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Variation of growth and reproduction in a marine bryozoan.

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Award date:
1991

Awarding institution:
Bangor University

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VARIATION OF GROWTH AND REPRODUCTION IN A MARINE BRYOZOAN

A thesis

submitted to the University of Wales

in candidature for the degree of Philosophiae Doctor

by

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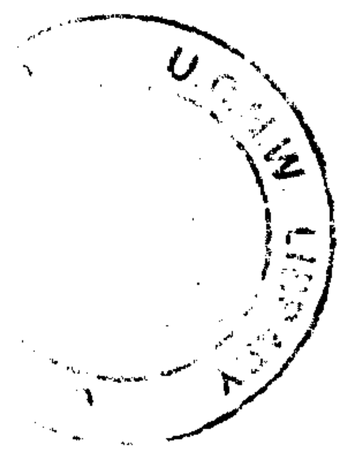
October 1991



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Acknowledgements

I would like to thank my supervisor, Prof. R.N. Hughes for his support and encouragement throughout the course of this project. I am also grateful to Dr. J. Davenport and Prof. E. Naylor for providing the research facilities. Special thanks are due to Mr. M.D. Budd, whose help and advice was invaluable in setting up and maintaining the culture method.

I would also like to thank Dr. M.T. Burrows, Mr. C. Whittaker and Dr. C. Gliddon, all of whom provided advice on computing and statistics at some stage in this project, Mr G. Allen for his assistance with the graphics, and Dr. D.J.Hughes for his advice on the completion of this thesis.

I am grateful to Mr. R.L. Roberts and Mr. G. Jones, both of whom provided transport to and from the School of Ocean Sciences raft. Mr. R.G. Hickson, Mr. M. Thomas, Miss S. Perks, Mr. A. Davies, Mr. E. Evans, Mr. B. Tilley, Mr. L. Tilley, Mr. A. Jones, and Mr. G. Edwards all provided technical assistance at some stage of this work, and Mr. E.W. Pritchard and Mr D.J. Roberts provided help with the photography.

Finally, I would like to thank all the people who made my stay in Bangor bearable, especially Andy, Jane and Caroline.

Summary

This thesis investigates aspects of growth and reproduction in the marine bryozoan Celleporella hyalina (L.).

It was found that C. hyalina could live and grow in the laboratory on a range of algal diets. Chlorophyte algae proved unsuitable, but Rhodomonas baltica proved outstanding as a foodstuff. Astogeny of Rhodomonas fed colonies was the same as that reported for naturally occurring colonies, and resulted in the production of viable larvae. It was further noted that female zooids were occasionally produced in the basal layer.

C. hyalina colonies were able to grow and attain sexual maturity in cell concentrations ranging from 10 to 300 cells μl^{-1}. Growth was greatest at around 100 cells μl^{-1}, and was suppressed at low and extremely high cell concentrations. Food supply had a highly significant effect on growth parameters, but sexual parameters were largely unaffected. Cell ingestion rate increased as a function of cell concentration, whereas feeding episode length declined with increasing food supply. Feeding behaviour in C. hyalina is described.

Highly significant genotype-environment interactions were found at all levels for both somatic and sexual parameters in 26 C. hyalina clones grown in four temperature/food supply combinations (macroenvironments). The number of autozooids per unit area was found to be strongly temperature dependent. The number of ovicells was consistently greater than the number of embryos produced. Male investment was favoured under conditions of low resource. Ranking of clonal performance varied considerably, both between macroenvironments, and according to the measure of fitness used.

Significant levels of intraclonal variation occurred within replicates of a single genotype grown in four macroenvironments. Variance was more prominent in somatic rather than sexual parameters. The presence of one statistical outlyer suggested that somatic mutations may sometimes occur.

C. hyalina colonies grown in isolation produced embryos sometimes as early as five weeks, but the number was low, and a high incidence of abortion was observed. When removed from isolation, the number of embryos increased significantly. There was no evidence of settlement from larvae produced in isolation.

General introduction

Bryozoans are an extremely widespread group of primarily marine, exclusively colonial organisms (Ryland 1976). Like most other colonial benthic invertebrates, bryozoans have the capacity for sexual reproduction while simultaneously growing asexually by means of vegetative propagation. Such clonal organisms of modular construction (Harper and Bell 1980, Hughes 1989), can often present an array of characters suitable for biometric study, allowing comparisons of morphological variation both within and between clones based on a range of complex zooecial features (eg Jackson and Cheetham 1990, D.J.Hughes and Jackson 1990). Studies of this type have provided evidence supporting the hypothesis that environmental stability favours local genetic differentiation (Schopf 1976, Schopf and Dutton 1976). Morphometric studies of fossil bryozoan species have yielded similar results (Farmer and Rowell 1973, Pachut and Anstey 1979, Pachut 1982). Comparable studies on variability in reproductive strategy, however, present more difficulties in that many bryozoans possess hermaphroditic ramets (sensu Harper 1976).

Celleporella hyalina is a common, temperate-zone algal epiphyte (Marcus 1938), and can be found in abundance on laminarian and fucoid algae around the British coast (Hayward and Ryland 1979). Shallow-water, algal epiphyte communities not only inhabit ephemeral substrata, but also experience wide temporal fluctuations in prevailing environmental conditions (Seed and O'Connor 1981, Cancino and Hughes 1987). C. hyalina is of special interest because it shows complete

modular partitioning of male, female and feeding morphs (Marcus 1938). The ease with which Celleporella can be cultivated has facilitated the study of life-history characteristics (Cancino 1986, Cancino and Hughes 1987,1988, D.J.Hughes 1989,1991, in press). However, in the absence of a suitable culture method for this bryozoan, previous studies have, of necessity, been carried out in the natural environment. These studies were, therefore, prone to the difficulties of achieving adequate experimental control under field conditions.

The laboratory culture of marine invertebrates has been well documented for commercial species (eg Bardach et al 1972), but in other cases, the development of culture techniques has been restricted on account of the cost and difficulties involved in constructing elaborate apparatus, and finding suitable diets. In this thesis, the possibility of the controlled laboratory culture of C. hyalina was investigated by rearing colonies on a range of algal diets, while monitoring subsequent growth and reproduction (chapter 1). In order for strictly controlled conditions to be maintained in laboratory-cultured colonies however, it was first necessary to gather information on feeding mechanism and behaviour (chapter 2).

Previous studies have inferred the heritability of life-history traits in C. hyalina (Cancino and Hughes 1987, D.J.Hughes 1989). If life-history patterns are assumed to be maintained by natural selection acting by differential reproductive success, then it must also be assumed that heritable variation exists for these traits within the

population (Pianka 1976, Stearns 1976, D.J.Hughes 1989). Although C. hyalina does not reproduce clonally in nature, the division and transplantation of individual colonies to provide genetically identical replicates provides a means by which the genotypic and environmental components of life-history variation can be determined (chapter 3).

The serial propagation of individual genotypes can then be used to determine the levels of variation possible within a single clone (chapter 4). Celleporella further provides a range of zooid morphs, all of which are potentially competing features in terms of resource allocation. As such, these can be taken as relative measures of clonal fitness both within and between environments (D.J.Hughes 1991, in press). Determination of the level of variation in clonal performance between treatments may help to answer certain fundamental questions with regards the maintenance of sexual reproduction in populations (Bell 1982, 1987).

Bierzychudek (1989), noted that apomictic taxa tend to occupy a broader geographical range, and tend to be more tolerant of environmental extremes than their sexual counterparts, and that such organisms may possess 'general purpose' genotypes, where an organism's performance is relatively unaffected by the environment that it experiences. Evidence of clonal variation in growth rate, sex ratio and reproductive allocation suggests that this is not the case in C. hyalina (D.J. Hughes 1989), but the simultaneous presence of male and female zooids on the same colony suggests that opportunities for self fertilisation must arise (Cancino 1983). Selfing would provide a means whereby successful gene

combinations in a particular environment could be conserved, rather than being lost as a result of genetic recombination involving different genomes (Templeton 1982). A previous study (Cancino 1983) suggested that self fertilisation was indeed possible in C. hyalina colonies, but these results were largely inconclusive, on account of the experimental method employed. Controlled laboratory culture would facilitate the cultivation of isolated colonies, and would therefore, allow determination of the occurence and extent of selfing in C. hyalina populations (chapter 5).

Chapter 1

Laboratory culture of *Celleporella hyalina* (L.)

1.1 Introduction

Studies of diet in bryozoans can be divided broadly into early accounts of gut contents in a number of marine species (Hasper 1912, Hentschel 1922, Hunt 1925), and later attempts at laboratory culture (Schneider 1959, 1963, Jebram 1968, Bullivant 1968, Winston 1976, Kitamura and Hirayama 1984). The early gut analyses revealed a wide variety of ingested items, ranging from diatoms (Hasper 1912, Hentschel 1922, Hunt 1925, Marcus 1941), which were for a long time thought to be the main food source for marine bryozoans, coccolithophores (Hentschel 1922), bacteria (Hunt 1925), and even nematocysts (Hentschel 1922).

Schneider (1959, 1963) first cultivated marine bryozoans in the laboratory, rearing Bugula avicularia on the colourless dinoflagellate Oxyrrhis marina, which had itself been fed on a green flagellate, Dunaliella sp. It was found that both species were ingested by the polypide, but Dunaliella cells left the gut undigested.

Jebram (1968) also used Oxyrrhis marina to culture several bryozoan species, including Alcyonidium sp., Bowerbankia gracilis, Farella repens, Electra crustulenta, E. monostachys, Conopeum seurati, C. reticulum and Bugula stolonifera. Jebram went on to look at the effects of growing Bryozoa in controlled water currents (1970, 1973). He found that changes in the proportional morphology of the gut could be produced according to the nutritional conditions. Further experiments investigated the qualitative and quantitative effects of many different algal diets (Jebram 1975, 1980a, 1980b, Jebram and Rummert 1978), leading to the conclusion

that variations in nutrition could frequently cause morphological modifications of zooids and zoaria in bryozoans (Jebram 1982). Similar conclusions were reached by Winston (1976), who looked at the effects of different unicellular algae on the growth of Conopeum tenuissimum. In this case, species of algae differing in food value were found to affect both the pattern and the amount of colony growth, rather than zooidal morphology.

The nutritional adaptability of bryozoans is another question about which little is known. Stephens and Schinske (1961) found Bugula turrita to be capable of removing amino acids directly from seawater. Winston (1978) noted that colonies of Flustrellidra hispida contain many irregular particles of sand, silt and organic detritus. It is quite possible that bacteria and other coating material could be digested from the surface of these inorganic particulates. Best (1985) also contemplated the availability as food of abraded algal particles and algal exudates. Dissolved organic matter, including exudate from a brown-algal substratum, has been demonstrated to serve as a nutrient source for Membranipora membranacea (De Burgh and Fankboner 1978). Indeed, Best and Thorpe (1991), have recently provided evidence that certain bryozoan species may be capable of the facultative absorption of dissolved organic matter in the absence of an alternative food source. Schopf (1969) found carbonate detritus in the rectum of deep-sea bryozoans, indicating that detrital material is ingested at least under certain conditions. However, phytoplankton would appear to

provide the main source of nutrition in most bryozoan species.

Clearance rates (Bullivant 1968a, Menon and Nair 1972), heat tolerance, growth and regeneration (Menon 1974), have also been examined in laboratory-reared bryozoans, but with the exception of Winston's (1976) study of Conopeum seurati, very few data exist regarding life-history strategies adopted under different nutritional regimes. Previous attempts to culture marine bryozoans have usually been handicapped by poor control of cell concentration and experimental design, frequently having been carried out under unnatural conditions over limited time periods. As a result, sexual maturity of colonies has rarely been attained in the laboratory (Jebram 1977).

To avoid the difficulties of laboratory culture, recent studies of the marine bryozoan Celleporella hyalina (L.) have relied upon manipulations of water flow in the natural environment (Cancino and Hughes 1987, D.J. Hughes 1989). This method relies on the untested assumption that a reduced flow rate is equivalent to a reduced food supply. Moreover, it is potentially confounded by the uncontrolled effects of other factors present under field conditions (Cancino and Hughes 1987). A more satisfactory experimental programme could be designed if selected environmental variables were controlled under laboratory conditions. This would be possible only if a suitable culture method and an acceptable algal diet could be found.

