P														P													
LOWER RIFFLE																														
1i			P	P													P	P												
1ii			P	P													P	P												
1iii			P				P										P				P									
2i			P														P													
2ii			P							P							P						P							
2iii			P														P													
3i			P	P													P	P												
3ii																														
3iii			P	P													P	P												
UPPER POOL																														
1i	1.2847	0.8527											100														213			
1ii	0.8035	0.5430											100														136			
1iii	2.5201	1.6269											100														407			
2i	0.7658	0.5035											100														126			
2ii	0.8161	0.5048											100														126			
2iii	0.8858	0.6090											100														152			
3i	0.1738	0.1328									10		P			90									3		P			30
3ii				P												P		P												P
3iii	1.7978	0.9626											100														241			
UPPER RIFFLE																														
1i	0.6159	0.1631		100		P												41		P										
1ii	0.8265	0.2098		100														52												
1iii	0.1663	0.0734	50	50		P	P										9	9	P	P										
2i			P								P						P								P					
2ii			P														P													
2iii			P	P													P	P												
3i				P														P												
3ii			P	P													P	P												
3iii			P	P													P	P												

Stream A

Sample taken

23/JUN/1980

SAMPLE	DRY WT (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.0345	0.0279														100													7
1ii	0.1812	0.1383														100													35
1iii	0.2481	0.1877	P										50			50	P									23			23
2i																P													P
2ii	0.1041	0.0853											50			50										11			11
2iii				P									P			P		P								P			P
3i	0.2124	0.1243														100													31
3ii	0.1928	0.1397											100													35			
3iii	0.5370	0.3672											50			50										46			46
LOWER RIFFLE																													
1i			P														P												
1ii			P			P											P			P									
1iii	0.2365	0.2086											100													52			
2i	0.0095	0.0019		P					100									P				P							
2ii			P	P													P	P											
2iii			P								P					P	P							P					P
3i			P	P													P	P											
3ii	0.0076	0.0053	P	100													P	1											
3iii			P	P		P											P	P		P									
UPPER POOL																													
1i	1.1014	0.7140		P									100					P								179			
1ii	0.8712	0.7646		5												95		10								182			
1iii	0.2097	0.1635											100													41			
2i	0.3667	0.2849		10									70			30		7								50			21
2ii	0.3071	0.2533		P												100		P											63
2iii	0.6108	0.4282											100													107			
3i	0.3732	0.1590		100														40											
3ii																P													P
3iii	0.1331	0.1130														100													28
UPPER RIFFLE																													
1i	0.0187	0.0102		100														3											
1ii			P	P		P											P	P		P									
1iii			P	P													P	P											
2i				P														P											
2ii				P		P												P		P									
2iii	0.0064	0.0000							100													P							
3i			P	P		P											P	P		P									
3ii			P	P													P	P											
3iii																													

Stream A

Sample taken

21/JUL/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.3907	0.3159											100													79			
1ii	0.1956	0.1262											100													32			
1iii																													
2i			P			P										P	P			P									P
2ii			P													P	P												P
2iii	0.5558	0.3818											100													95			
3i	0.6126	0.5161											100													129			
3ii	0.0573	0.0311														100													8
3iii	0.1468	0.0985											100													25			
LOWER RIFFLE																													
1i	0.0903	0.0614											100													15			
1ii	0.1103	0.0846											100													21			
1iii	1.1217	0.7151											100			P										179			P
2i																													
2ii																													
2iii			P														P												
3i																													
3ii	0.0661	0.0431											100													11			
3iii																													
UPPER POOL																													
1i	0.6868	0.5355											100													134			
1ii	0.1154	0.1024														100													26
1iii	1.1232	0.6043											100													151			
2i	0.5873	0.4211											100													105			
2ii	0.2202	0.1332														100													33
2iii	2.0848	0.8759											100													219			
3i	0.9072	0.5349														100													134
3ii	0.1658	0.1386											100													35			
3iii	0.5555	0.4534														100													113
UPPER RIFFLE																													
1i			P													P	P												P
1ii			P														P												
1iii																													
2i																													
2ii																													
2iii																													
3i																													
3ii			P													P	P												P
3iii																													

Stream A

Sample taken

11/AUG/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	1.4892	0.7024											100													176			
1ii	0.3322	0.2040											100													51			
1iii	1.6303	0.9388											100													235			
2i	0.4169	0.2473											100													62			
2ii	0.2123	0.1460											100													37			
2iii	1.6197	0.7426											100													186			
3i	0.4066	0.2428											100			P										61			P
3ii	3.8728	1.2107											100													303			
3iii	0.3485	0.2460											100													62			
LOWER RIFFLE																													
1i			P														P												
1ii			P														P												
1iii																P													P
2i																													
2ii			P														P												
2iii																													
3i			P														P												
3ii			P														P												
3iii			P														P												
UPPER POOL																													
1i	0.0172	0.0074											90			10										2			P
1ii	0.3402	0.2090											100													52			
1iii	1.1679	0.7700											100													193			
2i	0.6605	0.3520														100													88
2ii	2.0597	0.8944											100													224			
2iii	0.9288	0.5529											100													138			
3i	0.6988	0.5644														100													141
3ii																P													P
3iii	0.6352	0.5307														100													133
UPPER RIFFLE																													
1i	0.0271	0.0166	100														4												
1ii			P														P												
1iii	0.1507	0.1185	100														30												
2i	0.0489	0.0251	100													P	6												P
2ii	0.0221	0.0158	100														4												
2iii				P														P											
3i	0.0143	0.0087	100														2												
3ii	0.0357	0.0249	100														6												
3iii			P														P												

Stream A

Sample taken

8/SEP/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)												
CHANNEL A			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes		*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	0.4529	0.2566											100														64			
1ii	0.3723	0.2305											100														58			
1iii	0.4617	0.3259											100														81			
2i																P														P
2ii	0.5074	0.2898											100														72			
2iii	0.8214	0.3853											100														96			
3i	2.4574	1.4253											100														356			
3ii	0.7920	0.5516											100														138			
3iii	0.2708	0.1937											100														48			
LOWER RIFFLE																														
1i																														
1ii																														
1iii			P														P													
2i																														
2ii			P														P													
2iii			P							P							P							P						
3i			P							P							P							P						
3ii			P														P													
3iii										P														P						
UPPER POOL																														
1i	1.2310	0.7657											100														191			
1ii	0.7797	0.4357											100														109			
1iii	0.9367	0.6329											100			P											158			P
2i	0.6447	0.3530											100														88			
2ii	2.0427	1.4826											100														371			
2iii	1.2513	0.6091											100														152			
3i																														
3ii																														
3iii																														
UPPER RIFFLE																														
1i			P							P							P							P						
1ii			P							P							P							P						
1iii			P								P						P								P					
2i			P							P							P							P						
2ii			P														P													
2iii						P															P									
3i			P			P			P								P				P			P						
3ii				P						P								P						P						
3iii										P														P						

Stream B

Sample taken

29/JAN/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i																													
1ii	0.5814	0.2021	P									100					P									51				
1iii	0.4855	0.1353	100	P													34	P											
2i																													
2ii			P	P													P	P											
2iii	0.3733	0.1404	100	P													35	P											
3i	0.1110	0.0395	100														10												
3ii																													
3iii	0.0554	0.0400	70													30	7												3
LOWER RIFFLE																													
1i			P														P												
1ii																													
1iii	0.0420	0.0183	100		P												5		P										
2i																													
2ii																													
2iii																													
3i	0.0701	0.0119	100		P												3		P										
3ii	0.0393	0.0070	100		P												2		P										
3iii			P														P												
UPPER POOL																													
1i	0.4783	0.2561	P										100				P									64			
1ii	0.2872	0.1479											100													37			
1iii	0.9237	0.4372											100													109			
2i	1.3670	0.5674											100													142			
2ii	1.2888	0.5838											100													146			
2iii	0.9620	0.4171											100													104			
3i	0.9062	0.4122									P		100												P	103			
3ii	1.2391	0.7132											100													178			
3iii	1.2061	0.7015											100													175			
UPPER RIFFLE																													
1i			P	P							P						P	P							P				
1ii				P														P											
1iii				P														P											
2i				P														P											
2ii				P														P											
2iii				P														P											
3i			P														P												
3ii	0.0218	0.0046	P	100													P	1											
3iii	0.0747	0.0037		100														1											

Stream B

Sample taken

26/FEB/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.0256	0.0140	10								P					90	P							P					3
1ii	0.1202	0.0416	50								P					50	5							P					5
1iii	0.3738	0.1341	5													95	2												32
2i	0.6654	0.3652	P										100				P									91			
2ii	1.2674	0.2662	100								P					P	67							P					P
2iii	0.6815	0.1253	95													5	30												2
3i	0.4919	0.0748	100														19												
3ii	0.2873	0.0687	100														17												
3iii	0.3176	0.0941	80													20	19												5
LOWER RIFFLE																													
1i	0.2370	0.0350	75								P					25	7							P					2
1ii	0.1154	0.0318	40													60	3												5
1iii	0.0182	0.0116	5													95	P												3
2i																													
2ii			P														P												
2iii			P													P	P												P
3i			P													P	P												P
3ii	0.0449	0.0183	100														5												
3iii			P													P	P												P
UPPER POOL																													
1i																P													P
1ii	0.0872	0.0412														100													10
1iii																													
2i			P								P						P							P					
2ii	0.0606	0.0210	5								25					70	P							1					4
2iii			P								P					P	P							P					P
3i			P													P	P												P
3ii	1.2174	0.3811	100								P					P	95							P					P
3iii	0.4284	0.0591	100								P						15							P					
UPPER RIFFLE																													
1i	0.2460	0.0620	10								90					P	2							14					P
1ii			P								P						P							P					
1iii			P														P												
2i	0.0110	0.0032	10								90						P							1					
2ii	0.1610	0.0159	P								100						P							4					
2iii	0.1975	0.0417	70								30						7							3					
3i	0.0607	0.0094									100													2					
3ii	0.0258	0.0057	5								60					35	P							1					P
3iii	0.0528	0.0115														100													3

Stream B

Sample taken

25/MAR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.1209	0.0264	60								10					30	4								1					2
1ii			P														P												
1iii	0.4817	0.1935	70	P												30	34	P											15
2i	0.3828	0.0991	30										50			20	7										12		5
2ii	0.2212	0.1025	20									20	40			20	5									5	10		5
2iii	0.1391	0.0598	20													80	3												12
3i			P													P	P												P
3ii			P													P	P												P
3iii	0.0591	0.0473														100													12
LOWER RIFFLE																													
1i			P								P						P								P				
1ii	0.0299	0.0099	100													P	2												P
1iii			P								P						P								P				
2i			P														P												
2ii			P														P												
2iii			P														P												
3i			P													P	P												P
3ii			P														P												
3iii			P														P												
UPPER POOL																													
1i	0.0466	0.0172	P								100					P	P								4					P
1ii			P								P					P	P								P					P
1iii			P													P	P													P
2i	2.3454	0.7950	P									30				70	P									60				139
2ii	1.1042	0.5665	10									20				70	14									28				99
2iii	0.6113	0.1151	10									60				30	3									17				9
3i	0.8221	0.5360	P								10					90	P								13					121
3ii																													
3iii																													
UPPER RIFFLE																													
1i			P													P	P													P
1ii			P													P	P													P
1iii	0.0952	0.0173	60								40						3								2					
2i	0.2859	0.0531	P								100						P								13					
2ii			P								P						P								P					
2iii																														
3i			P													P	P													P
3ii			P				P									P	P				P									P
3iii			P													P	P													P

Stream B

Sample taken

22/APR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes		*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	0.3587	0.1754	80													20		35												9
1ii			P															P												
1iii	0.1475	0.0601	60													40		9												6
2i	0.4421	0.1233														100														31
2ii	1.1978	0.8411	20													80		42												168
2iii	0.9820	0.7331	90													10		165												18
3i	0.6802	0.2653														100														66
3ii	0.1904	0.0927														100														23
3iii	1.0524	0.7654	50													50		96												96
LOWER RIFFLE																														
1i	0.0175	0.0110	100															3												
1ii	0.0054	0.0047	100															1												
1iii																P														P
2i	0.0119	0.0041	100															1												
2ii	0.0514	0.0282	100															7												
2iii	0.1099	0.0526	100															13												
3i	0.1661	0.0761	100															19												
3ii			P						P									P						P						
3iii	0.0314	0.0132	100			P												3			P									
UPPER POOL																														
1i	0.4785	0.2629	20							·	5	35				40		13								3	23			26
1ii	0.9125	0.5455	15									85						20									116			
1iii	1.7868	0.7008	10									90				P		18									158			P
2i	0.2030	0.1212	5								45					50		2								14				15
2ii			P													P		P												P
2iii	0.0680	0.0518														100														13
3i											P															P				
3ii																P														P
3iii	0.0420	0.0285														100														7
UPPER RIFFLE																														
1i	0.0126	0.0103	100	P														3	P											
1ii	0.0680	0.0518	100															13												
1iii											P															P				
2i	0.0657	0.0333	100													P		8												P
2ii	0.0035	0.0026	90	10														1	P											
2iii	0.0224	0.0070	100															2												
3i	0.0056	0.0025	35								65							P								P				
3ii	0.5012	0.0914	100			P												23			P									
3iii			P	P						P								P	P					P						