Jebram (1977) has reported C. hyalina to be one of those species in which "rearing could be maintained for more or

less short times only." And although culture methods suitable for a variety of bryozoans are already available (Wood 1971, Jebram 1977, Emscherman 1987a), there remains a need for a cheap and effective method, which is both spatially economical, and maintainable over extended periods of time.

1.2 Materials and methods

Colonies of Celleporella hyalina growing on the seaweed Fucus serratus were collected from Church Island in the Menai Straits between March and June, 1988. Fronds bearing colonies were kept in complete darkness in plastic tanks containing 10μm filtered, running seawater for 24 h. Larvae are initially positively phototactic (Ryland 1960), and rapidly swim to the surface on exposure to a bright light source. These larvae were collected by pipette and transferred to glass dishes containing 0.2μm filtered, U.V.-irradiated seawater. Glass slides, which were conditioned in natural seawater for approximately 14 days in order to make them suitable as settlement substrata (Cancino 1983), were then added to the dishes. Widespread settlement was achieved by placing the dishes on a turntable and applying a jet of air contrary to the direction of rotation (Crisp 1976). Alternatively, when the available number was limited, larvae were placed directly onto slides in a water droplet. Slides were placed in petri dishes, covered by an opaque sheet, to prevent evaporation (Campbell and Maturo, pers. comm. 1988).

It was found subsequently that addition of an appropriate algal diet at this stage enhanced survival of the settled larvae, allowing feeding to begin immediately on completion of metamorphosis. The young colonies were transferred to plastic boxes containing the experimental diet in 1.25l of 0.2μm-filtered, U.V.-irradiated seawater. The slides were suspended in plastic, histological staining racks, allowing a relatively large number of animals to be held in a relatively small volume of water. Each rack was

tilted so that the bryozoans were facing downwards, encouraging faecal material to drop away from the growing colonies (Wood 1971). Culture vessels were held in a water bath maintained at a constant temperature of 18°C±1°C.

Experimental diets were added to each container to achieve a final concentration of approximately 100 cells.μl^{-1} and this medium was changed three times weekly. Slides were rotated in position every time the medium was changed, and it was assumed that the position of the colonies had no effect on growth, as long as there was no build up of debris. Circulation in each culture vessel was maintained by aeration. All colonies were carefully cleaned with a soft, artist's brush at least weekly, and the plastic racks cleaned and changed, in order to prevent the accumulation of adhering algae, and in certain cases, heterotrich ciliates, which could reach troublesome densities if not constantly removed.

The choice of algae for these experiments was limited to cultures which were available in the laboratory as standard invertebrate diets. These cultures were maintained in 20l glass vessels, and were harvested at a level of 5l daily, so maintaining an optimum growth rate. Two cultures of any one alga were generally grown simultaneously as insurance against frequent population crashes, which characterise large-scale culture and widespread use, with the associated problems of infection. Cell counts were carried out using a haemocytometer, which also allowed the condition of the algae to be monitored, and cell densities were calculated according to the method of Cassell (1965).

The algae used in this series of experiments were:

Pavlova lutheri-Chrysophyta

Skeletonema costatum-Bacillariophyta

Rhodomonas baltica-Cryptophyta

Tetraselmis chuii-Chlorophyta

Chlorella japonica-Chlorophyta

Dunaliella tertiolecta-Chlorophyta

Two different food combinations were also tested:

R. baltica + P. lutheri

R. baltica + S. costatum

Two control conditions were set up, with colonies grown both in filtered seawater and in filtered seawater to which algal culture medium had been added (Conway medium).

Slides were selected with no more than three centrally located, widely spaced colonies, and ten colonies were used per dietary treatment.

Weekly counts were taken of feeding autozooids, degenerated autozooids (brown bodies), fully formed buds, basal males, frontal males and female zooids. Counts were undertaken with a Wild M4A dissection microscope. Each colony was drawn weekly using a camera lucida, and area and perimeter were then calculated electronically using a digitiser. Experiments ran for between 7 to 12 weeks according to algal availability and colony survival. This time scale under natural conditions would in any case be long enough for this species to complete its life cycle. Twelve weeks was chosen as an apt time to terminate experiments, as the colony centre tends to senesce at this stage, often leading to problems with infection. By this time, therefore,

it is often difficult to determine whether aberrant growth patterns are due to diet, or some external factor.

1.3 Results

Celleporella survived and grew on both Pavlova lutheri and Skeletonema costatum (figure 1.1a,b), but Rhodomonas baltica proved to be outstanding as a diet (figure 1.1d). Colonies were unable to survive on seawater and culture medium alone (see table 1.1), and were also unable to persist when given the chlorophyte Dunaliella tertiolecta. Individual zooids were able to survive for short periods when fed on a diet of Tetraselmis chuii or Chlorella japonica (figure 1.1c, table 1.1), but in both cases, little or no growth occurred, resulting in eventual colony death. Colonies fed on Rhodomonas outperformed all other colonies in terms of growth rate, final colony size, and the total number of sexual zooids produced (table 1.2). Astogeny in Rhodomonas-fed colonies (plate 1.1) was the same as that seen in naturally occurring colonies (Cancino 1983), where distal budding forms an underlying layer of autozooids, to which sexual zooids are added after 2-3 weeks (figure 1.1d).

The proportion of autozooids that were active was found to be greater as the level of nutritional adequacy increased (figure 1.1). With diets such as Tetraselmis and Chlorella, the entire colony tended to degenerate within 1-2 weeks, leaving a single active autozooid. In colonies fed on Pavlova, a large proportion of active autozooids degenerated to brown bodies, but in this case, the process was more gradual, leaving the colony with only a few active feeding zooids, often resulting in colony death. Rhodomonas-fed colonies tended to develop a compact, circular form, whereas colonies maintained on less suitable diets tended to become

lobate, resulting in a high perimeter:area ratio (figure 1.2).

Basal male zooids (plate 1.2) were invariably the first sexual zooids to be produced, irrespective of diet (figure 1.1, table 1.3). These zooids were extremely variable in size, ranging from the largest autozooids (1200 μm), to less than 400 μm in length. The smaller basal males were clearly distinct under the dissection microscope on account of their small orifice relative to that of the autozooids, milky white coloration during sperm production, and the lack of a digestive tract. Male zooids possess a lophophore, but this is colourless, and not normally visible, even at high-power magnification. The male lophophore only becomes apparent when extended, and this was only observed on a few occasions during three years of research. In healthy colonies, basal males were normally produced after 2-3 weeks, although in certain colonies they occurred as early as the first week of growth (see table 1.3). Very slow-growing colonies, especially those on inadequate diets, frequently produced one or more basal male zooid at a relatively small colony size.

Frontal budding gives rise to frontal male zooids, female zooids, and occasionally frontal autozooids (plate 1.2) (Cancino and Hughes 1987) (although due to the infrequency of their occurrence, frontal autozooids were not counted in the current study). Frontal budding started in older parts of the colony, progressing outwards as colony size increased. Frontal male and female production started near but not usually at, the colony centre, and in older colonies approached the growing peripheral meristem.

Frontal male zooids (plate 1.2) were generally more uniform in shape and size than their basal counterparts, and were not usually greater than 500 μm in length. They were never observed before three weeks of growth, and were never produced where the diet was found to be 'inadequate' (table 1.2), even where substantial numbers of basal males had been produced.

Females were the final sexual zooids to be produced (plate 1.2), but these were numerous only when Rhodomonas was present in the diet (table 1.3). However, a single Pavlova-fed colony produced three female zooids in its sixth week of growth, and a single Skeletonema-fed colony produced 1 female zooid after eleven weeks (table 1.3). In Rhodomonas-fed colonies, female zooids were produced after four weeks, often showing a spectacular exponential increase. On very rare occasions, female zooids were seen in the basal layer of the colony. This was, however, generally associated with extremely lobate, 'unhealthy' colonies. The basal females were found to occur along the colony edge, where growth had, for the most part, ceased suggesting malnutrition. Later observations, however, suggested that the production of basal females was not related to malnutrition, but was a feature of specific genotypes (own unpublished results).

The single ovicell in the Skeletonema-fed colony contained an embryo on the final week of observation (table 1.3), but the three ovicells in the Pavlova-fed colony remained empty until the colony died. Despite embryos having been produced in both of the mixed diets (table 1.3), no embryos were observed throughout the concurrent Rhodomonas

treatment. The reasons for this remain obscure, as subsequent observations revealed profuse production of viable larvae on a monospecific diet of Rhodomonas (eg see chapter 2).

Levels of sexual investment were found to be widely different between experimental diets (table 1.3). Generally, basal male production was favoured in diets of low nutritional adequacy, with the production of frontal males often being omitted, and female zooids being produced only where colony growth was extensive.

The lack of significant differences between the effect on colonial growth of Rhodomonas when presented alone, and when combined with Pavlova or Skeletonema (table 1.4), suggests that these last two algae are less nutritious than Rhodomonas, but not themselves detrimental to colony growth. Basal males were the only zooid type found to be affected by the proportion of Rhodomonas in the diet (ANOVA, $P<0.05$), with fewer basal male zooids being produced by colonies which were fed Rhodomonas alone. In most cases, the greatest component of variation was the colony factor (clonal identity), which suggests that colony growth may not simply be dependent on diet, but also on colonial genotype. Rhodomonas-fed colonies also appeared to be very clean in comparison with other diets, in that little algal settlement occurred on the colony surface, a phenomenon which was found to lead in other cases to invasion by protozoans, and fouling by heterotrich ciliates.

In general, Rhodomonas baltica proved outstanding as a diet, enabling colonies of Celleporella hyalina to complete

their life cycle by the production of viable larvae in as little as six weeks. A Celleporella population was maintained in the laboratory for over two years with no signs of malnutrition. This method has also proved successful with other species (plate 1.3), notably Electra pilosa, Conopeum reticulum, Cryptosula pallasiana (Cheilostomata) and Bowerbankia gracilis (Ctenostomata).

1.4 Discussion

A cheap and effective method involving simple apparatus has been designed for the laboratory culture of marine bryozoans. Rhodomonas baltica proved to be an excellent diet, enabling colonies of Celleporella hyalina to complete their life cycle within as little as six weeks. Rhodomonas sp. has already been described as a suitable diet for Bugula neritina (Kitamura and Hirayama 1984), but this appears to be the first laboratory culture of a bryozoan that has resulted in the production of viable larvae.

It is clear that different algal species are not of uniform food value, both within and between bryozoan species. Chlorophyte species proved unsuitable as diets, promoting little or no growth, and eventual colony death. Species of Dunaliella have previously been used in the culture of a number of bryozoan species (Jebram 1968, Winston 1976), yet this alga was unable to sustain even limited growth in C. hyalina.

Promotion of growth, albeit of limited extent, by Skeletonema was surprising, as it has previously been assumed that bryozoans such as C. hyalina, not possessing a gizzard, could not digest thick-walled diatoms (Bullivant 1968a), although these have in the past been found in the guts of a number of marine species (Harper 1912, Hentschel 1922, Hunt 1925, Marcus 1941). However, Markham and Ryland (1987) found that although gizzard-bearing bryozoans were efficient in crushing the frustules of diatoms, gizzardless species were actually more successful at separating the frustule valves, providing evidence that even gizzardless bryozoans have some

potential to exploit diatoms, and perhaps other armoured phytoplankton as a food resource (McKinney 1990).

Jebram (1977) has also listed Skeletonema as one of those species "unable to sustain growth, or with toxic effects if eaten by bryozoans as monofoods over extended periods." Jebram's work has indeed shown that certain algal species may not only be nutritionally deficient, but also harmful to the organisms (Jebram 1968). This was probably not the case for Skeletonema or Pavlova in the present study, given that colonies fed mixed diets still performed relatively well, although Rhodomonas alone was superior as a diet.

Five characters have been described as being distinctive of C. hyalina by Ryland and Gordon (1977). These are (1) a schizoporelloid ancestrula, (2) the unilateral initial budding pattern, (3) sexual zooids usually frontally budded and larger than autozooids, (4) orifice of autozooids and male zooids almost orbicular, with a broad, shallow sinus and (5) ovicells with numerous frontal pores. Cancino and Hughes (1988), have already noted some modifications to characters 2 and 3, and scanning electron micrographs revealed that the nine to twelve frontal 'pores' on the ovicells were actually closed over by the cuticle, and are therefore 'windows' (Banta 1973), rather than true pores.

Early patterns of astogeny have previously been thought to be specifically distinct (Ryland and Gordon 1977, Hastings 1979, Gordon and Hastings 1979, Ryland 1979), and although most colonies observed by these authors showed a unilateral budding pattern, Cancino and Hughes (1987) found that two

simultaneous disto-lateral zooids or a single disto-medial zooid can sometimes also be produced by the ancestrula. Moreover, it was also noted that males were often produced in the basal layer, being commoner in winter and near the growing colony edges (Cancino and Hughes 1987). Basal males can be as large as autozooids and never feed. It was also reported that certain autozooids, on degeneration after two to three months, would start to function as males (Cancino and Hughes 1987). This has never been observed in laboratory-grown colonies, perhaps due to the relatively short periods over which the colonies were observed (present study).

The current set of results however suggest a further modification: the production, on rare occasions, of female zooids in the basal layer of certain colonies. These colonies tended to be highly lobate, in which expansion for the most part had ceased, but the basal gynozooids were functional and were seen to bear embryos. Marcus (1937, 1938) believed that C. hyalina produced sexual zooids by frontal or lateral budding according to season, and these could be as large as autozooids. There is however, no evidence for such features in European specimens of this species, and later work (eg see chapter 3) suggests that the production of basal females may be a feature of specific genotypes.

Cancino and Hughes (1988) also found a deviation from the spiral astogenetic pattern reported as typical for European and Californian colonies (Hayward and Ryland 1979, Pinter 1973). Thus, colonies recruited in late autumn-early winter gave rise to elongated, fan-shaped colonies, which eventually attained a circular shape, but at a size three-

four times bigger than spring-summer recruited colonies. Temperature was postulated as the main factor influencing budding pattern, although it was noted that the observed effect was minimised under conditions of restricted water flow, at a time during the winter when feeding of unprotected colonies could have been suppressed by rough seas (Spratt 1980).

In the above studies, however, the quality of diet may have had an equally important influence on colonial growth pattern. This dietary effect has been recorded in a number of species, notably Conopeum tenuissimum (Winston 1976), Bowerbankia gracilis (Jebram 1973) and Electra pilosa (Jebram 1980b). Slow growth was observed in bryozoans cultured in a medium of poor nutritional quality, resulting in elongated colonies, whereas when colonies were cultured in a favourable medium, dense mats of fast-growing zooids were produced (B. gracilis), or colonies grew with zooids arranged in a continuous sheet (C. tenuissimum). This was reflected by results from the current study, where colonies grown on less favourable diets tended to have extremely high perimeter to area ratios (PARs), whereas Rhodomonas-fed colonies tended to adhere to a tight, circular form, with classic, spiral budding (Hayward and Ryland 1979, Moyano 1986). A high number of zooids per unit area has usually been interpreted as a response allowing maximum use of resources at a favourable site, while an elongated colony, with fewer zooids per unit area maximises substratum covered, facilitating the location of more favourable sites (Winston 1976, Buss 1979a, 1986, Jackson 1983). However, uniserial growth cannot always be

regarded as a general fugitive strategy adopted by competitively inferior forms (Bishop 1989). Competitive ability in certain hydroid species has also been shown to be a simple deterministic function of growth form, where morphological variation is continuous, but nevertheless results in discrete competitive phenotypes (Buss and Grosberg 1990). As such, a low zooid number per unit area can result in competitive superiority. This undoubtedly is not the case in Celleporella, however. Although modular polymorphism allows considerable flexibility in colonial construction, this species is completely lacking in defensive structures and is a poor competitor (Cancino and Hughes 1987). Lobate growth in C. hyalina is probably best interpreted as a response to low food availability or malnutrition. As such, PAR values may provide a useful index of colony health.

Where clonal organisms are faced with temporary, sub-lethal stress, it is assumed that maintenance of the soma takes precedence over sexual investment under conditions of resource limitation (Hughes and Cancino 1985). In plants, this has generally been found to result in increased proportional investment in the less costly male sex, where ovule and seed production is a greater drain on resources than pollen release (Willson 1979). Relative costs of sexual investment in Celleporella appear to be reflected in the sequence in which the gonozooid types are produced. Basal males are invariably the first, followed by frontal males, then females. This sequence may also be regarded as an increase in complexity of form. Cancino (1983) reported basal males as being formed when two distal buds fail to fuse to

form a normal feeding autozooid. The relatively simple morphological derivation of basal males may provide a way in which colonies can achieve reproductive success at a very low colony size and at relatively low cost, with no additional sacrifice to further colonial growth. These energetic considerations may explain why basal males were produced preferentially when colonies were grown on poor or moderate diets. Similarly, Cancino and Hughes' (1987) observation that basal males were more numerous during the winter months could be explained as a result of low food availability. It is interesting however, that one Skeletonema-grown colony managed to produce a single ovicell and embryo, and one of the Pavlova-grown colonies produced three ovicells, although none of these ever bore embryos. In the latter, the production of frontal males was completely omitted.

The full significance of sexual investment in C. hyalina is considered in chapter 3. Having found a suitable culture method and diet for Celleporella it was then necessary to find out exactly how much food the bryozoans were consuming in order for a quantified diet to be administered. This is attempted in the next chapter.

Table 1.1

Characteristics of C. hyalina colonies grown on a range of algal diets.

Abbreviations: Ch-Chlorella japonica, Du-Dunaliella tertiolecta, Pa-Pavlova lutheri, Ro-Rhodomonas baltica, Sk-Skeletonema costatum, Te-Tetraselmis chuii, C1-Rhodomonas + Skeletonema, C2-Rhodomonas + Pavlova, Fs-filtered seawater, Fs + Co-filtered seawater + Conway medium (algal growth medium).

Diet	Growth	Shape
Pa	: Relatively poor, prone to infection	Lobate
Sk	: Fair, but slow	Lobate
Ro	: Excellent	Circular
Te	: Sustenance of individual zooids only, leading to colony death.	-
Ch	: As above	-
Du	: No growth	-
C1	: Good	Loosely circular
C2	: Good	Loosely circular
Fs	: No growth	-
Fs + Co	: No growth	-

Table 1.2

Performance of C. hyalina colonies grown on a range of algal diets, after 56 days.

Abbreviations: Ch-Chlorella japonica, Du-Dunaliella tertiolecta, Pa-Pavlova lutheri, Ro-Rhodomonas baltica, Sk-Skeletonema costatum, Te-Tetraselmis chuii, C1-Rhodomonas + Skeletonema, C2-Rhodomonas + Pavlova, Fs-filtered seawater, Fs + Co-filtered seawater + Conway medium (algal growth medium).

	$\bar{x}$ size (mm^2)	$\bar{x}$ total zooids	sexual zooids
Pa :	3.69±2.91	36.2±27.89	3.2±6.38
Sk :	1.48±0.66	313±16.58	1.1±1.77
Ro :	40.21±3484	590.7±481.93	180.3±282.19
Te :	0.40±0.13	6.0±0.82	-
Ch :	0.46±0.21	10.8±6.40	0.4±0.52
Du :	-	-	-
C1 :	10.65±9.05	156.7±100.68	12.9±33.29
C2 :	16.49±16.03	214.5±195.74	14.5±18.56
Fs :	-	-	-
Fs + Co:	-	-	-

Table 1.3

Production of sexual zooids by colonies of C. hyalina grown on a range of algal diets, after a period of 56 days.

Abbreviations: Ch-Chlorella japonica, Du-Dunaliella tertiolecta, Pa-Pavlova lutheri, Ro-Rhodomonas baltica, Sk-Skeletonema costatum, Te-Tetraselmis chuii, C1-Rhodomonas + Skeletonema, C2-Rhodomonas + Pavlova, Fs-filtered seawater, Fs + Co-filtered seawater + Conway medium (algal growth medium).

The numbers in brackets denote the week in which the sexual zooids were first recorded for each experimental diet. Embryos are recorded as either present or absent.

Diet	Basal males	Frontal males	Females	Embryos
Pa	1.7±4.37 (6)	-	1.5±2.01 (6)	no
Sk	0.6±0.69 (2)	0.5±1.08 (6)	-	yes
Ro	4.9±5.11 (3)	40.6±51.30 (3)	134.8±236.34 (4)	yes
Te	-	-	-	no
Ch	0.4±0.40 (3)	-	-	no
Du	-	-	-	no
C1	7.1±4.46 (1)	3.4±9.03 (3)	2.4±6.90 (5)	yes
C2	7.2±4.21 (3)	2.1±2.26 (3)	5.2±11.75 (7)	yes
Fs	-	-	-	no
Fs + Co	-	-	-	no

Table 1.4

Comparison of growth parameters between colonies when grown Rhodomonas baltica when fed as a monofood, and when fed in combination with Pavlova lutheri or Skeletonema costatum.

For each variable, the data are treated as a 3 factor analysis of variance (ANOVA), with repeated measures on one factor (time). The 'colony' factor (C) is nested within 'diet' (D).

Abbreviations for sources of variation D: DIET (n=3)

C: COLONY (n=10)

T: TIME (n=8 obs.)

a) Analysis of Variance for total autozooids, transformed to natural logs.

Source	DF	SS	MS	F	P
D	2	1.515	0.758	0.17	0.845
C(D)	27	120.537	4.464	2.79	0.000
T	7	50.998	7.285	4.55	0.000
D*T	14	132.031	9.431	5.89	0.000
Error	189	302.512	1.601		
Total	239	607.594			

Table 1.4 (cont.)

b) Analysis of Variance for basal male zooids, transformed to natural logs.

Source	DF	SS	MS	F	P
D	2	161.384	80.692	4.67	0.018
C(D)	27	466.493	17.278	2.32	0.001
T	7	91.014	13.002	1.74	0.101
D*T	14	255.694	18.264	2.45	0.003
Error	189	1408.844	7.454		
Total	239	2383.430			

c) Analysis of Variance for frontal male zooids, transformed to natural logs.

Source	DF	SS	MS	F	P
D	2	20.707	10.353	0.27	0.767
C(D)	27	1043.574	38.651	7.38	0.000
T	7	82.636	11.805	2.25	0.032
D*T	14	243.383	17.385	3.32	0.000
Error	189	989.902	5.238		
Total	239	2380.202			

d) Analysis of Variance for female zooids, transformed to natural logs.