Stream B

Sample taken

26/MAY/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.2138	0.1267	95	5		P		P									30	2		P		P							
1ii	1.0097	0.3327	100	P		P											83	P		P									
1iii	0.3632	0.1748	90			P						10					39			P						4			
2i	1.2721	0.6451	80			P										20	129			P									32
2ii	1.0373	0.5339	95			P										5	127			P									7
2iii	0.0089	0.0055	50	50		P											1	1		P									
3i	0.1734	0.1060	5	60		P		P								35	1	16		P		P							9
3ii			P	P												P	P	P											P
3iii	0.1151	0.0691		50		P										50		9		P									9
LOWER RIFFLE																													
1i	0.2124	0.1206	100			P											30			P									
1ii	0.2647	0.1705	100			P											43			P									
1iii	0.1074	0.0563	100			P	P										14			P	P								
2i	0.1550	0.1039	100			P										P	26			P									P
2ii	0.1308	0.1013	100			P											25												
2iii	0.3490	0.1511	95			5											36			2									
3i	0.0254	0.0152	80	10		10											3	P		P									
3ii	0.0713	0.0450	90	P		10											10	P		1									
3iii	0.5070	0.2566	100														64												
UPPER POOL																													
1i	1.8968	1.1696	100			P											292			P									
1ii	1.0556	0.7314	30			P						70					55			P						128			
1iii	0.8407	0.4776	45	15								40					54	18								48			
2i	0.1226	0.0662	30								30					40	5								5				6
2ii	0.2387	0.1682	100	P		P											42	P		P									
2iii	0.5430	0.3514	100			P											88			P									
3i	1.5009	0.8536	40			P						50				10	85			P						107			21
3ii	2.3278	0.8824	90			5						5					199			11						11			
3iii	2.2161	1.2048	40			10						50					120			30						151			
UPPER RIFFLE																													
1i	0.0110	0.0078	100			P	P										2			P	P								
1ii	0.0094	0.0016	100														P												
1iii	0.3601	0.1466	95			5											35			2									
2i	0.1318	0.0805	85								15						17								3				
2ii	0.1082	0.0657	85	5		P					10						14	1		P					2				
2iii	0.0752	0.0472	100														12												
3i	1.3452	0.2998		95		P					5							71		P					4				
3ii	0.0672	0.0192	20	80		P											1	4		P									
3iii	0.7371	0.2344	100	P		P											59	P		P									

Stream B

Sample taken

23/JUN/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	1.7656	0.8557	50			5						45					107			11					96				
1ii	1.0302	0.6095	35			5						60					53			8					91				
1iii																													
2i	2.4960	1.0734	60											40			161										107		
2ii	0.4075	0.2636	25			15								60			16			10							40		
2iii	0.9722	0.3991	40			50						10					40			50					10				
3i	0.3808	0.1917	10	80		10		P									5	38		5		P							
3ii	0.0516	0.0440														100													11
3iii	0.5586	0.2900	10	90		P											7	65		P									
LOWER RIFFLE																													
1i	0.2856	0.1731	100			P											43			P									
1ii	0.2022	0.0980	95			5										P	23			1									P
1iii	0.2173	0.1519	95			5											36			2									
2i	0.2282	0.1543	100			P											39			P									
2ii	0.2522	0.1613	95			5											38			2									
2iii	0.2587	0.1668	70	P		30											29	P		13									
3i	0.2735	0.1942	100			P											49			P									
3ii	0.3222	0.2473	90			10											56			6									
3iii	0.2762	0.1977	100			P											49			P									
UPPER POOL																													
1i	0.2550	0.1513	10			P						10				80	4			P					4				30
1ii	0.1921	0.1136	90	P		P						10					26	P		P					3				
1iii																													
2i	0.3830	0.2942	70			30											51			22									
2ii	0.4570	0.3135	5	15		65						5				10	4	12		51					4				8
2iii	0.0494	0.0209	70			30											4			2									
3i	0.9027	0.6952	100			P											174			P									
3ii	0.4154	0.3344	100	P		P											84	P		P									
3iii	1.1051	0.6537	80			5						15					131			8					25				
UPPER RIFFLE																													
1i	0.2745	0.1169	40			60											12			18									
1ii			P			P											P			P									
1iii	0.2060	0.1020	40	P		60											10	P		15									
2i	0.2688	0.1082	100			P											27			P									
2ii	0.0324	0.0179	100			P											4			P									
2iii	0.1202	0.0376	95			5											9			P									
3i	0.2199	0.0769	85			5					10						16			1				2					
3ii	0.3157	0.1471	45			5					50						17			2				18					
3iii	0.6020	0.2239	15			5					80						8			3				45					

Stream B

Sample taken

21/JUL/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)										
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i			P			P										P	P			P									P
1ii	0.1320	0.0191	100			P											5			P									
1iii	0.7627	0.2585	100			P											65			P									
2i	0.6412	0.3886	60			P						40					58			P					39				
2ii	1.3914	1.0052	30			P						10				60	75			P					25				151
2iii	0.0508	0.0360	90			10											8			1									
3i	0.2524	0.0837	90			P										10	19			P									2
3ii	1.1534	0.7925	100			P											198			P									
3iii	0.0934	0.0529	100														13												
LOWER RIFFLE																													
1i	0.1654	0.0721	100			P											18			P									
1ii	0.4657	0.2906	90			5										5	65			4									4
1iii	0.4837	0.2728	90			10											61			7									
2i			P			P											P			P									
2ii	0.1495	0.1202	100			P											30			P									
2iii	1.3190	0.5332	90			P						10					120			P					13				
3i	0.3795	0.1174	60			20										20	18			6									6
3ii	0.1306	0.0876	100			P										P	22			P									P
3iii	0.0924	0.0471	100			P											12			P									
UPPER POOL																													
1i	0.7257	0.4949	65			5				P		30					80			6				P	37				
1ii	0.5373	0.2856	50			5						45					36			4					32				
1iii	0.3978	0.2065	20			20						60					10			10					31				
2i	1.0788	0.7436	95			P						5					177			P					9				
2ii	0.9902	0.6872	90			P						10				P	155			P					17				P
2iii	0.5028	0.3366	95			P						5					80			P					4				
3i	1.2393	0.5916	40			P						60					59			P					89				
3ii	1.8058	0.5581	75			5						20					105			7					28				
3iii	1.0687	0.3278	95			5										P	78			4									P
UPPER RIFFLE																													
1i	0.2162	0.0627	50			50											8			8									
1ii																													
1iii	0.0862	0.0425	95			5											10			1									
2i	0.0201	0.0088	P			10					90						P			P				2					
2ii	0.1312	0.0391	20			80											2			8									
2iii			P			P											P			P									
3i	0.0228	0.0070	P								100						P							2					
3ii	0.0521	0.0501	P			P					100						P			P				13					
3iii	0.4621	0.1342	95			5					P						32			2				P					

Stream B

Sample taken

11/AUG/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.2006	0.1301	50			P										50	16			P									16
1ii	0.7161	0.4626	50			P						50					58			P					58				
1iii	0.3189	0.2234	50			P						50					28			P					28				
2i	0.3369	0.2216	80			P										20	44			P									11
2ii	0.0777	0.0449	95			5											11			1									
2iii	0.7098	0.3307	50			P						50					41			P					41				
3i	0.1511	0.1103	90			10											25			3									
3ii	0.2667	0.1708	60			P						40					26			P					17				
3iii	0.3712	0.2528	100			P											63			P									
LOWER RIFFLE																													
1i	0.1354	0.0999	90			10											22			2									
1ii	0.0415	0.0229	85			15											5			1									
1iii			P			P					P						P			P					P				
2i						P														P									
2ii			P			P											P			P									
2iii			P			P											P			P									
3i	0.1293	0.0259	95			5											6			P									
3ii			P			P											P			P									
3iii						P														P									
UPPER POOL																													
1i	0.5313	0.3593	40			P						60					36			P					54				
1ii	0.5471	0.3443	70			5						25					60			4					22				
1iii	1.0606	0.4027	60			P						40					60			P					40				
2i	1.0522	0.7395	100			P											185			P									
2ii	1.0391	0.6051	25			P						75					38			P					113				
2iii	1.8119	0.8405	30			P						70					63			P					147				
3i	0.7439	0.3343	100			P										P	84			P									P
3ii																P													P
3iii	1.1815	0.5189	50			50										P	65			65									P
UPPER RIFFLE																													
1i	0.0140	0.0072	20			80											P			1									
1ii	0.0268	0.0152	40			60					P						2			2					P				
1iii	0.1716	0.1142	30			70											9			20									
2i	0.0115	0.0053	P			100											P			1									
2ii	0.0398	0.0176	30			70											1			3									
2iii	0.0762	0.0571	95			5											14			1									
3i	0.0140	0.0072	P			P					100						P			P					2				
3ii			P			P											P			P									
3iii	0.9682	0.2966	45			50					5						33			37					4				

Stream B

Sample taken

11/SEP/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL B			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachiospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachiospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
11	0.6091	0.3752	100														94												
111	0.7845	0.5295	60			P						40					79			P					53				
1111	0.5131	0.3493	90									10					79								9				
21	0.7302	0.5145	80									5	15				103								6	19			
211	0.5046	0.3560	90			P						10					80			P					9				
2111	0.4864	0.3505	95			P										5	83			P									4
31	0.5861	0.4386	90			P					5					5	99			P					5				5
311	0.1922	0.1346	100			P											34			P									
3111	0.3359	0.2208	100														55												
LOWER RIFFLE																													
11	0.0846	0.0543	95			P					5						13			P					1				
111	0.4054	0.2960	85			5					10						63			4					7				
1111	0.5101	0.3179	100			P											79			P									
21	0.0402	0.0330	90			10											7			1									
211	0.2201	0.0981	50			50											12			12									
2111	0.0824	0.0596	90			10											13			1									
31	0.0645	0.0305	100			P											8			P									
311	0.0669	0.0548	100			P											14			P									
3111	0.1802	0.1124	95			5											27			1									
UPPER POOL																													
11	0.2321	0.1644	100			P											41			P									
111	1.3169	0.9038	100			P											226			P									
1111	0.4762	0.3487	95			5							P				83			4						P			
21	0.6576	0.4325	90			P				P	10						97			P				P	11				
211	0.9562	0.6581	95			5											156			8									
2111	0.3117	0.1593	90			P					10						36			P					4				
31	0.7555	0.4376	20			P					P	80					22			P					P	88			
311	0.5790	0.3417	100			P											85			P									
3111	0.1134	0.0854	80													20	17												4
UPPER RIFFLE																													
11			P			P											P			P									
111	0.3252	0.1289	20			80											6			26									
1111	0.0392	0.0301	20			80											2			6									
21	0.0920	0.0492	20			80											2			10									
211	0.0659	0.0171	60			40											3			2									
2111	0.3434	0.1298	95			5											31			2									
31						P														P									
311	0.1053	0.0423	P	P		P					100						P	P		P					11				
3111	0.0539	0.0240	P			80					20						P			5					1				

Stream C

Sample taken

29/JAN/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potomogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potomogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	1.5795	0.4061	90													10	91												10
1ii	2.1909	0.5900	95													5	140												7
1iii	1.1997	0.3136	95										P			5	74									P			4
2i	2.7125	0.9787	90													10	220												24
2ii	1.8270	0.4676	95													5	111												6
2iii	2.2135	0.4295	95										P			5	102									P			5
3i	2.3046	0.6250	90										P			10	141									P			16
3ii	2.8674	0.8559	95													5	203												11
3iii	3.8798	1.0029	95													5	238												13
LOWER RIFFLE																													
1i	0.0171	0.0038	100									P					1								P				
1ii	0.0063	0.0007	100									P					P								P				
1iii	1.9311	0.4151	95													5	99												5
2i	0.0645	0.0200	100														5												
2ii	1.6959	0.8025	95													5													
2iii	0.4985	0.1433	90													10	32												4
3i	0.0026	0.0009	100														P												
3ii	0.0024	0.0001	100														P												
3iii	0.0220	0.0016	100														P												
UPPER POOL																													
1i	3.6088	2.6049	100														651												
1ii	1.0355	0.4300	85													15	91												16
1iii	2.1765	1.2312	10													90	31												277
2i	1.1545	0.5717	100														143												
2ii																	191												10
2iii	0.8154	0.3351	100														84												
3i	0.3769	0.1101	90													10	25												3
3ii	0.3655	0.2059	100														51												
3iii	1.1686	0.8548	95													5	203												11
UPPER RIFFLE																													
1i			P								P						P							P					
1ii	0.0643	0.0557	95								P	5					13							P	1				
1iii	0.0396	0.0103	100														3												
2i			P														P												
2ii			P								P						P							P					
2iii	0.0266	0.0117	60													40	2												1
3i			P														P												
3ii	0.0753	0.0500	100								P						13							P					
3iii	0.0919	0.0201	100														5												