Source	DF	SS	MS	F	P
D	2	27.301	13.651	0.39	0.681
C(D)	27	944.397	34.978	6.28	0.000
T	7	92.138	13.163	2.36	0.025
D*T	14	329.356	23.525	4.22	0.000
Error	189	1053.302	5.573		
Total	239	2446.494			

Figure 1.1

Growth of colonies of C. hyalina on a variety of algal diets. (N=10). Data points are mean values ±95% confidence limits

solid lines - somatic parameters

broken lines - sexual parameters

Solid circles - Feeding autozooids

Solid triangles - Fully formed buds

Solid boxes - Degenerated autozooids (brown bodies)

Open circles - Basal male zooids

Open boxes - Frontal male zooids

Open triangles - Female zooids

a) Pavlova lutheri

b) Skeletonema costatum

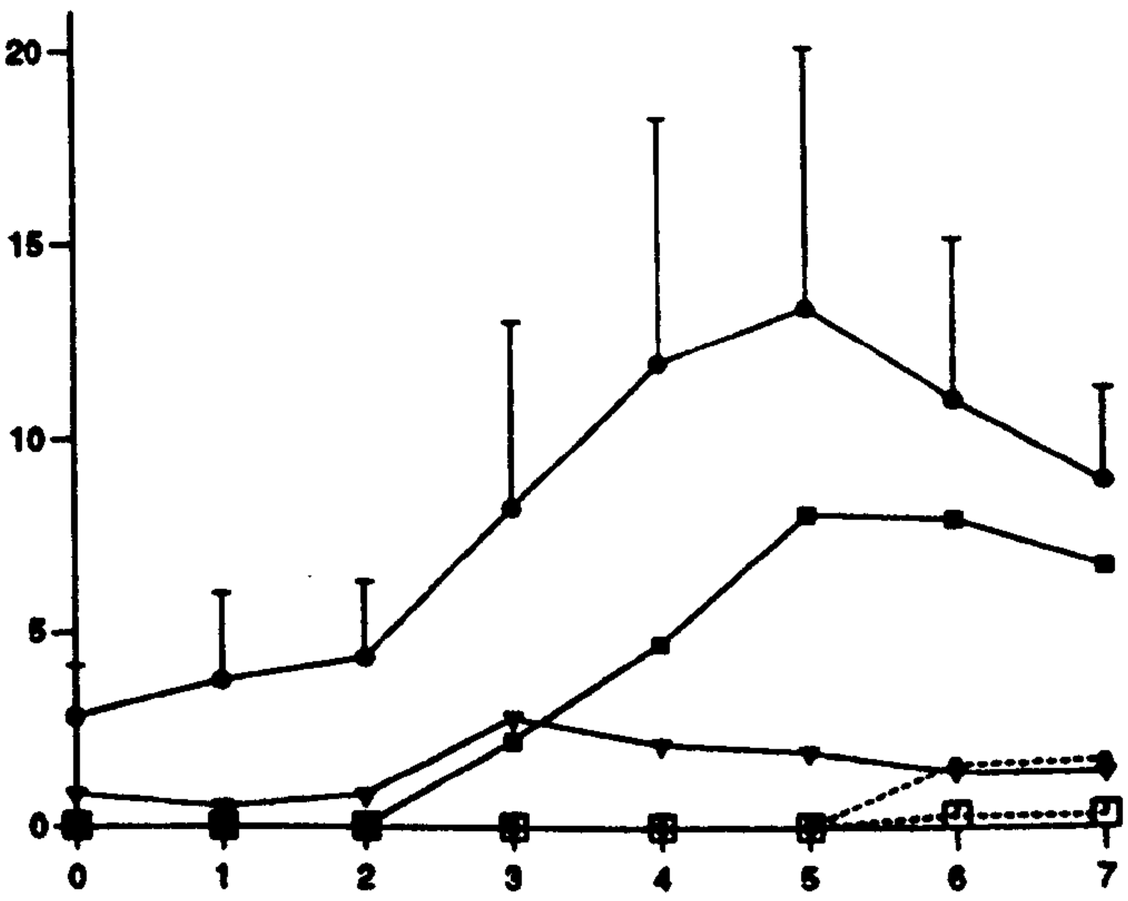
c) Tetraselmis chuii

d) Rhodomonas baltica

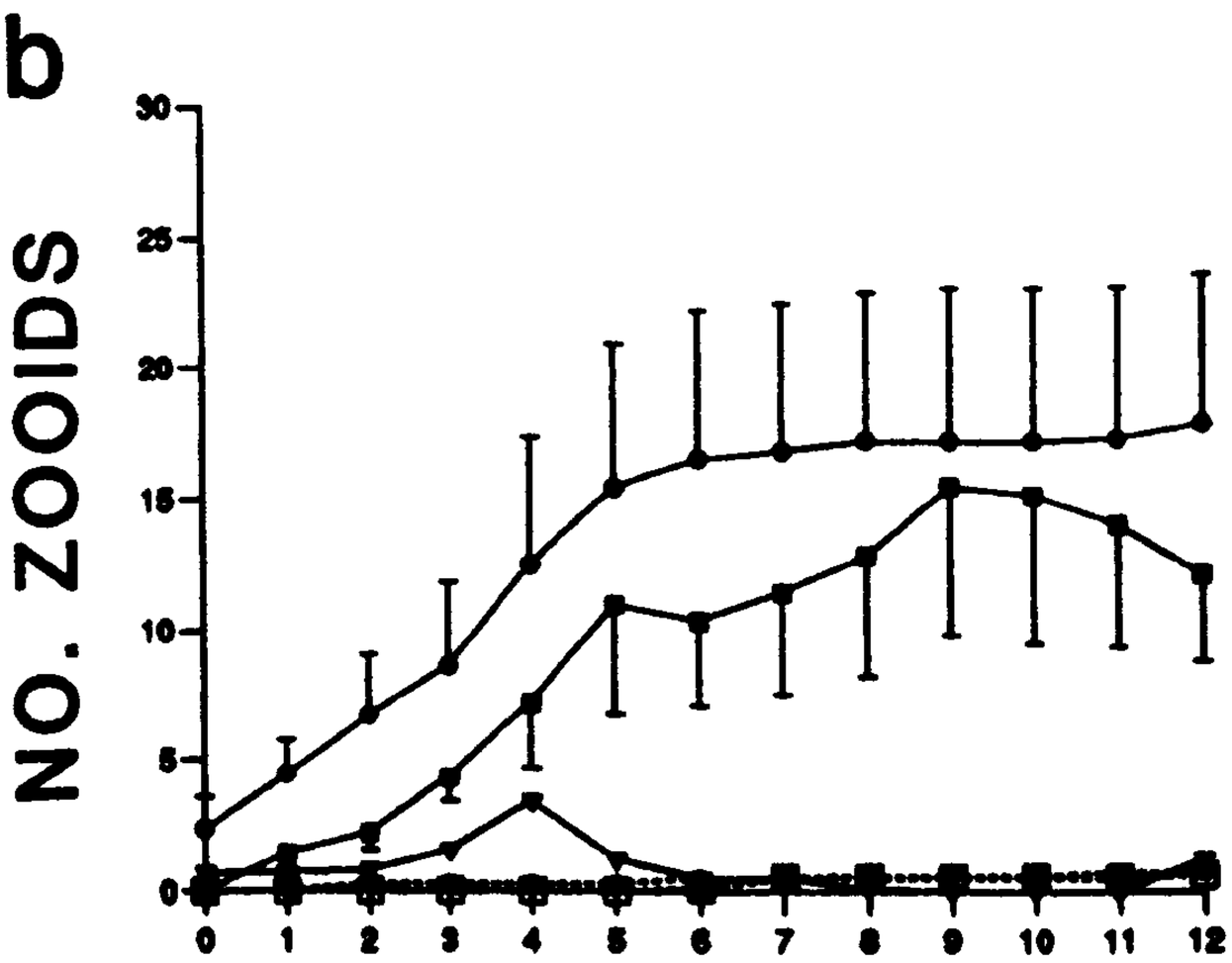
e) Rhodomonas baltica + Skeletonema costatum

f) Rhodomonas baltica + Pavlova lutheri

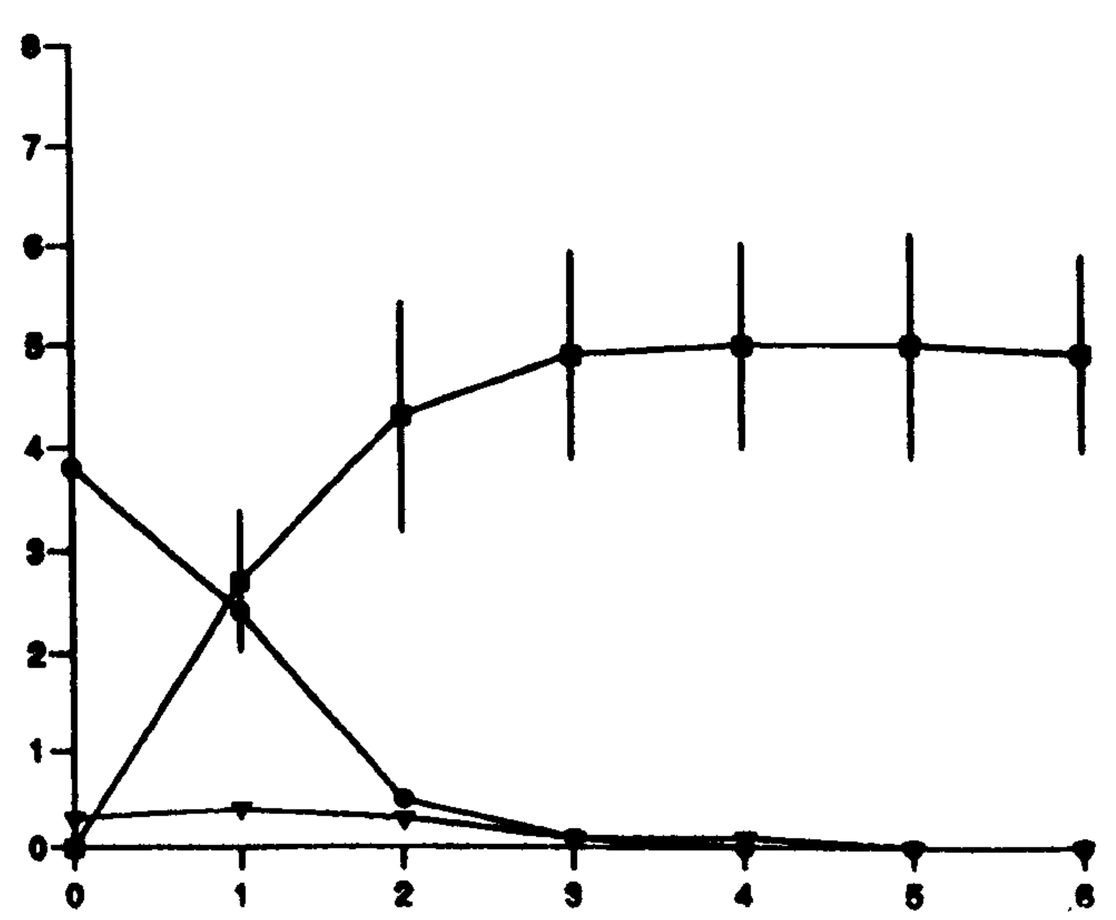
a


20
15
10
5
0
0
1
2
3
4
5
6
7

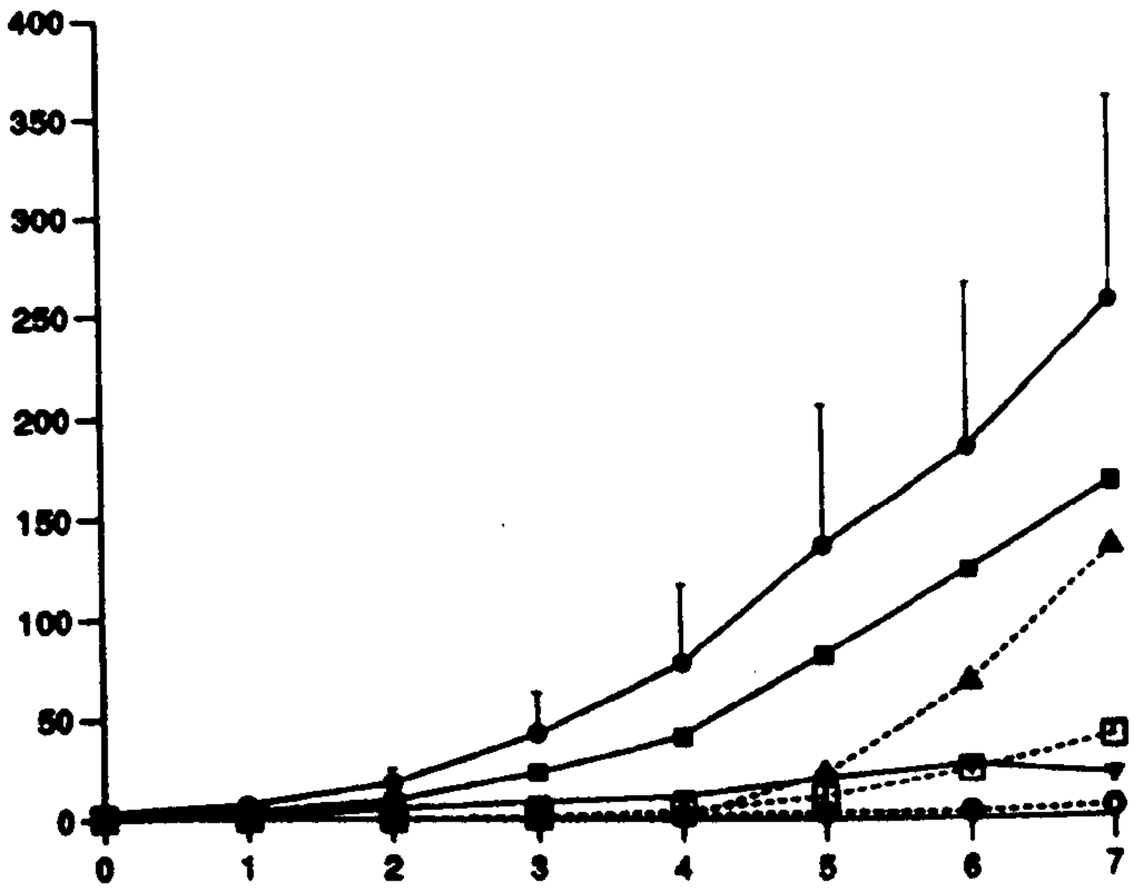
b


NO. ZOOIDS
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
11
12

C


8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
ELAPSED TIME (WEEKS)