Stream C

Sample taken

26/FEB/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.4902	0.0988	50								50						12								12				
1ii	0.3360	0.1001	80													20	20												5
1iii																													
2i	0.7900	0.2694	50													50	34												34
2ii			P													P	P												P
2iii	1.1679	0.8013	95													5	190												10
3i	3.1715	0.7241	100								P					P	181								P				P
3ii	4.2492	1.2760	80								10		P			10	255								32		P		32
3iii			P														P												
LOWER RIFFLE																													
1i			P													P	P												P
1ii			P														P												
1iii	0.0069	0.0061	80													20	1												P
2i			P													P	P												P
2ii			P														P												
2iii	0.0285	0.0182	70								10					20	3								P				1
3i			P														P												
3ii			P													P	P												P
3iii	0.3691	0.0964	60								40					P	14								10				P
UPPER POOL																													
1i	1.5473	0.4082	100														102												
1ii	0.9807	0.3735	100														93												
1iii	0.6473	0.2877	90													10	65												7
2i	0.5484	0.2760	100								P						69								P				
2ii	0.6060	0.2996	25													75	19												56
2iii	0.8038	0.5003	100														125												
3i	2.7487	1.4447	50								20		20			10	181								72		72		36
3ii	1.0900	0.5544	P								P		P			100	P								P		P		139
3iii	0.3356	0.2156	75													25	40												13
UPPER RIFFLE																													
1i	0.0756	0.0207	40								60						2								3				
1ii			P														P												
1iii	0.0738	0.0400	95								5					P	10								1				P
2i	0.0127	0.0080	100														2												
2ii			P								P						P								P				
2iii			P														P												
3i			P								P					P	P								P				P
3ii			P								P					P	P								P				P
3iii			P														P												

Stream C

Sample taken

25/MAR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	1.1767	0.3827	50								P					50	48							P					48
1ii	0.0586	0.0516														100													13
1iii	0.5748	0.4069	95													5	97												5
2i	1.0191	0.6388	80								20						128							32					
2ii	1.6670	0.6957	95								5						165							9					
2iii	0.4550	0.2856	95								P					5	68							P					4
3i	0.2718	0.0759	100														19												
3ii	0.1303	0.0560	90								10						13							1					
3iii	0.1074	0.0262	100														7												
LOWER RIFFLE																													
1i			P														P												
1ii	1.1949	0.9358	60													40	140												94
1iii	3.4417	2.4648	P								100						P							616					
2i			P								P						P							P					
2ii			P														P												
2iii	0.1250	0.0264	100														7												
3i	0.1629	0.0822	100								P						21							P					
3ii			P								P						P							P					
3iii	0.5673	0.1983	100								P						50							P					
UPPER POOL																													
1i	0.0600	0.0165														100													4
1ii	2.9344	1.9201	P													100	P												480
1iii	2.8393	2.3921	30								P					70	179							P					419
2i	0.0636	0.0382	P								P					100	P							P					10
2ii	0.0117	0.0098														100													2
2iii	1.1323	0.8071	P								P					100	P							P					202
3i	0.3540	0.1586														100													40
3ii	1.1115	0.6850														100													171
3iii	0.0745	0.0588	P													100	P												15
UPPER RIFFLE																													
1i	0.2752	0.1265	5								95						2							30					
1ii	0.1293	0.0976	100														24												
1iii	0.0361	0.0207	80								P					20	4							P					1
2i			P								P						P							P					
2ii			P								P					P	P							P					P
2iii											P													P					
3i			P								P						P							P					
3ii			P														P												
3iii			P								P						P							P					

Stream C

Sample taken

22/APR/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL C			Cladophora	Vaucheria	Stigeoclonium	Oedogonium	Microspora	Spirogyra	Batrachospermum	Rhodochorton	Amblystegium	Elodea	Potamogeton	Callitriche	Ranunculus	Other Macrophytes	Cladophora	Vaucheria	Stigeoclonium	Oedogonium	Microspora	Spirogyra	Batrachospermum	Rhodochorton	Amblystegium	Elodea	Potamogeton	Callitriche	Ranunculus	Other Macrophytes
LOWER POOL																														
1i																P														P
1ii	0.2971	0.1803	10													90	5													41
1iii	0.1619	0.0975	P													100	P													24
2i	1.4035	0.8139	100														203													
2ii	0.0963	0.0575	20													80	3													12
2iii																P														P
3i	0.0408	0.0183														100														5
3ii	1.2807	0.8506	100														213													
3iii	0.6758	0.4193	25													75	26													79
LOWER RIFFLE																														
1i	0.0239	0.0137	100														3													
1ii			P														P													
1iii			P														P													
2i	0.2950	0.2479														100														62
2ii			P														P													
2iii	0.0029	0.0011	30	30												40	P	P												P
3i	0.0047	0.0034	100														1													
3ii	0.2501	0.1165	100														29													
3iii	0.0015	0.0011	100														P													
UPPER POOL																														
1i	0.1918	0.1191	P													100	P													30
1ii	0.6120	0.2965	5								5					90	4								4					67
1iii	0.1323	0.0627	P													100	P													16
2i																P														P
2ii	0.4293	0.2446	P													100	P													61
2iii	0.9355	0.7067	P													100	P													177
3i			P														P													
3ii	0.2486	0.1974	P													100	P													49
3iii	0.3902	0.2654	P													100	P													66
UPPER RIFFLE																														
1i	0.3097	0.0713	100														18													
1ii	0.3872	0.1058	100														26													
1iii																65	2													4
2i	0.0318	0.0238	35														23													
2ii	0.3413	0.0927	100														2													
2iii	0.0082	0.0079	100														4									1				
3i	0.0619	0.0183	80									20					1	P												
3ii	0.0171	0.0046	95	5												P		P												P
3iii				P													1													
	0.0128	0.0057	100																											

Stream C

Sample taken

26/MAY/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes		*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	0.2776	0.1640	100	P			P									P		41	P			P								P
1ii	0.3180	0.2227	50				P									50		28				P								28
1iii	0.9998	0.7860	50	P	P	P	P									50		98	P	P	P	P								98
2i			P													P		P												P
2ii	0.1560	0.1002	30													70		8												18
2iii	0.0611	0.0431	100				P											11				P								
3i	0.0642	0.0444	100				P									P		11				P								P
3ii	0.0521	0.0324	50			P										50		4			P									4
3iii	0.7342	0.6825	P	P												100		P	P											171
LOWER RIFFLE																														
1i			P			P												P			P									
1ii	0.0590	0.0492	40				P				60							5				P				7				
1iii	0.0041	0.0034	90		P						10							1		P						P				
2i	0.0899	0.0636	100							•								16												
2ii	0.1005	0.0673	100				P											17				P								
2iii	0.0258	0.0154	10				P				P					90		P				P				P				3
3i	0.1340	0.1120	100															28												
3ii	0.0360	0.0228	95	5		P	P			P								5	P		P	P			P					
3iii	0.1478	0.0596	100															15												
UPPER POOL																														
1i	2.6316	1.8998									P					100										P				475
1ii	0.4269	0.2951	100				P				P							74				P				P				
1iii	1.3003	0.9131	50													50		114												114
2i																P														P
2ii	0.3188	0.2469	P													100		P												62
2iii																														
3i																														
3ii																														
3iii	0.4111	0.2874												100														72		
UPPER RIFFLE																														
1i	0.0421	0.0193	100															5												
1ii	0.0883	0.0387	100				P											10				P								
1iii	0.0193	0.0121	100															3												
2i	0.4474	0.2598	100															65												
2ii	0.1217	0.0296	95	5			P											7	P			P								
2iii	0.0214	0.0079	P	100			P											P	2			P								
3i	0.0793	0.0422	90	10														9	1											
3ii	0.0772	0.0211	20	80			P											1	4			P								
3iii	1.1766	0.3481	100						P									87						P						

Stream C

Sample taken

23/JUN/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g.	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)												
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i	0.3779	0.2739	100														68												
1ii	0.2111	0.1653	95	5		P	P										39	2		P	P								
1iii	0.6308	0.4964	100			P											124			P									
2i	0.4252	0.3086	100			P											77			P									
2ii	0.1687	0.1111	65	5		P										30	18	1		P									8
2iii	0.4500	0.3727	90			5										5	84			5									5
3i	0.1822	0.1358	95			5											32			2									
3ii	0.6444	0.5349	100	P		P											134	P		P									
3iii	0.6492	0.5428	100			P											136			P									
LOWER RIFFLE																													
1i	0.0340	0.0167	100			P											4			P									
1ii	0.3012	0.2332	100			P	P			P							58			P	P			P						
1iii	0.1963	0.1360	100			P											34			P									
2i	0.1580	0.1132	100			P											28			P									
2ii	0.1647	0.1216	100			P											30			P									
2iii	0.0991	0.0818	100			P											20			P									
3i	0.2169	0.1493	100			P											37			P									
3ii	0.2821	0.1944	100														49												
3iii	0.0864	0.0631	100			P											16			P									
UPPER POOL																													
1i	1.7478	1.2141	90			P	5				P					5	273			P	15			P					15
1ii	1.1953	0.8756	70	P												30	153	P											66
1iii	0.1327	0.0848	70				P				P					30	15				P			P					6
2i	0.7556	0.3564	90				P									10	80				P								9
2ii	0.3354	0.1488	100	P		P											37	P		P									
2iii	0.2992	0.1560	30				P				5					65	12				P			2					25
3i	0.8712	0.5486	P								P					100	P							P					137
3ii	0.8331	0.3035	50			P	P				P					50	38			P	P			P					38
3iii	1.6416	0.8503	55	5		P										40	117	11		P									85
UPPER RIFFLE																													
1i	0.2054	0.1083	100			P											27			P									
1ii	0.5980	0.3020	100			P	P										76			P	P								
1iii	0.1783	0.0763	100			P											19			P									
2i	0.2302	0.1422	100														36												
2ii	0.3685	0.1124	100			P										P	28			P									P
2iii	0.6074	0.3973	90	P		P					10						89	P		P				10					
3i			P														P												
3ii	0.0910	0.0698	100			P											17			P									
3iii																													

Stream C

Sample taken

21/JUL/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)															BIOMASS ($g\ m^{-2}$)												
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes		*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																														
1i	0.1418	0.0599	90			10												13			1									
1ii			P			P					P					P		P			P					P				P
1iii	0.8080	0.4161	100															104			P					P				P
2i											P					P										P				P
2ii																														
2iii	0.8486	0.5441	100			P												136			P									
3i	0.7890	0.5750	100			P												144			P									
3ii	0.6235	0.4423	100															111												
3iii	2.0401	0.9586	100			P												240			P									
LOWER RIFFLE																														
1i	0.5010	0.3611	100			P												90			P									
1ii	0.1403	0.0952	100			P												24			P									
1iii	0.2456	0.1864	90			P								10				42			P								5	
2i	0.2319	0.1817	100															45												
2ii			P								P							P								P				
2iii	0.0139	0.0085	80			20												2			P									
3i	0.0354	0.0192	100															5												
3ii	0.2255	0.1488	100			P												37			P									
3iii	0.4273	0.2436	100			P										P		61			P									P
UPPER POOL																														
1i	0.0558	0.0186	100			P										P		5			P									P
1ii	0.2309	0.0966	100			P												24			P									
1iii	0.6945	0.3508														100														88
2i	0.4837	0.1324	95			5												31			2									
2ii	0.4318	0.1194	60			40												18			12									
2iii																														
3i	1.3998	0.8277	100			P										P		207			P									P
3ii	1.1425	0.7750									P					100										P				194
3iii	1.4869	0.5934	50			50												74			74									
UPPER RIFFLE																														
1i	0.8936	0.6136	100			P												153			P									
1ii	0.4385	0.3156	100			P												79			P									
1iii	0.2990	0.1852	100			P												46			P									
2i			P								P							P								P				
2ii			P								P							P								P				
2iii	0.1440	0.0733	10								90							2								16				
3i			P													P		P												P
3ii			P								P							P								P				
3iii			P			P												P			P									