d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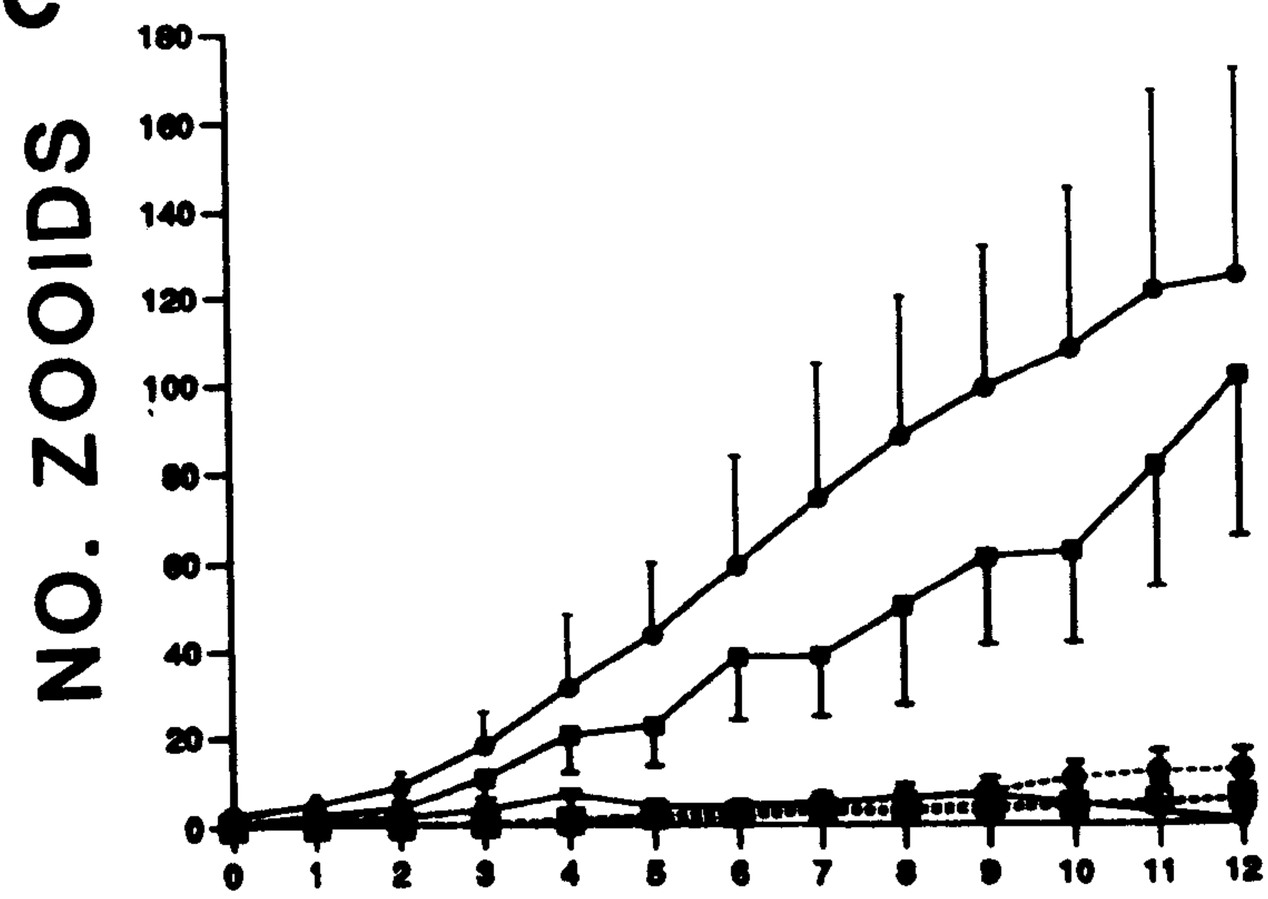
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7

e


NO. ZOOIDS
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
11
12

f

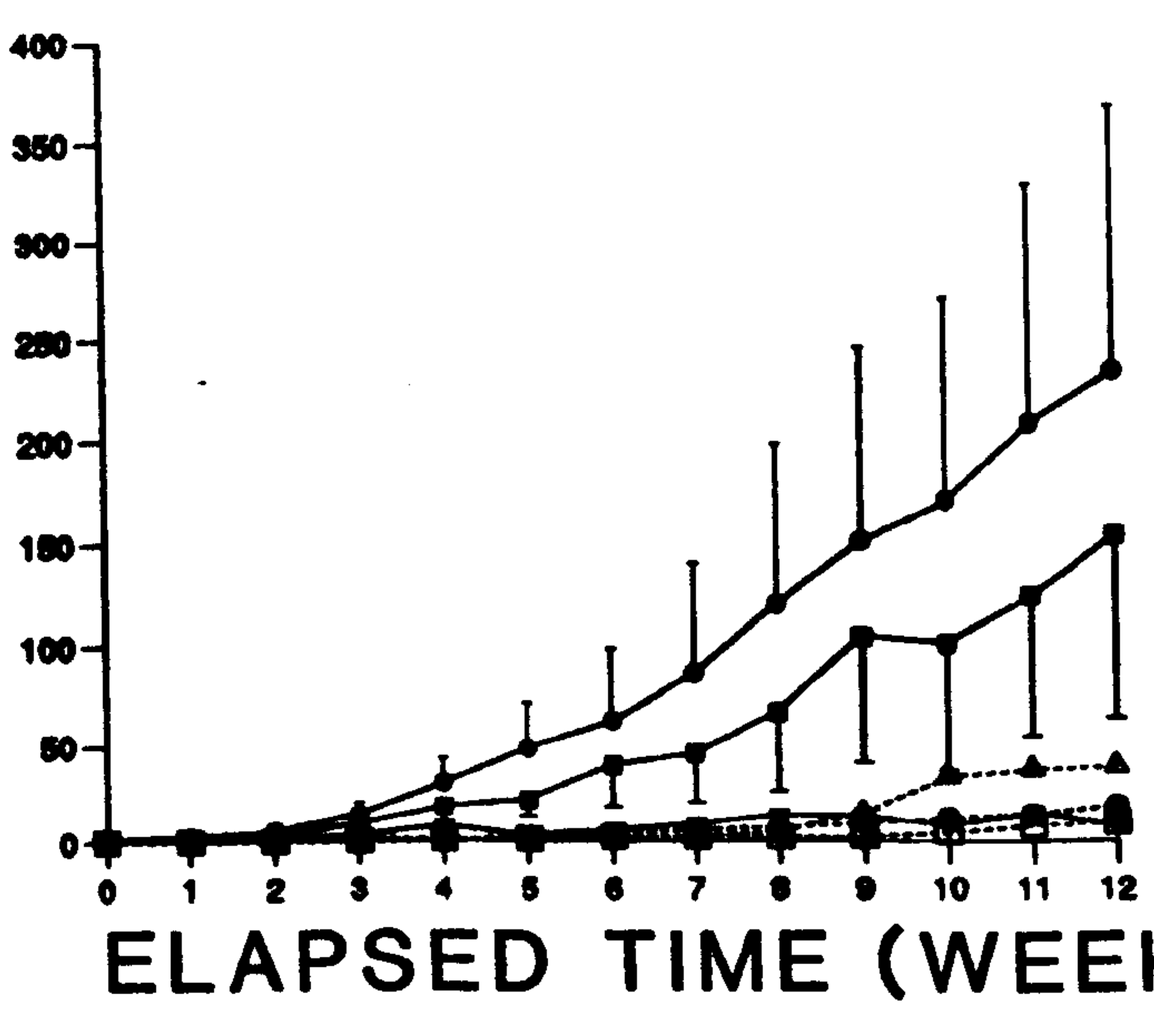

400
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
11
12
ELAPSED TIME (WEEKS)

Figure 1.2

Perimeter:area ratios for *C. hyalina* colonies grown on 6 different algal diets. Data points are mean values for each treatment group.

Solid circles - *Pavlova lutheri*

Solid boxes - *Skeletonema costatum*

Solid triangles - *Tetraselmis chuii*

Open circles - *Rhodomonas baltica*

Open boxes - *Rhodomonas baltica* + *Skeletonema costatum*

Open triangles - *Rhodomonas baltica* + *Pavlova lutheri*

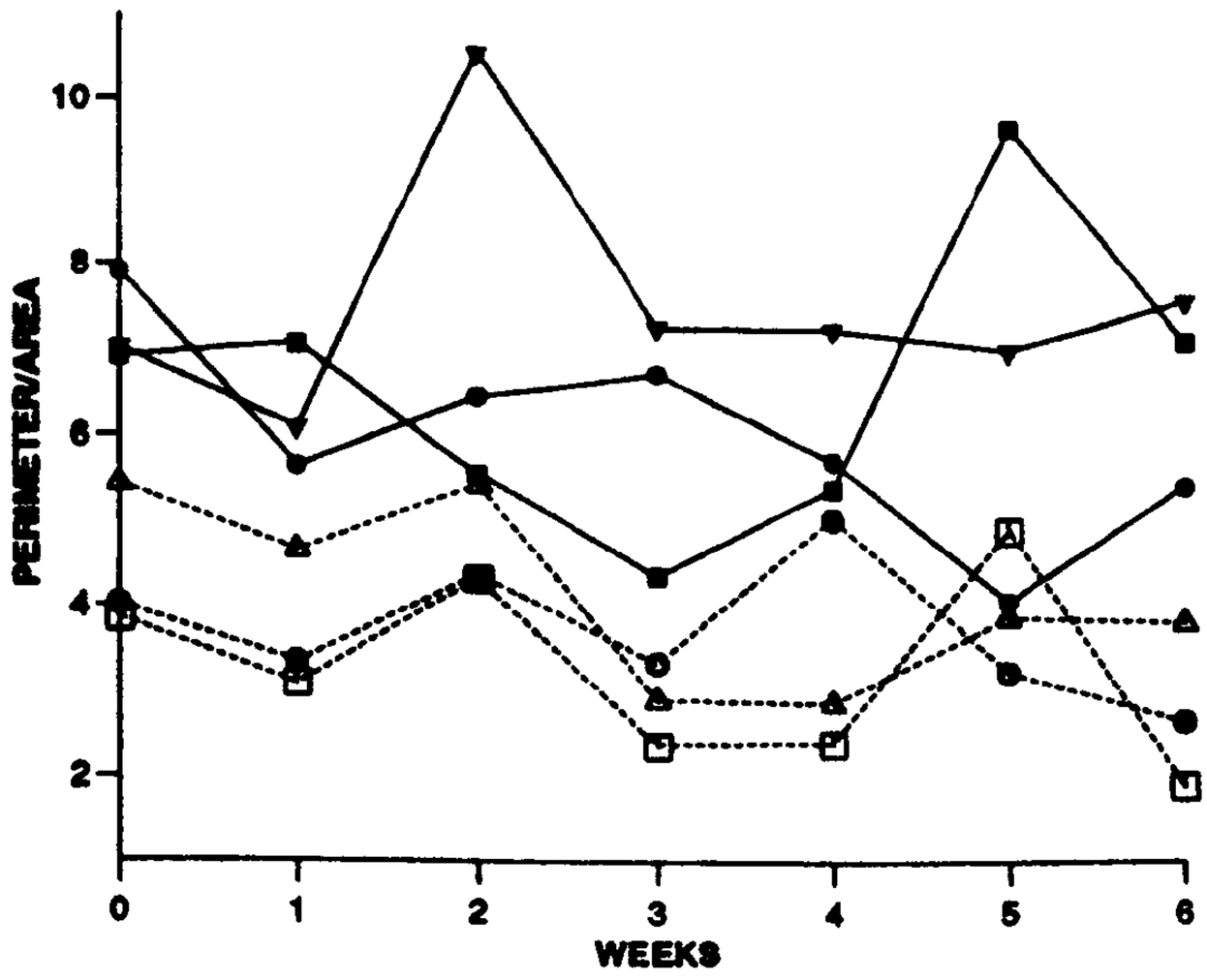

PERIMETER/AREA
10
8
6
4
2
0
1
2
3
4
5
6
WEEKS

Plate 1.1

Colony astogeny of C. hyalina. (a) Primary zooid (ancestrula), having completed metamorphosis, begins to feed approximately 2 days after settlement, after which, new autozooids are budded frontally (b). Autozooids are budded continually throughout the colonial lifetime from the growing colony edge (c), and zooids can be seen at all stages of development in zones of astogenetic change. Rudimentary polypides (rp) form in the most recent buds. The stomach becomes visible on the polypide's first feeding (ff), after which the autozooid becomes fully operational (au). After 3-4 weeks, autozooids degenerate (deg), resulting in the formation of a residual brown body (bb), which is expelled before a new polypide is formed.

Scale bars = 100 μm

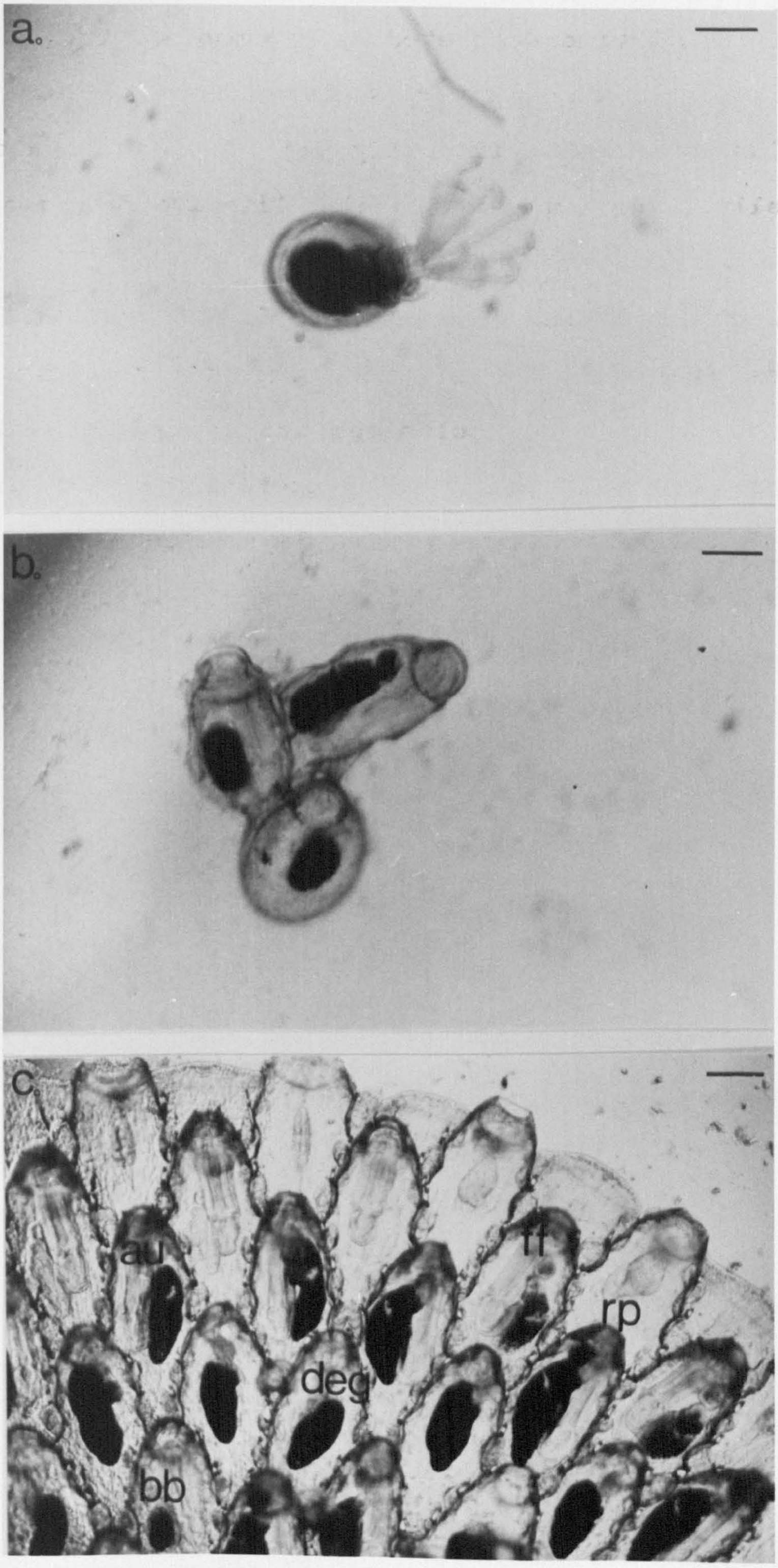

a.
b.
c.
au
ff
rp
deg
bb

Plate 1.2

Zooid morphology of C. hyalina.

(a) The feeding lophophore of a newly formed autozooid at the growing colony edge. Also present is a basal male zooid (mb), easily distinguished from autozooids by their relatively small orifice size, and milky coloration. This is further demonstrated in (b), which shows a mixture of autozooids at various stages of degeneration, and basal males near the colony interior, prior to the commencement of frontal budding (fb). Frontal budding (c) results in the production of frontal autozooids (af), frontal males (mf), and females bearing ovicells (ov). The 3 females demonstrated can clearly be seen to bear embryos in the lower left hand section of the ovicell.

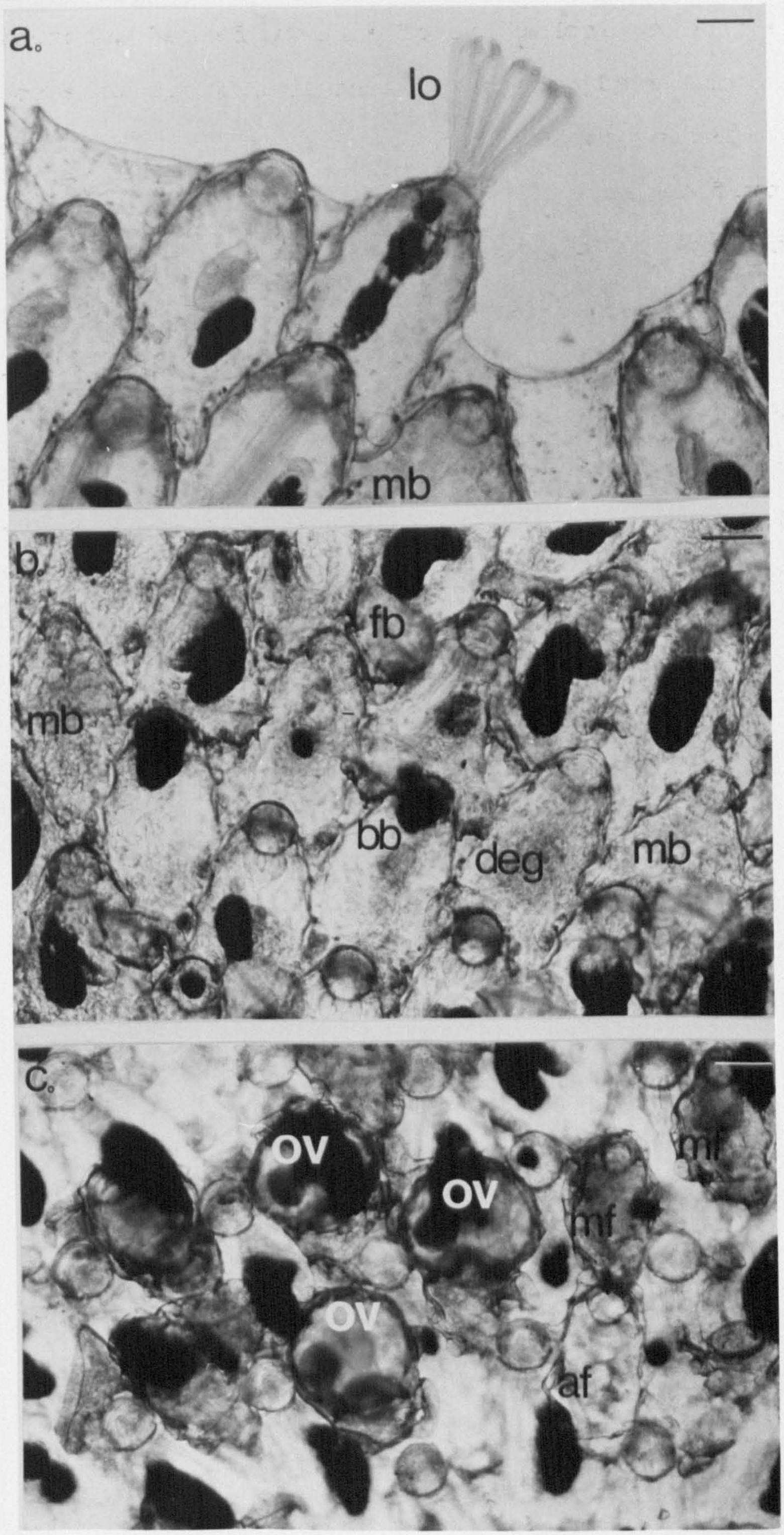

a.
lo
mb
b.
fb
mb
bb
deg
mb
c.
ov
ov
ov
mf
mf
af

Plate 1.3

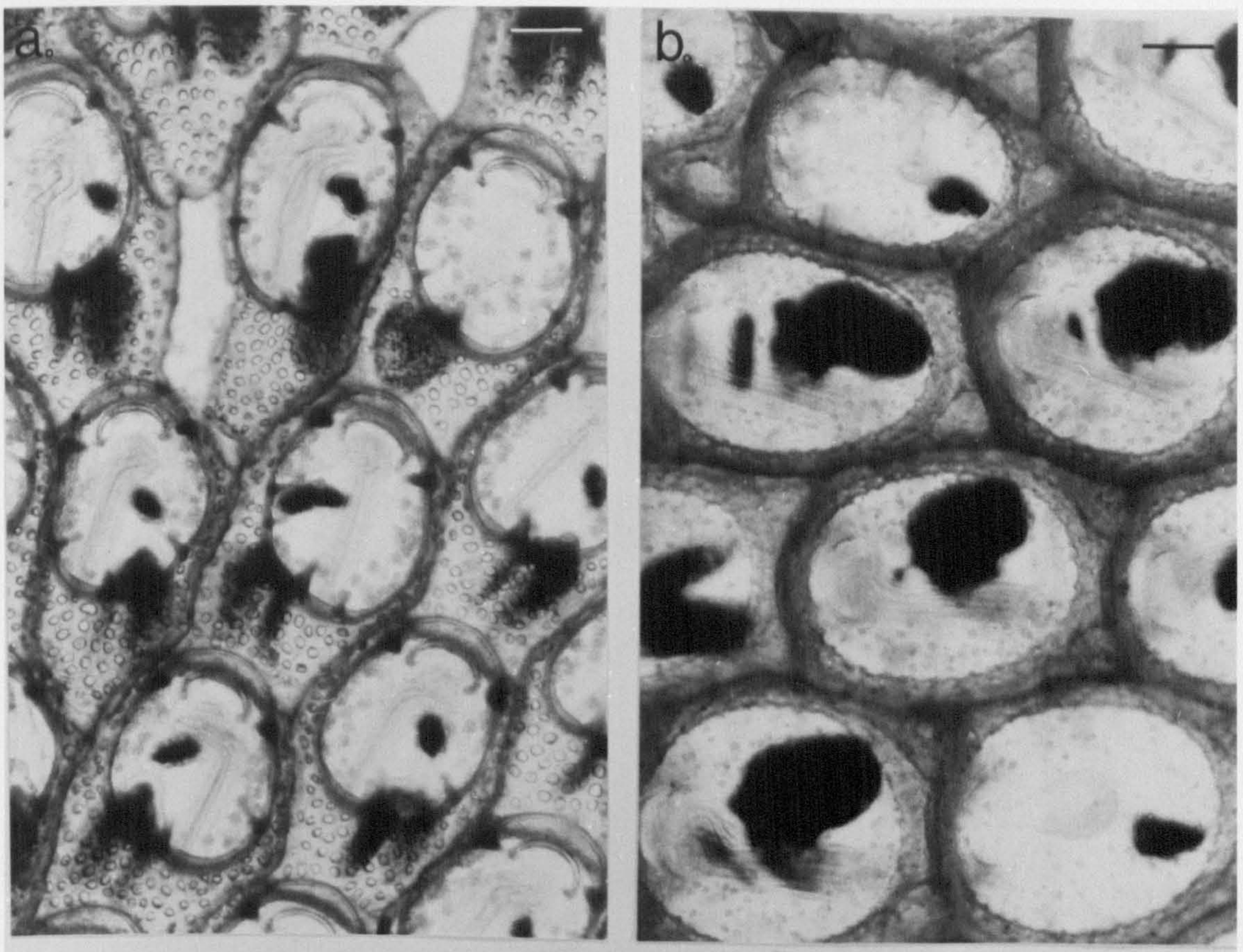
Bryozoan species readily cultured on a diet of Rhodomonas baltica.