Stream C

Sample taken

11/AUG/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)														BIOMASS ($g\ m^{-2}$)											
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i			P			P										P	P			P									P
1ii	0.1748	0.1169	70			30										P	20			9									P
1iii	0.8021	0.5955	95			5											141			7									
2i	0.0801	0.0531	90			10											12			1									
2ii	0.0600	0.0499	90			10										P	11			1									P
2iii	0.2204	0.1261	90			10											28			3									
3i	0.2124	0.1236	100			P										P	31			P									P
3ii	0.3964	0.2305	55			5										40	32			3									23
3iii	0.3215	0.2091	100			P											52			P									
LOWER RIFFLE																													
1i	0.0684	0.0344	50			10					40						4			1					3				
1ii	0.0649	0.0246	90			10											6			1									
1iii	0.0694	0.0522	90			10											12			1									
2i			P			P											P			P									
2ii	0.0119	0.0071	80			20											1			P									
2iii			P			P											P			P									
3i	0.0304	0.0190	100			P											5			P									
3ii	0.0226	0.0164	90			10											4			P									
3iii	0.0496	0.0363	70			30											6			3									
UPPER POOL																													
1i	0.3485	0.2460	100													P	62												P
1ii	0.3484	0.1622	100			P					P					P	41			P					P				P
1iii	0.4732	0.3337	20			P										80	17			P									67
2i	0.5615	0.2101	100			P										P	53			P									P
2ii	0.7970	0.5683	100			P											142			P									
2iii	0.9288	0.5659	95			5											134			7									
3i	1.2396	1.0950														100													274
3ii	0.5914	0.3403	100			P										P	85			P									P
3iii	1.1045	0.6402	80			10										10	128			16									16
UPPER RIFFLE																													
1i			P			P											P			P									
1ii	0.0070	0.0041	100			P											1			P									
1iii	0.2435	0.1846	95			5											44			2									
2i	0.2495	0.1673	100			P											42			P									
2ii	0.2481	0.1402	30			P					70						11			P					25				
2iii	0.1474	0.1321	100			P											33			P									
3i	0.0270	0.0222	100			P											6			P									
3ii	0.1406	0.0743	95			5											18			1									
3iii	0.1826	0.1160	100			P											29			P									

Stream C

Sample taken

8/SEP/1980

SAMPLE	DRY WT. (g)	VOLATILE SOLIDS (g)	PROPORTION OF CONSTITUENT IN SAMPLE (%)													BIOMASS ($g\ m^{-2}$)											
CHANNEL C			*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes	*Cladophora*	*Vaucheria*	*Stigeoclonium*	*Oedogonium*	*Microspora*	*Spirogyra*	*Batrachospermum*	*Rhodochorton*	*Amblystegium*	*Elodea*	*Potamogeton*	*Callitriche*	*Ranunculus*	Other Macrophytes
LOWER POOL																													
1i			P													P	P												P
1ii	0.5270	0.3850	90			10											87			10									
1iii	0.8144	0.5645	100			P											141			P									
2i	0.3714	0.2447	80			20											49			12									
2ii	0.2022	0.1397	90			10											31			3									
2iii	0.2347	0.1823	70			30											32			14									
3i	0.4637	0.3262	90			10											73			8									
3ii	0.3354	0.1632	60			40											24			16									
3iii	0.3466	0.2428	95			5											58			3									
LOWER RIFFLE																													
1i	0.1777	0.1067	85			15											23			4									
1ii	0.0739	0.0471	P			20					80						P			2				9					
1iii	0.0425	0.0289	30			40					30						2			3				2					
2i	0.1299	0.0655	30			40					30						5			7				5					
2ii	0.3001	0.2130	70			30											37			16									
2iii	0.0373	0.0197	5			95										P	P			5									P
3i	0.0496	0.0334	55			5					40						5			P				3					
3ii	0.1630	0.1137	45			5					50						13			1				14					
3iii	0.0864	0.0760	90			10											17			2									
UPPER POOL																													
1i	2.0448	1.5081	5			5					P					90	19			19				P					339
1ii	0.2756	0.1635	20			80										P	8			33									P
1iii	0.1634	0.1222	70			30											21			9									
2i	0.2512	0.1492	40			60					P						15			22				P					
2ii	0.3404	0.1822	70			30											32			14									
2iii	0.1717	0.0905	70			30											16			7									
3i	0.5170	0.3865	15			15										70	14			14									68
3ii	0.3623	0.1914	80			20											38			10									
3iii	0.4253	0.2833	70			10										20	50			7									14
UPPER RIFFLE																													
1i	0.1838	0.0908	70			30											16			7									
1ii			P			P											P			P									
1iii	0.1623	0.0616	95			5											15			1									
2i	0.0634	0.0249	5			5					90						P			P				6					
2ii	0.1023	0.0568	50			P					50						7			P				7					
2iii	0.3429	0.2302	100			P											58			P									
3i			P			P											P			P									
3ii	0.3381	0.1335	50			40					10						17			13				3					
3iii	0.0598	0.0406	100			P											10			P									

APPENDIX 2

Basic computer programs.

Written in Hewlett-Packard HP2000 BASIC

Program SPAN : Split-plot AOV

ANOV 1 : 1-way AOV

ANOV 2 : 2-way AOV

Program SPAN

```
10  PRINT "A TWO WAY (SPLIT PLOT) A.O.V.  INCORPORATING LOG(X+1) OR"
20  PRINT "ROOT(X+0.5) OF DATA BEFORE ANALYSIS"
30  PRINT
40  PRINT "DATA MUST BE INPUT FROM A FILE AND A FILE MUST EXIST FOR"
50  PRINT "A.O.V.  TABLE (ie OUTPUT OF PROGRAM). "
60  PRINT
70  PRINT "A BLOCKED OR REPLICATED DESIGN IS ACCOMODATED"
80  PRINT
90  PRINT "POLYNOMIAL ANALYSIS IS PERFORMED IN THE FORM - "
100  PRINT "A v (B+C)/2   AND   B v C"
110  PRINT
120  FILES *,*
130  DIM A[200,10],A$[6],B[200],C[20,10],D[10]
140  DIM E[20],F[20]
150  PRINT "HOW MANY MAIN-PLOT TREATMENTS ?"
160  INPUT L
170  PRINT "HOW MANY SUB-PLOT TREATMENTS ?"
180  INPUT T
190  PRINT "ARE THERE ANY BLOCKS ?"
200  PRINT "YES=1  NO=0"
210  INPUT A1
220  IF A1=1 THEN 250
230  IF A1=0 THEN 280
240  GOTO 190
250  PRINT "HOW MANY BLOCKS ?"
260  INPUT R
270  GOTO 300
280  PRINT "OK HOW MANY REPLICATES THEN ?"
290  INPUT R
300  Y=L*T
310  MAT A=ZER[Y,R]
320  MAT B=ZER[Y]
330  MAT C=ZER[L,R]
340  MAT D=ZER[R]
350  MAT E=ZER[L]
360  MAT F=ZER[T]
370  PRINT "ENTER NAME OF FILE FOR A.O.V. TABLE STORAGE"
380  DIM F$[6]
390  INPUT F$
400  ASSIGN F$,2,V1
410  PRINT "ENTER NAME OF FILE FOR SPLIT PLOT A.O.V. "
420  INPUT A$
430  ASSIGN A$,1,V1
440  MAT  READ #1;A
450  PRINT "ENTER TRANSFORMATION REQUIRED"
460  PRINT "NONE=0  LOG(X+1)=1  ROOT(X+0.5)=2"
470  INPUT F1
480  IF F1>3 THEN 450
490  IF F1=0 THEN 580
500  FOR I=1 TO 27
510  FOR J=1 TO 9
520  IF F1=2 THEN 550
530  A[I,J]=LOG(A[I,J]+1)/2.30259
540  GOTO 560
550  A[I,J]=SQR(A[I,J]+.5)
560  NEXT J
570  NEXT I
580  S1=S2=B2=0
590  FOR I=1 TO Y
600  R1=0
610  FOR J=1 TO R
620  R1=R1+A[I,J]
630  S1=S1+A[I,J] ** 2
640  S2=S2+A[I,J]
650  NEXT J
660  B2=B2+R1 ** 2
670  B[I]=R1
680  NEXT I
690  Z1=0
700  C3=0
710  P1=(L-1)*T
720  P=P1+1
730  FOR X=1 TO P STEP T
740  Z1=Z1+1
750  FOR J=1 TO R
```

```
760  C1=0
770  F=T-1
780  FOR I=X TO X+F
790  C1=C1+A[I,J]
800  NEXT I
810  C[Z1,J]=C1
820  C3=C3+C1 ** 2
830  NEXT J
840  NEXT X
850  D2=0
860  FOR J=1 TO R
870  C2=0
880  FOR I=1 TO L
890  C2=C2+C[I,J]
900  NEXT I
910  D[J]=C2
920  D2=D2+C2 ** 2
930  NEXT J
940  C5=0
950  FOR I=1 TO L
960  C4=0
970  FOR J=1 TO R
980  C4=C4+C[I,J]
990  NEXT J
1000  C5=C5+C4 ** 2
1010  E[I]=C4
1020  NEXT I
1030  T2=0
1040  FOR X=1 TO T
1050  T1=0
1060  FOR I=X TO X+((L-1)*T) STEP T
1070  T1=T1+B[I]
1080  NEXT I
1090  F[X]=T1
1100  T2=T2+T1 ** 2
1110  NEXT X
1120  S2=S2 ** 2
1130  G1=G2=G3=G4=G5=G6=G7=G8=G9=0
1140  S3=S4=S6=S7=S9=0
1150  S2=S2/(L*T*R)
1160  G1=S1-S2
1170  G2=(C3/T)-S2
1180  G3=(C5/(T*R))-S2
1190  IF A1=0 THEN 1220
1200  G4=(D2/(L*T))-S2
1210  S4=R-1
1220  G5=(B2/R)-S2
1230  G6=(T2/(L*R))-S2
1240  G7=G5-(G3+G6)
1250  G8=G2-(G3+G4)
1260  G9=G1-(G2+G6+G7)
1270  REM DEGREES OF FREEDOM
1280  S3=L-1
1290  S8=((L*R)-1)-(S3+S4)
1300  S6=T-1
1310  S7=S3*S6
1320  S9=((L*T*R)-1)-(S3+S4+S7+S6+S8)
1330  REM TOTAL DEGREES OF FREEDOM
1340  D1=(L*T*R)-1
1350  REM POLYNOMIALS
1360  PRINT
1370  DIM G[2,3]
1380  G[1,1]=0
1390  G[1,2]=1
1400  G[1,3]=-1
1410  G[2,1]=-2
1420  G[2,2]=1
1430  G[2,3]=1
1440  FOR I=1 TO 2
1450  H[I]=I[I]=J[I]=0
1460  FOR J=1 TO 3
1470  H[I]=H[I]+(E[J]*G[I,J])
1480  I[I]=I[I]+G[I,J] ** 2
1490  NEXT J
1500  H[I]=H[I] ** 2
1510  I[I]=(I[I]*(R*T))
1520  J[I]=H[I]/I[I]
1530  NEXT I
1540  K[I]=0
1550  FOR I=1 TO 2
1560  K[I]=J[I]/(G8/S8)
1570  NEXT I
1580  PRINT
1590  PRINT #2
1600  PRINT "SOURCE OF","DEGREES OF","SUM OF","MEAN","F-"
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```
1610  PRINT #2;"SOURCE OF","DEGREES OF","SUM OF","MEAN","F-"
1620  PRINT "VARIATION","FREEDOM","SQUARES","SQUARE","RATIO"
1630  PRINT #2;"VARIATION","FREEDOM","SQUARES","SQUARE","RATIO"
1640  PRINT "-------------------------------------------------------------------------------"
1650  PRINT #2;"-------------------------------------------------------------------------------"
1660  PRINT "MAIN-PLOT"
1670  PRINT #2;"MAIN-PLOT"
1680  PRINT "TREATMENT",S3,G3,(G3/S3),(G3/S3)/(G8/S8)
1690  PRINT #2;"TREATMENT",S3,G3,(G3/S3),(G3/S3)/(G8/S8)
1700  PRINT "A v (B+C)/2"," 1",J[2],J[2],K[2]
1710  PRINT #2;"A v (B+C)/2"," 1",J[2],J[2],K[2]
1720  PRINT "B v C"," 1",J[1],J[1],K[1]
1730  PRINT #2;"B v C"," 1",J[1],J[1],K[1]
1740  PRINT
1750  PRINT #2
1760  IF A1=0 THEN 1790
1770  PRINT "BLOCKS",S4,G4,(G4/S4),(G4/S4)/(G8/S8)
1780  PRINT #2;"BLOCKS",S4,G4,G4/S4,(G4/S4)/(G8/S8)
1790  PRINT "MAIN-PLOT ERROR",S8,G8,(G8/S8)
1800  PRINT #2;"MAIN-PLOT ERROR",S8,G8,(G8/S8)
1810  PRINT
1820  PRINT #2;""
1830  PRINT "SUB-PLOT"
1840  PRINT #2;"SUB-PLOT"
1850  PRINT "TREATMENT",S6,G6,(G6/S6),(G6/S6)/(G9/S9)
1860  PRINT #2;"TREATMENT",S6,G6,(G6/S6),(G6/S6)/(G9/S9)
1870  PRINT
1880  PRINT #2
1890  PRINT "INTERACTION",S7,G7,(G7/S7),(G7/S7)/(G9/S9)
1900  PRINT #2;"INTERACTION",S7,G7,G7/S7,(G7/S7)/(G9/S9)
1910  PRINT
1920  PRINT #2
1930  PRINT "SUB-PLOT ERROR",S9,G9,(G9/S9)
1940  PRINT #2;"SUB-PLOT ERROR",S9,G9,G9/S9
1950  PRINT "-------------------------------------------------------------------------"
1960  PRINT #2;"-------------------------------------------------------------------------------"
1970  PRINT "TOTAL",D1
1980  PRINT #2;"TOTAL",D1
1990  PRINT "-------------------------------------------------------------------------"
2000  PRINT #2;"-------------------------------------------------------------------------------"
2010  PRINT
2020  PRINT #2
2030  PRINT #2;"DATA IN FILE ",A$
2040  END
```

Progam ANOV 1

```
10  FILES *,*
20  PRINT "THIS IS A ONE-WAY A.O.V. FOR EVEN NUMBERS OF REPLICATES"
30  PRINT "FOR A BLOCKED OR REPLICATED DESIGN"
40  PRINT "WITH THE OPTION OF PARTITIONING THE TREATMENT S.O.S. "
50  PRINT
60  PRINT "DO YOU WANT A.O.V. TABLE STORING IN AN ASCII FILE?"
70  PRINT "YES=1 NO=0"
80  INPUT G9
90  IF G9=1 THEN 120
100  IF G9=0 THEN 160
110  GOTO 50
120  PRINT "INPUT NAME OF ASCII FILE FOR STORAGE"
130  DIM G$[6]
140  INPUT G$
150  ASSIGN G$,2,V1
160  DIM A[20,10],B[20,50]
170  DIM C[25]
180  DIM D[25]
190  PRINT "INPUT NUMBER OF TRT.LEVELS"
200  INPUT L
210  PRINT "INPUT NUMBER OF REPLICATES"
220  INPUT R
230  PRINT "DO YOU WANT TO PARTITION TRT.S.O.S? YES=1 NO=0"
240  INPUT A1
250  IF A1=1 THEN 280
260  IF A1=0 THEN 340
270  GOTO 230
280  MAT A=ZER[L,5]
290  L1=L2=L3=0
300  FOR I=1 TO L
310  PRINT "INPUT VALUE OF TRT.LEVEL",I
320  INPUT A[I,1]
330  NEXT I
340  PRINT "DOES THE DESIGN CONSIST OF REPLICATES =1"
350  PRINT "                          OR BLOCKS     =2"
360  INPUT A7
370  IF A1=0 THEN 380
380  N=0
390  MAT B=ZER[L,R]
400  PRINT
410  FOR I=1 TO L
420  PRINT "ENTER VALUES FOR CELL"I
430  FOR J=1 TO R
440  INPUT B[I,J]
450  N=N+1
460  N9=N
470  NEXT J
480  NEXT I
490  C=D=0
500  MAT D=ZER[L]
510  X=0
520  FOR I=1 TO L
530  FOR J=1 TO R
540  LET C=C+B[I,J] ** 2
550  D=D+B[I,J]
560  D[I]=D[I]+B[I,J]
570  NEXT J
580  NEXT I
590  PRINT
600  MAT  PRINT B
610  IF G9=0 THEN 640
620  MAT  PRINT #2;B
630  PRINT #2
640  PRINT
650  REM CORRECTION TERM
660  E=(D ** 2)/N
670  REM TOTAL S.O.S.
680  F=C-E
690  REM TOTAL DEG. FREEDOM
700  G=N-1
710  REM TRT.S.O.S
720  H=0
730  FOR I=1 TO L
740  C[I]=0
750  FOR J=1 TO R
```

```
760  C[I]=C[I]+B[I,J]
770  NEXT J
780  C[I]=C[I] ** 2
790  H=H+(C[I]/R)
800  NEXT I
810  H=H-E
820  IF A7=1 THEN 970
830  REM BLOCKS DEG FREEDOM
840  G=R-1
850  REM BLOCKS S.O.S.
860  DIM F[50]
870  P=0
880  FOR J=1 TO R
890  F[J]=0
900  FOR I=1 TO L
910  F[J]=F[J]+B[I,J]
920  NEXT I
930  F[J]=F[J] ** 2
940  P=P+(F[J]/L)
950  NEXT J
960  P=P-E
970  REM TRT. DEG. FREEDOM
980  K=L-1
990  GOTO 1000
1000  REM WITHIN GROUPS S.O.S
1010  M=F-H
1020  REM WITHIN GROUPS DEG. FREEDOM
1030  IF A7=2 THEN 1060
1040  N=G-K
1050  GOTO 1070
1060  N=G-(K+G)
1070  IF A1=0 THEN 1910
1080  REM POLYNOMIALS
1090  IF L<2 THEN 1910
1100  FOR I=1 TO L
1110  A[I,2]=A[I,1]-A[1,1]
1120  NEXT I
1130  FOR I=L TO 2 STEP -1
1140  A[I,2]=A[I,2]/A[2,2]
1150  NEXT I
1160  FOR I=1 TO L
1170  L1=L1+A[I,2]
1180  L2=L2+(A[I,2] ** 2)
1190  L3=L3+(A[I,2] ** 3)
1200  NEXT I
1210  REM LINEAR POLYNOMIALS
1220  A1=-L1/L
1230  FOR I=1 TO L
1240  A[I,3]=A1+A[I,2]
1250  NEXT I
1260  IF L<3 THEN 1520
1270  REM QUADRATIC POLYNOMIALS
1280  Q1=Q2=0
1290  FOR I=1 TO L
1300  Q1=Q1+(A[I,2]*A[I,3])
1310  Q2=Q2+((A[I,2] ** 2)*A[I,3])
1320  NEXT I
1330  B1=-Q2/Q1
1340  A2=((-(L1*B1))-L2)/L
1350  FOR I=1 TO L
1360  A[I,4]=A2+(A[I,2]*B1)+(A[I,2] ** 2)
1370  NEXT I
1380  IF L<4 THEN 1520
1390  REM CUBIC POLYNOMIALS
1400  C1=C2=C3=0
1410  FOR I=1 TO L
1420  C1=C1+((A[I,2] ** 3)*A[I,3])
1430  C2=C2+((A[I,2] ** 2)*A[I,4])
1440  C3=C3+((A[I,2] ** 3)*A[I,4])
1450  NEXT I
1460  C4=-C3/C2
1470  B2=((-(Q2*C4))-C1)/Q1
1480  A3=(-(L1*B2)-(L2*C4)-L3)/L
1490  FOR I=1 TO L
1500  A[I,5]=A3+(B2*A[I,2])+(C4*(A[I,2] ** 2))+(A[I,2] ** 3)
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```
1510  NEXT I
1520  PRINT "TREATMENT","REDUCED"," ","POLYNOMIALS"," "
1530  PRINT "LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
1540  MAT  PRINT A
1550  IF G9=0 THEN 1590
1560  PRINT #2;"TREATMENT","REDUCED"," ","POLYNOMIALS"," "
1570  PRINT #2;"LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
1580  MAT  PRINT #2;A
1590  DIM E[5]
1600  MAT E=ZER
1610  DIM H[6]
1620  MAT H=ZER
1630  IF L=2 THEN 1690
1640  IF L=3 THEN 1670
1650  B7=5
1660  GOTO 1700
1670  B7=4
1680  GOTO 1700
1690  B7=3
1700  FOR J=3 TO B7
1710  T6=T7=T8=T9=0
1720  FOR I=1 TO L
1730  T9=T9+(D[I]*A[I,J])
1740  T6=T6+(A[I,J] ** 2)
1750  NEXT I
1760  T6=T6*R
1770  N[J]=T6
1780  T8=T8+T9
1790  H[J]=T8
1800  H[J]=H[J]/N[J]
1810  T8=T8 ** 2
1820  IF T6=0 THEN 1840
1830  GOTO 1860
1840  T7=0
1850  GOTO 1890
1860  T7=T8/T6
1870  O1=SQR(T8)
1880  O2=O1/T6
1890  E[J]=T7
1900  NEXT J
1910  REM TABLE ORGANISATION
1920  PRINT
1930  IF G9=0 THEN 1950
1940  PRINT #2
1950  PRINT "SOURCE OF","DEGREES OF","SUM OF","MEAN"," F-"
1960  IF G9=0 THEN 1980
1970  PRINT #2;"SOURCE OF","DEGREES OF","SUM OF","MEAN"," F-"
1980  PRINT "VARIATION"," FREEDOM","SQUARES","SQUARE","RATIO"
1990  IF G9=0 THEN 2010
2000  PRINT #2;"VARIATION","FREEDOM","SQUARES","SQUARE","RATIO"
2010  PRINT "-------------------------------------------------------------------------------"
2020  IF G9=0 THEN 2040
2030  PRINT #2;"-------------------------------------------------------------------------------"
2040  PRINT "TREATMENT",K,H,H/K,(H/K)/(M/N)
2050  IF G9=0 THEN 2070
2060  PRINT #2;"TREATMENT",K,H,H/K,(H/K)/(M/N)
2070  PRINT "----------"
2080  IF G9=0 THEN 2100
2090  PRINT #2;"----------"
2100  IF A1=0 THEN 2360
2110  PRINT "LINEAR EFF."," 1",E[3],E[3],(E[3]/(M/N))
2120  IF G9=0 THEN 2140
2130  PRINT #2;"LINEAR EFF."," 1",E[3],E[3],(E[3]/(M/N))
2140  IF L=2 THEN 2320
2150  PRINT "QUAD EFF."," 1",E[4],E[4],(E[4]/(M/N))
2160  IF G9=0 THEN 2180
2170  PRINT #2;"QUAD EFF."," 1",E[4],E[4],(E[4]/(M/N))
2180  IF L=3 THEN 2270
2190  PRINT "CUBIC EFF."," 1",E[5],E[5],(E[5]/(M/N))
2200  IF G9=0 THEN 2220
2210  PRINT #2;"CUBIC EFF."," 1",E[5],E[5],(E[5]/(M/N))
2220  T3=(H-(E[3]+E[4]+E[5]))
2230  PRINT "RESIDUAL",K-3,T3,"",""
2240  IF G9=0 THEN 2260
2250  PRINT #2;"RESIDUAL",K-3,T3,"",""
2260  GOTO 2360
2270  T2=(H-(E[3]+E[4]))
2280  PRINT "RESIDUAL",K-2,T2,"",""
2290  IF G9=0 THEN 2360
2300  PRINT #2;"RESIDUAL",K-2,T2,"",""
```

```
2310  GOTO 2360
2320  T2=(H-(E[3]))
2330  PRINT "RESIDUAL"," 0","","",""
2340  IF G9=0 THEN 2360
2350  PRINT #2;"RESIDUAL"," 0","","",""
2360  IF A7=1 THEN 2430
2370  PRINT
2380  IF G9=0 THEN 2400
2390  PRINT #2
2400  PRINT "BLOCKS",G,P,P/G,(P/G)/(M/N)
2410  IF G9=0 THEN 2430
2420  PRINT #2;"BLOCKS",G,P,P/G,(P/G)/(M/N)
2430  PRINT "ERROR",N,M,M/N," "
2440  IF G9=0 THEN 2460
2450  PRINT #2;"ERROR",N,M,M/N
2460  PRINT "-------------------------------------------------------------------------------"
2470  IF G9=0 THEN 2490
2480  PRINT #2;"-------------------------------------------------------------------------------"
2490  PRINT "TOTAL",G,F
2500  IF G9=0 THEN 2520
2510  PRINT #2;"TOTAL",G,F
2520  PRINT
2530  IF G9=0 THEN 2550
2540  PRINT #2
2550  IF A1=0 THEN 3820
2560  PRINT "DO YOU WISH TO PUT LINE OF BEST FIT ON MAIN TRTS ?"
2570  PRINT "YES=1  NO=0"
2580  INPUT A9
2590  IF A9=1 THEN 2620
2600  IF A9=0 THEN 3820
2610  GOTO 2550
2620  PRINT "UPTO WHICH LEVEL IS SIGNIFICNT"
2630  PRINT "LINEAR=1 QUADRATIC=2 CUBIC=3"
2640  INPUT M1
2650  H[2]=0
2660  FOR I=1 TO L
2670  H[2]=H[2]+D[I]
2680  NEXT I
2690  H[2]=H[2]/N9
2700  DIM G[30]
2710  MAT G=ZER
2720  IF M1=2 THEN 2840
2730  IF M1=3 THEN 2940
2740  FOR I=1 TO L
2750  A[I,2]=1
2760  G1=0
2770  FOR J=2 TO 3
2780  G=H[J]*A[I,J]
2790  G1=G1+G
2800  NEXT J
2810  G[I]=G1
2820  NEXT I
2830  GOTO 3090
2840  FOR I=1 TO L
2850  A[I,2]=1
2860  G1=0
2870  FOR J=2 TO 4
2880  G=H[J]*A[I,J]
2890  G1=G1+G
2900  NEXT J
2910  G[I]=G1
2920  NEXT I
2930  GOTO 3090
2940  FOR I=1 TO L
2950  A[I,2]=1
2960  G1=0
2970  FOR J=2 TO 5
2980  G=H[J]*A[I,J]
2990  G1=G1+G
3000  G[I]=G1
3010  NEXT J
3020  NEXT I
3030  DIM X[30]
3040  DIM Z[30]
3050  DIM U[30]
```

```
3060  MAT X=ZER[L]
3070  MAT Z=ZER[L]
3080  MAT U=ZER[L]
3090  FOR I=1 TO L
3100  X1=Z1=U1=0
3110  X1=X1+A[I,3] ** 2
3120  X[I]=X1
3130  Z1=Z1+A[I,4] ** 2
3140  Z[I]=Z1
3150  U1=U1+A[I,5] ** 2
3160  U[I]=U1
3170  NEXT I
3180  DIM V[30]
3190  MAT V=ZER[L]
3200  IF M1=2 THEN 3280
3210  IF M1=3 THEN 3340
3220  FOR I=1 TO L
3230  V[I]=((1/N9)+(X[I]/N[3]))
3240  V[I]=V[I]*(M/N)
3250  V[I]=V[I] ** .5
3260  NEXT I
3270  GOTO 3390
3280  FOR I=1 TO L
3290  V[I]=((1/N9)+(X[I]/N[3])+(Z[I]/N[4]))
3300  V[I]=V[I]*(M/N)
3310  V[I]=V[I] ** .5
3320  NEXT I
3330  GOTO 3390
3340  FOR I=1 TO L
3350  V[I]=((1/N9)+(X[I]/N[3])+(Z[I]/N[4])+(U[I]/N[5]))
3360  V[I]=V[I]*(M/N)
3370  V[I]=V[I] ** .5
3380  NEXT I
3390  DIM W[30]
3400  MAT W=ZER[L]
3410  PRINT "INPUT VALUE OF t (SEE ERROR TERM FOR THE NO OF DEGREES OF FREEDOM)"
3420  INPUT T
3430  PRINT
3440  PRINT "t="T
3450  IF G9=0 THEN 3480
3460  PRINT #2;"t="T
3470  PRINT #2
3480  FOR I=1 TO L
3490  W[I]=V[I]*T
3500  NEXT I
3510  PRINT
3520  PRINT "TREATMENT","  POINT","   S.E.","  UPPER","  LOWER"
3530  IF G9=0 THEN 3550
3540  PRINT #2;"TREATMENT","  POINT","   S.E.","  UPPER","  LOWER"
3550  PRINT " LEVEL ","         ","   +/-","  LIMIT","  LIMIT"
3560  IF G9=0 THEN 3580
3570  PRINT #2;" LEVEL","         ","   +/-","  LIMIT","  LIMIT"
3580  PRINT
3590  IF G9=0 THEN 3610
3600  PRINT #2
3610  FOR I=1 TO L
3620  PRINT A[I,1],G[I],V[I],(G[I]+W[I]),(G[I]-W[I])
3630  IF G9=0 THEN 3650
3640  PRINT #2;A[I,1],G[I],V[I],(G[I]+W[I]),(G[I]-W[I])
3650  NEXT I
3660  PRINT
3670  IF G9=0 THEN 3690
3680  PRINT #2
3690  IF M1=1 THEN 3750
3700  IF M1=2 THEN 3790
3710  PRINT "RESPONSE CURVE IS CUBIC"
3720  IF G9=0 THEN 3740
3730  PRINT #2;"RESPONSE CURVE IS CUBIC"
3740  GOTO 3820
3750  PRINT "RESPONSE CURVE IS LINEAR"
3760  IF G9=0 THEN 3780
3770  PRINT #2;"RESPONSE CURVE IS LINEAR"
3780  GOTO 3820
3790  PRINT "RESPONSE CURVE IS QUADRATIC"
3800  IF G9=0 THEN 3820
3810  PRINT #2;"RESPONSE CURVE IS QUADRATIC"
3820  END
```