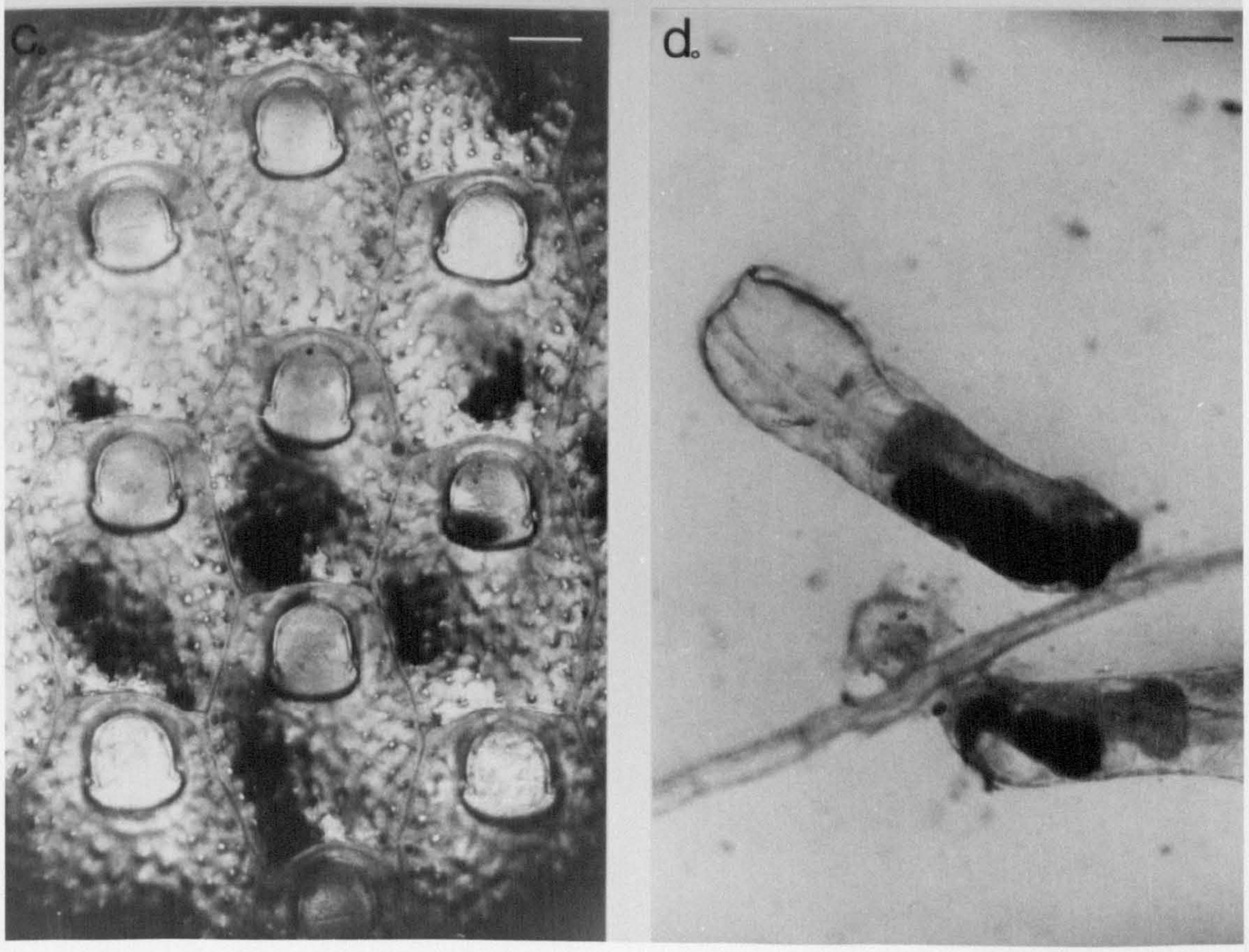
a) Electra pilosa

b) Conopeum reticulum

c) Cryptosula pallasiana

d) Bowerbankia gracilis


a.
b.


c.
d.

Chapter 2

The effect of cell concentration on colony growth and feeding in *C. hyalina*.

2.1 Introduction

Bryozoans capture food particles by means of a ciliated funnel of tentacles known as the lophophore. The mechanism of particle capture, however, has been a matter of some controversy (Strathmann 1973, 1982, Ryland 1976). Early workers gave descriptive accounts of feeding in the Bryozoa (Borg 1926, Atkins 1932), cilia having been observed to draw food particles into the feeding crown, and towards the mouth by means of a feeding current, generated by the lateral cilia.

Using the ctenostome Zoobotryon verticillatum, Bullivant (1968a) was the first to experimentally investigate bryozoan feeding mechanisms, naming it 'impingement feeding', after an industrial process. Lateral cilia were found to beat outwards, causing water to flow in through the open end of the inverted cone. This water current was directed into a shallow 'cup' at the base of the cone in which the mouth is located, and was deflected upward and outward to exit between the tentacles. A sharp deflection of this current over the mouth was thought to cause particles to be thrown towards the mouth, or into an eddy of comparatively still water, from which they could be sucked in by rapid muscular dilation of the pharynx.

This synthesis was challenged by Strathmann (1971, 1973), who claimed that impingement could not account for particle capture, since the physical density of food particles is too close to that of seawater. Instead, he proposed that bryozoans captured particles by brief reversals of the lateral cilia, triggered by contact with other particles.

Best and Thorpe (1983) published evidence of a relatively simple, filter-system of particle collection, not dissimilar to that originally proposed by Atkins (1932), rather than the more complex mechanisms suggested by other workers (Bullivant 1968a, Strathmann 1973, 1982, Gilmour 1978). It is possible, however, that capture methods may vary between species (Winston 1978), and may vary for different classes of food particle (Winston 1978, Best and Thorpe 1983, Best 1985), and flow velocity (Okamura 1987a).

Irrespective of the actual feeding mechanism, the rate at which a filter-feeding organism can clear a suspension may be an important parameter in determining its success in the marine ecosystem. The few workers attempting experimentally to estimate feeding rates in bryozoa have tended to rely on indirect methods, the reliability of which may be questionable (Bullivant 1968a, Menon 1974). Bullivant (1968a) found relatively high clearance rates in Z. verticillatum, when compared with other invertebrate species. High clearance rates can be expected in smaller organisms on account of their higher metabolic rate, and although bryozoan colonies as a whole may reach a relatively large size, individual zooids behave as metabolic individuals (D.J.Hughes and Hughes 1986a, Cancino et al 1991).

As pointed out by Best and Thorpe (1983), Bullivant's (1968a) measurements were simplistic and probably inaccurate, in that feeding rate was assumed to be constant throughout the experiment, and no allowance was made for those zooids within the colony that were not feeding. Depending on species, bryozoans may need to maintain structural, defensive

and sexual zooid types, none of which is usually involved in food capture, as well as areas of active budding, all of which require a continual supply of nutrients. Also using methods similar to those employed by Bullivant (1968a), Menon (1974) found fluctuation in the feeding rates of the common bryozoans Electra pilosa and Conopeum reticulum.

Strathmann (1973) used direct measurements of clearance rates, but on dissected lophophores rather than intact colonies. More recently, Strathmann (1982) employed high-speed cinemicography in an attempt to measure clearance rates and particle velocities in Flustrellidra hispida. It was considered however, that the particles were collected only by ciliary reversal, or by 'tentacle flicking', and food intake was probably therefore underestimated (Best and Thorpe 1986a). In both cases, constant feeding-current velocity was assumed.

Direct measurements of feeding-current velocities within the lophophores of intact, living colonies of F. hispida were carried out by Best and Thorpe (1983), who found fluctuations with time, food-particle concentration, and along different parts of the lophophore. This was subsequently found to be true in five other marine bryozoans (Best and Thorpe 1986a). It was concluded that feeding in bryozoans is an active process, with colonies responding to changing environmental conditions, and not merely the passive filtration of seawater.

It was assumed for many years that competition for food was insignificant among sessile marine invertebrates living on hard substrata, spatial competition being regarded as of

primary importance (eg Connell 1961, Knight Jones and Moyse 1961, Dayton 1971, Paine 1974, Jackson 1977, 1979a, 1979b, 1984, Buss 1979a, 1980, Buss and Jackson 1979, Winston and Jackson 1981, 1984, Jackson and Winston 1982). Recent evidence however, suggests that feeding may be an important aspect of competition among sessile animals (eg. Buss 1979b, 1981, Buss and Jackson 1981, Jackson and Winston 1982, Best and Thorpe 1986a, 1986b, Thorpe et al 1985, Okamura 1984, 1985, 1987a, 1990). Moreover, feeding currents may be effective in the prevention of colony fouling (Lidgard 1981, Palumbi and Jackson 1982).

Buss (1979b) provided the first, unequivocal evidence that feeding competition can occur among sessile suspension feeders, and that this may interact with competition for space. Thus, by capturing more of the available food resource, colonies may gain more energy with which to overgrow competitors. Best and Thorpe's (1986a) study provided evidence that competitive success, and the ability to overgrow other species, may be linked to clearance rate or feeding-current velocity. These authors also suggested that the adjustment of feeding rate in response to fluctuations in food supply may be a significant component of competitive ability. In certain cases, spatial and feeding competition may be functionally linked, to the point of being inseparable.

In any case, the food resources of suspension feeders are highly variable in time, space and composition (Okamura 1990). Past studies have also tended to ignore the effects of external water currents. It is clear however, that variation

in the physical environment can significantly influence ingestion of different particle types by suspension feeders. In a series of experiments investigating the effects of flow rate on the feeding of bryozoans, Okamura (1984, 1985, 1987, 1990) found that both inter- and intra-specific competition for food among bryozoans can occur. The adverse effects of such competition may be expected to vary depending on such factors as the local flow regime, the density of colonies, and the position of a particular colony with respect to other colonies and to the prevailing ambient flow.

Having found an acceptable algal food for Celleporella hyalina, in order to administer a controlled, strictly quantified diet, it was first necessary to obtain some information on feeding in this species. Bearing in mind the sometimes complex nature of feeding interactions in this group, it was decided to study the effects of algal cell concentration on feeding and growth using direct methods. Since C. hyalina exhibits complete division of labour among its polymorphic zooids, it was also desirable to ascertain whether allocation into male, female and feeding function was adjusted in colonies, according to the level of food supply.

2.2 Materials and methods

2.2.1 Colony growth over a range of cell concentrations

The effect of food supply on colony growth in C. hyalina was examined by rearing colonies over a range of algal cell concentrations, using the culture method described in chapter 1.

The colonies were grown on glass slides, in 0.2μm-filtered, U.V.-irradiated seawater at 18°C under common garden conditions, with a diet of approximately 100 cells.μl^{-1} of the cryptophyte alga Rhodomonas baltica. This medium was changed three times weekly.