Program ANOV 2

```
10  PRINT "THIS IS A 2-WAY ANOVAR WITH THE OPTION OF ANALYSING"
20  PRINT "THE TWO TREATMENTS AND THEIR INTERACTION"
30  PRINT "USING ORTHOGONAL POLYNOMIAL ANALYSIS. "
40  PRINT
50  PRINT "DATA MUST BE INPUT FROM THE TERMINAL AND THE RESULTS OF"
60  PRINT "THE ANALYSIS MAY BE RUN INTO AN ASCII FILE FOR SUBSEQUENT"
70  PRINT "PRINT OUT. "
80  PRINT
90  PRINT "NOTE - IF THE TWO TRTS DIFFER IN NUMBER OF LEVELS ARRANGE"
100  PRINT "----   DATA SO THAT TRT A HAS LESS LEVELS THAN TRT B. "
110  FILES *,*
120  DIM A$[6]
130  DIM A[30,30],B[30],C[30]
140  DIM E[10,5],F[10,5],G[15,25],H[1,25],K[20]
150  PRINT
160  PRINT "DO YOU WANT A PRINT OUT?"
170  PRINT "YES=1 NO=0"
180  INPUT P
190  IF P=0 THEN 250
200  IF P=1 THEN 220
210  GOTO 150
220  PRINT "INPUT NAME OF ASCI FILE"
230  INPUT A$
240  ASSIGN A$,2,V1
250  PRINT "INPUT NUMBER OF LEVELS OF VARIABLE A"
260  INPUT A
270  PRINT "INPUT NUMBER OF LEVELS OF VARIABLE B"
280  INPUT B
290  PRINT "INPUT NUMBER OF REPLICATES"
300  INPUT C
310  MAT A=ZER[A,(B*C)]
320  MAT B=ZER[A]
330  MAT C=ZER[B]
340  MAT D=ZER[A,B]
350  MAT E=ZER[A,5]
360  MAT F=ZER[B,5]
370  MAT G=ZER[15,(A*B)]
380  MAT H=ZER[1,A*B]
390  MAT K=ZER[15]
400  A1=A2=A3=0
410  FOR I=1 TO A
420  X=0
430  FOR J=1 TO (B*C) STEP C
440  X=X+1
450  FOR D=1 TO C
460  PRINT "INPUT VALUE AT LEVEL A"I"LEVEL B"X"REPLICATE"D
470  INPUT A1
480  A[(I),(J+D-1)]=A1
490  A2=A2+A1
500  A3=A3+(A1 ** 2)
510  NEXT D
520  NEXT J
530  NEXT I
540  A4=(A2 ** 2)/(A*B*C)
550  A5=A3-A4
560  FOR I=1 TO A
570  FOR J=1 TO (B*C)
580  B[I]=B[I]+A[I,J]
590  NEXT J
600  NEXT I
610  Y=0
620  FOR X=1 TO (B*C) STEP C
630  Y=Y+1
640  FOR J=X TO (X+C)-1
650  FOR I=1 TO A
660  C[Y]=C[Y]+A[I,J]
670  NEXT I
680  NEXT J
690  NEXT X
700  F=0
710  FOR I=1 TO A
720  F=F+(B[I] ** 2)
730  NEXT I
740  F=F/(B*C)
750  F=F-A4
```

```
760  G=A-1
770  H=0
780  FOR J=1 TO B
790  H=H+(C[J] ** 2)
800  NEXT J
810  H=H/(A*C)
820  H=H-A4
830  K=B-1
840  FOR I=1 TO A
850  X=0
860  FOR J=1 TO (B*C) STEP C
870  X=X+1
880  FOR Z=0 TO (C-1)
890  D[I,X]=D[I,X]+A[I,(J+Z)]
900  NEXT Z
910  NEXT J
920  NEXT I
930  L=0
940  FOR J=1 TO B
950  FOR I=1 TO A
960  L=L+D[I,J] ** 2
970  NEXT I
980  NEXT J
990  L=L/C
1000  L=L-F-H-A4
1010  M=G*K
1020  N=A5-L-H-F
1030  O=((A*B*C)-1)-(M+G+K)
1040  MAT  PRINT A
1050  IF P=0 THEN 1070
1060  MAT  PRINT #2;A
1070  PRINT "DO YOU WANT A,B,OR A*B ANALYSING USING ORTHOGONAL POLYNOMIALS?"
1080  PRINT "YES=1 NO=0"
1090  INPUT P2
1100  IF P2=0 THEN 2870
1110  PRINT "ENTER TREATMENT LEVELS FOR VARIABLE A"
1120  MAT E=ZER[A,5]
1130  L1=L2=L3=0
1140  FOR I=1 TO A
1150  INPUT E[I,1]
1160  NEXT I
1170  FOR I=1 TO A
1180  E[I,2]=E[I,1]-E[1,1]
1190  NEXT I
1200  FOR I=A TO 2 STEP -1
1210  E[I,2]=E[I,2]/E[2,2]
1220  NEXT I
1230  FOR I=1 TO A
1240  L1=L1+E[I,2]
1250  L2=L2+(E[I,2] ** 2)
1260  L3=L3+(E[I,2] ** 3)
1270  NEXT I
1280  REM LINEAR POLYNOMIALS
1290  A1=-L1/A
1300  FOR I=1 TO A
1310  E[I,3]=A1+E[I,2]
1320  NEXT I
1330  IF A<3 THEN 1590
1340  REM QUADRATIC POLYNOMIALS
1350  G1=G2=0
1360  FOR I=1 TO A
1370  G1=G1+(E[I,2]*E[I,3])
1380  G2=G2+((E[I,2] ** 2)*E[I,3])
1390  NEXT I
1400  B1=-G2/G1
1410  A2=((-(L1*B1))-L2)/A
1420  FOR I=1 TO A
1430  E[I,4]=A2+(E[I,2]*B1)+(E[I,2] ** 2)
1440  NEXT I
1450  IF A<4 THEN 1590
1460  REM CUBIC POLYNOMIALS
1470  C1=C2=C3=0
1480  FOR I=1 TO A
1490  C1=C1+((E[I,2] ** 3)*E[I,3])
1500  C2=C2+((E[I,2] ** 2)*E[I,4])
```

```
1510  C3=C3+((E[I,2] ** 3)*E[I,4])
1520  NEXT I
1530  C4=-C3/C2
1540  B2=((-(Q2*C4))-C1)/Q1
1550  A3=(-(L1*B2)-(L2*C4)-L3)/A
1560  FOR I=1 TO A
1570  E[I,5]=A3+(B2*E[I,2])+(C4*(E[I,2] ** 2))+(E[I,2] ** 3)
1580  NEXT I
1590  PRINT "ENTER TREATMENT LEVELS FOR VARIABLE B"
1600  MAT F=ZER[B,5]
1610  L1=L2=L3=0
1620  FOR I=1 TO B
1630  INPUT F[I,1]
1640  NEXT I
1650  FOR I=1 TO B
1660  F[I,2]=F[I,1]-F[1,1]
1670  NEXT I
1680  FOR I=B TO 2 STEP -1
1690  F[I,2]=F[I,2]/F[2,2]
1700  NEXT I
1710  FOR I=1 TO B
1720  L1=L1+F[I,2]
1730  L2=L2+(F[I,2] ** 2)
1740  L3=L3+(F[I,2] ** 3)
1750  NEXT I
1760  REM LINEAR POLYNOMIALS
1770  A1=-L1/B
1780  FOR I=1 TO B
1790  F[I,3]=A1+F[I,2]
1800  NEXT I
1810  IF B<3 THEN 2070
1820  REM QUADRATIC POLYNOMIALS
1830  Q1=Q2=0
1840  FOR I=1 TO B
1850  Q1=Q1+(F[I,2]*F[I,3])
1860  Q2=Q2+((F[I,2] ** 2)*F[I,3])
1870  NEXT I
1880  B1=-Q2/Q1
1890  A2=((-(L1*B1))-L2)/B
1900  FOR I=1 TO B
1910  F[I,4]=A2+(F[I,2]*B1)+(F[I,2] ** 2)
1920  NEXT I
1930  IF B<4 THEN 2070
1940  REM CUBIC POLYNOMIALS
1950  C1=C2=C3=0
1960  FOR I=1 TO B
1970  C1=C1+((F[I,2] ** 3)*F[I,3])
1980  C2=C2+((F[I,2] ** 2)*F[I,4])
1990  C3=C3+((F[I,2] ** 3)*F[I,4])
2000  NEXT I
2010  C4=-C3/C2
2020  B2=((-(Q2*C4))-C1)/Q1
2030  A3=(-(L1*B2)-(L2*C4)-L3)/B
2040  FOR I=1 TO B
2050  F[I,5]=A3+(B2*F[I,2])+(C4*(F[I,2] ** 2))+(F[I,2] ** 3)
2060  NEXT I
2070  PRINT "                                   VARIABLE A"
2080  PRINT "                                   ----------"
2090  PRINT "TREATMENT","REDUCED"," ","POLYNOMIALS"," "
2100  PRINT "LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
2110  MAT  PRINT E
2120  PRINT "                                   VARIABLE B"
2130  PRINT "                                   ----------"
2140  PRINT "TREATMENT","REDUCED"," ","POLYNOMIALS"," "
2150  PRINT "LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
2160  MAT  PRINT F
2170  GOTO 2310
2180  IF P=0 THEN 2310
2190  PRINT #2," "                                VARIABLE A"
2200  PRINT #2," "                                ----------"
2210  PRINT #2,"TREATMENT","REDUCED"," ","POLYNOMIALS"," "
2220  PRINT #2,"LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
2230  MAT  PRINT #2,E
2240  PRINT #2
2250  PRINT #2," "                                VARIABLE B"
```

```
2260  PRINT #2;"                                          ----------"
2270  PRINT #2;"TREATMENT","REDUCED"," ","POLYNOMIALS"," "
2280  PRINT #2;"LEVELS","LEVELS","LINEAR","QUADRATIC","CUBIC"
2290  MAT  PRINT #2;F
2300  PRINT #2
2310  I=0
2320  FOR J=1 TO A*B STEP B
2330  I=I+1
2340  FOR X=0 TO B-1
2350  G[1,J+X]=E[I,3]
2360  G[2,J+X]=E[I,4]
2370  G[3,J+X]=E[I,5]
2380  NEXT X
2390  NEXT J
2400  X=-1
2410  FOR I=1 TO B
2420  X=X+1
2430  FOR J=1 TO A*B STEP B
2440  G[4,(J+X)]=F[I,3]
2450  G[5,J+X]=F[I,4]
2460  G[6,J+X]=F[I,5]
2470  NEXT J
2480  NEXT I
2490  FOR J=1 TO A*B
2500  G[7,J]=G[1,J]*G[4,J]
2510  G[8,J]=G[1,J]*G[5,J]
2520  G[9,J]=G[1,J]*G[6,J]
2530  G[10,J]=G[2,J]*G[4,J]
2540  G[11,J]=G[2,J]*G[5,J]
2550  G[12,J]=G[2,J]*G[6,J]
2560  G[13,J]=G[3,J]*G[4,J]
2570  G[14,J]=G[3,J]*G[5,J]
2580  G[15,J]=G[3,J]*G[6,J]
2590  NEXT J
2600  X=0
2610  FOR I=1 TO A
2620  FOR J=1 TO B
2630  X=X+1
2640  H[1,X]=D[I,J]
2650  NEXT J
2660  NEXT I
2670  IF P=0 THEN 2740
2680  PRINT #2;"                                  TREATMENT TOTALS"
2690  PRINT #2;"                                  ----------------"
2700  MAT  PRINT #2;H
2710  PRINT #2;"                                ALL POLYNIOMIALS"
2720  PRINT #2;"                                ----------------"
2730  MAT  PRINT #2;G
2740  MAT K=ZER[15]
2750  FOR I=1 TO 15
2760  R=G=0
2770  FOR J=1 TO (A*B)
2780  R=R+H[1,J]*G[I,J]
2790  G=G+G[I,J] ** 2
2800  NEXT J
2810  IF G=0 THEN 2840
2820  R=R ** 2/(G*C)
2830  GOTO 2850
2840  R=0
2850  K[I]=R
2860  NEXT I
2870  PRINT "SOURCE OF","DEGREES OF","SUM OF","MEAN"," F-"
2880  PRINT "VARIATION"," FREEDOM ","SQUARES","SQUARE","RATIO"
2890  PRINT "-------------------------------------------------------------------------------"
2900  PRINT
2910  PRINT "      A    ",G,F,F/G,(F/G)/(N/O)
2920  IF P2=0 THEN 2980
2930  PRINT "A linear","1",K[1],K[1],K[1]/(N/O)
2940  IF A<3 THEN 2980
2950  PRINT "A qudratic","1",K[2],K[2],K[2]/(N/O)
2960  IF A<4 THEN 2980
2970  PRINT "A cubic","1",K[3],K[3],K[3]/(N/O)
2980  PRINT "      B    ",K,H,H/K,(H/K)/(N/O)
2990  IF P2=0 THEN 3050
3000  PRINT "B linear","1",K[4],K[4],K[4]/(N/O)
```

```
3010  IF B<3 THEN 3050
3020  PRINT "B quadratic","1",K[5],K[5],K[5]/(N/O)
3030  IF B<4 THEN 3050
3040  PRINT "B cubic","1",K[6],K[6],K[6]/(N/O)
3050  PRINT "     A*B   ",M,L,L/M,(L/M)/(N/O)
3060  IF P2=0 THEN 3240
3070  PRINT "AL*BL","1",K[7],K[7],K[7]/(N/O)
3080  IF B<3 THEN 3120
3090  PRINT "AL*BQ","1",K[8],K[8],K[8]/(N/O)
3100  IF B<4 THEN 3120
3110  PRINT "AL*BC","1",K[9],K[9],K[9]/(N/O)
3120  IF A<3 THEN 3240
3130  PRINT "AQ*BL","1",K[10],K[10],K[10]/(N/O)
3140  IF B<3 THEN 3180
3150  PRINT "AQ*BQ","1",K[11],K[11],K[11]/(N/O)
3160  IF B<4 THEN 3180
3170  PRINT "AQ*BC","1",K[12],K[12],K[12]/(N/O)
3180  IF A<4 THEN 3240
3190  PRINT "AC*BL","1",K[13],K[13],K[13]/(N/O)
3200  IF B<3 THEN 3240
3210  PRINT "AC*BQ","1",K[14],K[14],K[14]/(N/O)
3220  IF B<4 THEN 3240
3230  PRINT "AC*BC","1",K[15],K[15],K[15]/(N/O)
3240  PRINT "    ERROR ",O,N,N/O
3250  PRINT "-------------------------------------------------------------------------------"
3260  PRINT "    TOTAL ",(A*B*C)-1,A5
3270  PRINT "-------------------------------------------------------------------------------"
3280  IF P=0 THEN 3700
3290  PRINT #2;"SOURCE OF","DEGREES OF","SUM OF","MEAN"," F-"
3300  PRINT #2;"VARIATION","FREEDOM","SQUARES","SQUARE","RATIO"
3310  PRINT #2;"-------------------------------------------------------------------------------"
3320  PRINT #2
3330  PRINT #2;"      A    ",Q,F,F/Q,(F/Q)/(N/O)
3340  IF P2=0 THEN 3400
3350  PRINT #2;"A linear","1",K[1],K[1],K[1]/(N/O)
3360  IF A<3 THEN 3400
3370  PRINT #2;"A quadratic","1",K[2],K[2],K[2]/(N/O)
3380  IF A<4 THEN 3400
3390  PRINT #2;"A cubic","1",K[3],K[3],K[3]/(N/O)
3400  PRINT #2;"      B    ",K,H,H/K,(H/K)/(N/O)
3410  IF P2=0 THEN 3470
3420  PRINT #2;"B linear","1",K[4],K[4],K[4]/(N/O)
3430  IF B<3 THEN 3470
3440  PRINT #2;"B quadratic","1",K[5],K[5],K[5]/(N/O)
3450  IF B<4 THEN 3470
3460  PRINT #2;"B cubic","1",K[6],K[6],K[6]/(N/O)
3470  PRINT #2;"     A*B   ",M,L,L/M,(L/M)/(N/O)
3480  IF P2=0 THEN 3660
3490  PRINT #2;"AL*BL","1",K[7],K[7],K[7]/(N/O)
3500  IF B<3 THEN 3540
3510  PRINT #2;"AL*BQ","1",K[8],K[8],K[8]/(N/O)
3520  IF B<4 THEN 3540
3530  PRINT #2;"AL*BC","1",K[9],K[9],K[9]/(N/O)
3540  IF A<3 THEN 3660
3550  PRINT #2;"AQ*BL","1",K[10],K[10],K[10]/(N/O)
3560  IF B<3 THEN 3600
3570  PRINT #2;"AQ*BQ","1",K[11],K[11],K[11]/(N/O)
3580  IF B<4 THEN 3600
3590  PRINT #2;"AQ*BC","1",K[12],K[12],K[12]/(N/O)
3600  IF A<4 THEN 3660
3610  PRINT #2;"AC*BL","1",K[13],K[13],K[13]/(N/O)
3620  IF B<3 THEN 3660
3630  PRINT #2;"AC*BQ","1",K[14],K[14],K[14]/(N/O)
3640  IF B<4 THEN 3660
3650  PRINT #2;"AC*BC","1",K[15],K[15],K[15]/(N/O)
3660  PRINT #2;"  ERROR ",O,N,N/O
3670  PRINT #2;"-------------------------------------------------------------------------------"
3680  PRINT #2;"   TOTAL ",(A*B*C)-1,A5
3690  PRINT #2;"-------------------------------------------------------------------------------"
3700  END
```

APPENDIX 3

Analysis of variance (AOV) summary tables for biomass data of taxa present in the Checkley streams.

AOV summary tables - *Cladophora* biomass.

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT TREATMENT	2	15.5593	7.77963	24.6468
A v (B+C)/2	1	12.5313	12.5313	39.7008
B v C	1	3.02792	3.02792	9.59284
MAIN-PLOT ERROR	24	7.57545	.315644	
SUB-PLOT TREATMENT	8	12.4807	1.56009	7.38205
INTERACTION	16	6.21383	.388364	1.83767
SUB-PLOT ERROR	192	40.5763	.211335	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT TREATMENT	2	30.7855	15.3928	109.108
A v (B+C)/2	1	30.6741	30.6741	217.427
B v C	1	.111362	.111362	.78937
MAIN-PLOT ERROR	24	3.38586	.141078	
SUB-PLOT TREATMENT	8	22.0213	2.75266	16.1483
INTERACTION	16	16.4587	1.02867	6.03465
SUB-PLOT ERROR	192	32.7284	.170461	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT TREATMENT	2	57.3292	28.6646	149.4
A v (B+C)/2	1	57.1607	57.1607	297.921
B v C	1	.168649	.168649	.878996
MAIN-PLOT ERROR	24	4.60477	.191865	
SUB-PLOT TREATMENT	8	26.256	3.282	12.8806
INTERACTION	16	54.219	3.38869	13.2994
SUB-PLOT ERROR	192	48.9217	.254801	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT TREATMENT	2	88.431	44.2155	130.868
A v (B+C)/2	1	84.6735	84.6735	250.615
B v C	1	3.75784	3.75784	11.1224
MAIN-PLOT ERROR	24	8.1087	.337863	
SUB-PLOT TREATMENT	8	7.1217	.890213	2.64233
INTERACTION	16	18.2294	1.13934	3.38178
SUB-PLOT ERROR	192	64.6857	.336905	
TOTAL	242			

AOV summary tables - *Vaucheria* biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.980112	.490056	4.79515
A v (B+C)/2	1	.955857	.955857	9.35297
B v C	1	2.42555E-02	2.42555E-02	.237337
MAIN-PLOT ERROR	24	2.45276	.102198	
SUB-PLOT				
TREATMENT	8	2.68487	.335609	5.45234
INTERACTION	16	1.98483	.124052	2.01537
SUB-PLOT ERROR	192	11.8182	6.15531E-02	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	7.45827E-04	3.72914E-04	.999992
A v (B+C)/2	1	7.45833E-04	7.45833E-04	2.
B v C	1	0	0	0
MAIN-PLOT ERROR	24	.00895	3.72917E-04	
SUB-PLOT				
TREATMENT	8	2.98333E-03	3.72916E-04	.999998
INTERACTION	16	5.96667E-03	3.72917E-04	1.
SUB-PLOT ERROR	192	.0716	3.72916E-04	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.274507	.137254	3.81347
A v (B+C)/2	1	.263859	.263859	7.33109
B v C	1	1.06501E-02	1.06501E-02	.295904
MAIN-PLOT ERROR	24	.863802	3.59917E-02	
SUB-PLOT				
TREATMENT	8	1.34537	.168171	3.94106
INTERACTION	16	1.38789	.086743	2.0328
SUB-PLOT ERROR	192	8.19295	4.26716E-02	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.196444	9.82222E-02	1.39058
A v (B+C)/2	1	1.69667E-05	1.69667E-05	2.40206E-04
B v C	1	.196428	.196428	2.78094
MAIN-PLOT ERROR	24	1.69521	7.06339E-02	
SUB-PLOT				
TREATMENT	8	.8293	.103662	2.27966
INTERACTION	16	1.82363	.113977	2.50648
SUB-PLOT ERROR	192	8.73079	4.54728E-02	
TOTAL	242			

AOV summary tables - *Oedogonium* biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	2.01162	1.00581	13.2314
A v (B+C)/2	1	830447	.830447	10.9245
B v C	1	1 18117	1.18117	15.5383
MAIN-PLOT ERROR	24	1.82441	7.60171E-02	
SUB-PLOT				
TREATMENT	8	2.38352	.29794	6.96787
INTERACTION	16	2.59498	.162186	3.79302
SUB-PLOT ERROR	192	8.20975	4.27591E-02	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.645684	.322842	14.6837
A v (B+C)/2	1	.590395	.590395	26.8527
B v C	1	5.52898E-02	5.52898E-02	2.51472
MAIN-PLOT ERROR	24	.527675	2.19864E-02	
SUB-PLOT				
TREATMENT	8	2.12753	.265942	9.62681
INTERACTION	16	2.41046	.150654	5.45351
SUB-PLOT ERROR	192	5.30402	2.76251E-02	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	2.03482	1.01741	9.1424
A v (B+C)/2	1	2.03292	2.03292	18.2677
B v C	1	1.89981E-03	1.89981E-03	1.70716E-02
MAIN-PLOT ERROR	24	2.67084	.111285	
SUB-PLOT				
TREATMENT	8	5.27836	.659795	8.25896
INTERACTION	16	8.41557	.525973	6.58385
SUB-PLOT ERROR	192	15.3386	7.98885E-02	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.891687	.445844	10.9831
A v (B+C)/2	1	.723413	.723413	17.8209
B v C	1	.168274	.168274	4.14534
MAIN-PLOT ERROR	24	.974243	4.05934E-02	
SUB-PLOT				
TREATMENT	8	2.74772	.343465	10.1672
INTERACTION	16	5.18177	.323861	9.5869
SUB-PLOT ERROR	192	6.48606	3.37816E-02	
TOTAL	242			

AOV summary tables - *Batrachospermum* biomass

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	7.45827E-04	3.72914E-04	.999992
A v (B+C)/2	1	7.45833E-04	7.45833E-04	2.
B v C	1	0	0	0
MAIN-PLOT ERROR	24	.00895	3.72917E-04	
SUB-PLOT				
TREATMENT	8	2.98333E-03	3.72916E-04	.999998
INTERACTION	16	5.96667E-03	3.72917E-04	1.
SUB-PLOT ERROR	192	.0716	3.72916E-04	
TOTAL	242			

AOV summary tables - *Microspora* biomass

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	1.19333E-02	5.96664E-03	.999996
A v (B+C)/2	1	2.98333E-03	2.98333E-03	.499999
B v C	1	8.94999E-03	8.94999E-03	1.5
MAIN-PLOT ERROR	24	.1432	5.96667E-03	
SUB-PLOT				
TREATMENT	8	4.77333E-02	5.96667E-03	1.
INTERACTION	16	9.54667E-02	5.96667E-03	1.
SUB-PLOT ERROR	192	1.1456	5.96666E-03	
TOTAL	242			

AOV summary tables - *Amblystegium* biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	1.40189	.700947	7.14781
A v (B+C)/2	1	1.13288	1.13288	11.5524
B v C	1	.26901	.26901	2.74319
MAIN-PLOT ERROR	24	2.35355	9.80645E-02	
SUB-PLOT				
TREATMENT	8	.858846	.107356	1.39447
INTERACTION	16	1..13957	.071223	.925134
SUB-PLOT ERROR	192	14.7814	7.69867E-02	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.641715	.320857	7.62838
A v (B+C)/2	1	.230076	.230076	5.47006
B v C	1	.411639	.411639	9.78671
MAIN-PLOT ERROR	24	1.00946	.042061	
SUB-PLOT				
TREATMENT	8	.927995	.115999	2.13361
INTERACTION	16	1.05531	6.59569E-02	1.21317
SUB-PLOT ERROR	192	10.4386	5.43675E-02	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.215387	.107694	2.00454
A v (B+C)/2	1	.142291	.142291	2.64852
B v C	1	7.30962E-02	7.30962E-02	1.36057
MAIN-PLOT ERROR	24	1.2894	5.37249E-02	
SUB-PLOT				
TREATMENT	8	.301309	3.76636E-02	.964894
INTERACTION	16	.725538	4.53461E-02	1.16171
SUB-PLOT ERROR	192	7.49452	3.90339E-02	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.205803	.102902	4.33989
A v (B+C)/2	1	8.77699E-02	8.77699E-02	3.7017
B v C	1	.118034	.118034	4.97809
MAIN-PLOT ERROR	24	.569056	2.37107E-02	
SUB-PLOT				
TREATMENT	8	.46421	5.80263E-02	2.08647
INTERACTION	16	.885083	5.53177E-02	1.98907
SUB-PLOT ERROR	192	5.33967	2.78108E-02	
TOTAL	242			

AOV summary tables - *Elodea* biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	2.98335E-03	1.49167E-03	2.28572
A v (B+C)/2	1	7.45834E-04	7.45834E-04	1.14286
B v C	1	2.23750E-03	2.23750E-03	3.42857
MAIN-PLOT ERROR	24	1.56625E-02	6.52604E-04	
SUB-PLOT				
TREATMENT	8	5.22084E-03	6.52605E-04	.861541
INTERACTION	16	1.04417E-02	6.52604E-04	.861538
SUB-PLOT ERROR	192	.145437	7.57486E-04	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	1.08115E-02	5.40574E-03	.999993
A v (B+C)/2	1	2.70289E-03	2.70289E-03	.5
B v C	1	8.10866E-03	8.10866E-03	1.5
MAIN-PLOT ERROR	24	.129739	5.40578E-03	
SUB-PLOT				
TREATMENT	8	4.32461E-02	5.40577E-03	.999998
INTERACTION	16	8.64925E-02	5.40578E-03	1.
SUB-PLOT ERROR	192	1.03791	5.40578E-03	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	16.3194	8.15968	67.6825
A v (B+C)/2	1	4.07984	4.07984	33.8412
B v C	1	12.2395	12.2395	101.524
MAIN-PLOT ERROR	24	2.8934	.120558	
SUB-PLOT				
TREATMENT	8	4.83207	.604009	3.50483
INTERACTION	16	9.66414	.604009	3.50483
SUB-PLOT ERROR	192	33.0885	.172336	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	3.94428	1.97214	11.4666
A v (B+C)/2	1	.986071	.986071	5.73328
B v C	1	2.95821	2.95821	17.1998
MAIN-PLOT ERROR	24	4.12778	.171991	
SUB-PLOT				
TREATMENT	8	1.59703	.199629	2.14596
INTERACTION	16	3.19406	.199629	2.14597
SUB-PLOT ERROR	192	17.8608	9.30252E-02	
TOTAL	242			

AOV summary tables - *Potamogeton* biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	8.22877E-02	4.11439E-02	2.2207
A v (B+C)/2	1	8.22881E-02	8.22881E-02	4.44141
B v C	1	0	0	0
MAIN-PLOT ERROR	24	.444659	1.85275E-02	
SUB-PLOT				
TREATMENT	8	.329152	.041144	2.2207
INTERACTION	16	.638305	.041144	2.22071
SUB-PLOT ERROR	192	3.55727	1.85274E-02	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.476048	.238024	3.7184
A v (B+C)/2	1	.476049	.476049	7.43681
B v C	1	0	0	0
MAIN-PLOT ERROR	24	1.5363	6.40125E-02	
SUB-PLOT				
TREATMENT	8	1.15305	.144132	4.08978
INTERACTION	16	2.30611	.144132	4.08979
SUB-PLOT ERROR	192	6.76643	3.52418E-02	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	84.4746	42.2373	47.2785
A v (B+C)/2	1	82.8082	82.8082	92.6916
B v C	1	1.66632	1.66632	1.8652
MAIN-PLOT ERROR	24	21.4409	.893373	
SUB-PLOT				
TREATMENT	8	6.12282	.765352	3.03124
INTERACTION	16	42.4778	2.65486	10.5148
SUB-PLOT ERROR	192	48.4777	.252488	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	131.128	65.5641	283.194
A v (B+C)/2	1	130.947	130.947	565.604
B v C	1	.181345	.181345	.783293
MAIN-PLOT ERROR	24	5.5564	.231517	
SUB-PLOT				
TREATMENT	8	6.15002	.768753	3.59107
INTERACTION	16	11.4648	.716553	3.34723
SUB-PLOT ERROR	192	41.1021	.214074	
TOTAL	242			

AOV summary tables - *Callitriche* biomass

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	4.98369E-03	2.49184E-03	1.
A v (B+C)/2	1	1.24592E-03	1.24592E-03	.5
B v C	1	3.73776E-03	3.73776E-03	1.5
MAIN-PLOT ERROR	24	5.98041E-02	2.49184E-03	
SUB-PLOT				
TREATMENT	8	1.99347E-02	2.49184E-03	.999999
INTERACTION	16	3.98694E-02	2.49184E-03	.999999
SUB-PLOT ERROR	192	.478433	2.49184E-03	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	2.85758E-02	1.42879E-02	.999999
A v (B+C)/2	1	7.14394E-03	7.14394E-03	.5
B v C	1	2.14318E-02	2.14318E-02	1.5
MAIN-PLOT ERROR	24	.342909	1.42879E-02	
SUB-PLOT				
TREATMENT	8	.114303	1.42879E-02	1.
INTERACTION	16	.228606	1.42879E-02	1.
SUB-PLOT ERROR	192	2.74327	1.42879E-02	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.109422	5.47109E-02	2.24726
A v (B+C)/2	1	2.73555E-02	2.73555E-02	1.12363
B v C	1	8.20666E-02	8.20666E-02	3.37089
MAIN-PLOT ERROR	24	.584295	2.43456E-02	
SUB-PLOT				
TREATMENT	8	.437688	.054711	2.24726
INTERACTION	16	.875377	.054711	2.24726
SUB-PLOT ERROR	192	4.67436	2.43456E-02	
TOTAL	242			

AOV Summary tables . - 'Other Macrophytes' biomass

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	1.04679E-02	5.23395E-03	.92308
A v (B+C)/2	1	7.45213E-03	7.45213E-03	1.31429
B v C	1	3.01579E-03	3.01579E-03	.531876
MAIN-PLOT ERROR	24	.136082	5.67009E-03	
SUB-PLOT				
TREATMENT	8	2.33279E-02	2.91599E-03	.716413
INTERACTION	16	6.39965E-02	4.12478E-03	1.01339
SUB-PLOT ERROR	192	.781491	4.07026E-03	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.234881	.11744	3.05284
A v (B+C)/2	1	.188	.188	4.88702
B v C	1	4.68815E-02	4.68815E-02	1.21868
MAIN-PLOT ERROR	24	.923261	3.84692E-02	
SUB-PLOT				
TREATMENT	8	.243851	3.04814E-02	.693747
INTERACTION	16	.880576	.055036	1.2526
SUB-PLOT ERROR	192	8.43597	4.39373E-02	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	23.6407	11.8203	15.189
A v (B+C)/2	1	4.48037	4.48037	5.75723
B v C	1	19.1603	19.1603	24.6207
MAIN-PLOT ERROR	24	18.6772	.778217	
SUB-PLOT				
TREATMENT	8	12.0965	1.51206	3.4905
INTERACTION	16	12.6355	.789716	1.82301
SUB-PLOT ERROR	192	83.173	.433193	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	3.11259	1.5563	7.48294
A v (B+C)/2	1	3.09479	3.09479	14.8803
B v C	1	1.78038E-02	1.78038E-02	8.56035E-02
MAIN-PLOT ERROR	24	4.9915	.207979	
SUB-PLOT				
TREATMENT	8	10.3885	1.29857	5.07655
INTERACTION	16	17.056	1.066	4.16738
SUB-PLOT ERROR	192	49.1129	.255797	
TOTAL	242			

AOV summary tables - Volatile solids (ash-free dry weight)

UPPER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	1.41942E-02	7.09709E-03	6.47582
A v (B+C)/2	1	1.08593E-02	1.08593E-02	9.90872
B v C	1	3.33481E-03	3.33481E-03	3.04289
MAIN-PLOT ERROR	24	2.63025E-02	1.09594E-03	
SUB-PLOT				
TREATMENT	8	2.56571E-02	3.20714E-03	4.1114
INTERACTION	16	2.27897E-02	1.42436E-03	1.82596
SUB-PLOT ERROR	192	.149772	7.80060E-04	
TOTAL	242			

LOWER RIFFLE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	4.00346E-02	2.00173E-02	7.80285
A v (B+C)/2	1	3.77548E-02	3.77548E-02	14.717
B v C	1	2.27989E-03	2.27989E-03	.888713
MAIN-PLOT ERROR	24	6.15692E-02	2.56538E-03	
SUB-PLOT				
TREATMENT	8	.066369	8.29612E-03	4.16453
INTERACTION	16	7.21849E-02	4.51156E-03	2.26473
SUB-PLOT ERROR	192	.382482	1.99209E-03	
TOTAL	242			

UPPER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	2.32658E-02	1.16329E-02	.917775
A v (B+C)/2	1	1.59912E-03	1.59912E-03	.126162
B v C	1	2.16676E-02	2.16676E-02	1.70946
MAIN-PLOT ERROR	24	.304203	1.26751E-02	
SUB-PLOT				
TREATMENT	8	.102958	1.28697E-02	1.22153
INTERACTION	16	.421981	2.63738E-02	2.50327
SUB-PLOT ERROR	192	2.02286	1.05358E-02	
TOTAL	242			

LOWER POOL

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
MAIN-PLOT				
TREATMENT	2	.119512	.059756	6.72936
A v (B+C)/2	1	9.11468E-02	9.11468E-02	10.2644
B v C	1	2.83676E-02	2.83676E-02	3.19458
MAIN-PLOT ERROR	24	.213118	8.87990E-03	
SUB-PLOT				
TREATMENT	8	4.31838E-02	5.39798E-03	.830933
INTERACTION	16	.463907	2.89942E-02	4.46319
SUB-PLOT ERROR	192	1.24729	6.49628E-03	
TOTAL	242			

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