At the start of an experiment, each colony was reduced to a cluster of < 10 autozooids, all sexual zooids being carefully removed. A maximum of three, well-spaced zooid clusters was retained per glass slide (76*39mm). The reduced colonies were then transferred to experimental conditions, where they were reared, using methods already described (chapter 1), under one of eight cell concentrations. The range of cell concentrations used was 10, 25, 50, 75, 100, 150, 200 and 300 cells.μl^{-1}. It was considered that this covered the range of cell concentrations that C. hyalina colonies could possibly encounter (Novarino, personal communication). In all cases, Rhodomonas baltica was used as the experimental diet. A single strain of Rhodomonas was used throughout, and colonies were always fed at the same time, with the same batch culture, such that the condition of the alga would be uniform, irrespective of cell concentration.

Rhodomonas, cultured on Conway medium, was grown in 5l flat-bottomed, round, glass flasks, with circulation being

maintained by aeration. Batch cultures were seeded and reared over a period of two weeks from a 250 ml subculture, at room temperature. A bank of subcultures was maintained at all times, and two batch cultures were always run concurrently, to insure against the possibility of algal population crashes as a result of infection. Despite all culture and harvesting procedures being carried out under conditions of sterile transfer, the risk of infection could not entirely be eliminated.

Batch cultures were changed after one month in order to keep the algal cells proportionately young and vigorous. As cells were counted using a haemocytometer, the condition of the cultures could be monitored and bacterial infection quickly detected. Cell densities were calculated according to the method of Cassell (1965).

Ten colonies were used per cell concentration and experiments were run for 7 weeks, after which time the largest colonies had started to come into contact with each other, and zooid counts became prohibitively time-consuming. In any case, healthy, laboratory-reared, and naturally occurring colonies would have completed their life cycle by the production of viable larvae within this time period. Weekly counts were made of feeding autozooids, degenerated autozooids (brown bodies), fully formed buds, basal male zooids, frontal male zooids, female zooids and embryos. Drawings of the colonies were made weekly, and the drawings digitised electronically to calculate area and perimeter.

2.2.2 Ingestion rates.

Attempts to monitor cell depletion by individual colonies from a restricted volume of seawater proved unreliable, so a direct method was chosen. Colonies which had been starved for a period of 24 hours were placed in a petri dish containing R. baltica at a concentration of 10, 25, 50, 75, 100, 150, 200 or 300 cells.μl^{-1} filtered seawater. This was placed on a water-cooled stage under a high-power dissection microscope. The stage maintained an ambient temperature of approximately 18°C. Circulation could be maintained by focusing a gentle air jet against the petri dish edge. Too vigorous a jet made ripples, which could obscure and disturb the feeding lophophores.

With the use of a fibre-optic light source, the feeding behaviour of individual polypides could be observed. By focusing on the pharynx of a feeding lophophore, counts could be made of the number of cells ingested during a timed feeding episode.

By this technique, the feeding behaviour of lophophores could be observed, and a picture of colonial response to different cell concentrations built up. Although R. baltica was used as the primary food, the response of C. hyalina to several other algal species was also observed, to determine whether discrimination is possible.

2.3 Results

2.3.1 Growth of colonies with a range of cell concentrations

Colonies were able to grow and attain sexual maturity at all levels of food supply, resulting in the production of viable larvae (table 2.1). In general, colonies performed best within the range of 50 to 150 cells.μl^{-1}. Both low, and extremely high cell concentrations suppressed colony growth, and resulted in small numbers of sexual zooids being produced. Surprisingly, the percentage of ovicells bearing embryos was found to be greatest at a level of 10 cells.μl^{-1}, but this could be explained by the small number of females present, nearly all of which came to bear embryos. Otherwise, the percentage of occupied ovicells was relatively constant about a mean value of 73.8 percent, with the exception of colonies grown in 300 cells.μl^{-1}, where the number of ovicells bearing embryos was reduced to 49.4 percent. A three-factor analysis of variance, using the experimental factors CONCENTRATION (C), representing the eight levels of cell concentration used in the experiment, COLONY (Col), nested within CONCENTRATION, representing the number of colonies used under each cell concentration, and TIME (T), the number of observations made, showed all measured parameters to be significantly different (table 2.2).

In all cases, the COLONY, TIME and COLONY*TIME interaction terms were found to be highly significant ($P<0.001$). A statistically significant COLONY effect indicated intercolonial variation in growth rate for all the measured parameters. As the colonies grew, all parameters changed significantly over the duration of the experiment, so

high significance of the TIME factor was also to be expected. The significant COLONY*TIME interaction meant that although colonies may have differed significantly for a character when the entire data set was considered, they were not necessarily different at all observations. This is also a predictable result, since all colonies began growth at approximately the same size, gradually diverging over time. CONCENTRATION was found to have a highly significant effect ($P<0.001$) on colony area, perimeter, feeding autozooids, degenerated autozooids, fully formed buds, total autozooids, frontal males, embryos and total zooids. This effect was reduced for females ($P<0.01$), and was only weakly significant for basal males, total males and total gonozooids ($P<0.05$).

The relative contribution of each source of variation to the total variability within the data can be evaluated by partitioning the total sum-of-squares for each character (table 2.3). In all cases, the TIME factor explained by far the greatest part of variability, ranging from 55% for frontal male production, to 80% for total zooids. The contribution of CONCENTRATION never exceeded 10%, and tended to be much lower in the case of sexual parameters. With the exception of area, total autozooids, and total zooids, the contribution of the CONCENTRATION effect was exceeded by the COLONY effect, which in most cases was itself exceeded by the contribution of TOTAL ERROR. This last source is due to the TIME*COLONY interaction term, and the relatively high values indicate that there was a tendency for the relative responses of colonies to vary according to the stage in the experiment.

Variability of this kind can be interpreted as due to genetic differences between colonies.

The perimeter:area ratio was found to show a highly significant CONCENTRATION effect ($P<0.001$), and although most variance was taken up by the TIME factor (table 2.4), CONCENTRATION contributed 13% of the total variation, compared with 9% from the COLONY factor. CONCENTRATION was also found to have a significant effect on the number of zooids per unit area (table 2.4, total autozooids/area, $P<0.05$). In this case, however, COLONY and TOTAL ERROR were predominant in their contributions to the total variance (41.3% and 26.4% respectively, compared with 10.6%), suggesting that the response of total zooids to food concentration may have largely been due to genetic differences between colonies.

Sex ratio, total reproductive allocation and male reproductive allocation were all found to be unaffected by cell concentration (table 2.4), although a weak effect was detected for female reproductive allocation ($P<0.05$). For each of these parameters, the TIME component of variation was greatly reduced, and despite being the principal component in male, female and total reproductive allocation, it amounted to only 13% for sex ratio (table 2.5). CONCENTRATION accounted for no more than 5% of total variation in each case, and the relatively large contributions from COLONY and the ERROR term suggest genetic constraints as being important in sex allocation in C. hyalina.

Considering sex allocation, it is useful to examine the final numbers of autozooids, basal males, frontal males and

females as percentages of the total number of zooids, when it can be seen that the relative allocation changed little between cell concentrations (table 2.6). Somatic investment was greatest at 10 cells.μl^{-1} (81.4%), where colony growth was poorest, and least at 50 cells.μl^{-1}, remaining relatively constant around a mean value of 73.3% for all other cell concentrations. Basal males were the least represented group, showing a peak of 11% at 10 cells.μl^{-1}, falling to 1.5% at 100 cells.μl^{-1}, then rising slightly again as final colony size decreased, to 6.3% at 300 cells.μl^{-1}. Contrastingly, frontal male investment was found to be greatest at intermediate cell concentrations, with a peak of 19.8% at 50 cells.μl^{-1}. Female allocation was relatively constant with a mean value of 14.3%, and a range of only 19.8% at 100 cells.μl^{-1}, to 4.8% at 10 cells.μl^{-1}. However, a linear discriminant analysis was unable to find any significant differences in the levels of relative allocation between different cell concentrations.

2.3.2 Cell ingestion rates.

In general, the number of cells ingested by C. hyalina per trial increased as a function of cell concentration (table 2.7). The number of cells ingested per minute was least at 10 cells.μl^{-1} (20.63±5.03), and was greatest at 300 cells.μl^{-1} (86.03±7.86). Conversely, the length of each feeding episode tended to decrease with increased cell concentration, the longest feeding episodes occurring at 10 cells.μl^{-1} (67.81s±12.45s), and the shortest at 300 cells.μl^{-1} (22.60s±4.68s). However, neither of these

parameters was found to be statistically significant (ANOVA, $P<0.802$ and $P<0.198$ respectively).

2.3.3 Feeding method and behaviour in C. hyalina.

Even after having been starved for 24h, the gut of autozooids was never seen to empty, despite a recorded gut-passage time of approximately 26 minutes when food was readily available (pers. obs.).

Food particles were drawn upwards into the feeding crown by a current generated by the lateral cilia. Particles continued to move upwards and out of the lophophore unless periodical ciliary reversal occurred, bringing particles down to the cilia-free region above the mouth (figure 2.1). Particles were drawn into the mouth on a current generated by cilia within the pharynx. Feeding was therefore discontinuous, relying on periodical ciliary reversal, so the number of algal cells passing through the lophophore always greatly exceeded the number of cells ingested.

Once a bolus of 3-4 cells had collected within the pharynx, a peristaltic contraction occurred, drawing the food particles straight down into the caecum, where ingested items were rapidly mixed. As cell concentrations increased above 100 cells.μl^{-1}, a large amount of congestion could occur around the mouth. Consequently, a food bolus considerably larger than four cells could build up within the pharynx. Polypides were observed to clear this by reversing the pharyngeal current, which had initially drawn food items in. However, the food bolus at this stage frequently did not break up, and would sometimes remain, travelling up and down

the inside length of the tentacles, thereby causing obstruction. Ultimately, when the bolus became too large, this resulted in retraction of the lophophore.

High cell concentrations were also associated with a large degree of tentacle 'behaviour', with tentacle 'flicking' (Winston 1978), being highly prominent. Also observed was a behaviour usually associated with the formation of large, external food boluses, in which the tentacles of the lophophore were observed to 'link arms'. In this behaviour, alternate tentacles intertwined and pressed down towards the mouth, possibly in an effort to ingest the bolus. When polypides had become sated after feeding in high cell concentrations, algae would frequently pass through the gut with only a limited amount of digestion taking place. The release of this partially digested material was generally simultaneous across the colony.

Also observed, was the response of C. hyalina to a number of algal species (see chapter 1). Feeding however, appeared to be indiscriminate, and no response was observed which could have been interpreted as 'taste'-mediated. Large detrital particles (20 μm+), did however result in retraction of the lophophore.

2.4 Discussion

The feeding mechanism described in the present study for C. hyalina confirms several aspects of feeding behaviour previously attributed to other bryozoan species, but there are also certain differences. Thus, although C. hyalina relies on a relatively simple filtering system, this differs from the mechanism originally described by Borg (1926), and subsequently by Atkins (1932), and Best and Thorpe (1983). In C. hyalina, the lateral cilia were found to draw food particles up into the feeding crown, with ciliary reversal, as first described by Strathmann (1973, 1982), bringing particles down over the mouth. This contrasts with the continual, downward current described by the above authors in the ctenostome Flustrellidra hispida. The mechanism by which cells are ingested has remained largely speculative (McKinney 1990), and although Borg (1926) originally noted a rapid dilation of the pharynx, which, aided by cilia on the pharyngeal epithelium, would suck food into the mouth, it appeared that cell ingestion in C. hyalina was accomplished solely by a ciliary-generated current within the pharynx.

Best and Thorpe (1983, 1986) were able to show that progressively higher cell concentrations resulted in the generation of faster feeding currents. Although not stated directly (McKinney 1990), higher feeding velocities were apparently generated by a faster ciliary beat. It was found that Flustrellidra hispida could fill the pharynx with about 1250 cells in as little as 7 seconds at cell concentrations of greater than 25 cells.μl^{-1}. Although in the present study, feeding current velocities were not measured directly, the

rate of cell ingestion by C. hyalina appeared to increase as a function of cell concentration, although this was not statistically significant, and was complemented by a decrease in the mean length of feeding episodes.

Although C. hyalina would not ingest more than four algal cells at a time under normal conditions, food boluses of up to 16 cells sometimes formed within the pharynx at unnaturally high cell concentrations. These were not normally swallowed, but were ejected by a reversal of the pharyngeal current. The ejected bolus would frequently remain within the lophophore, travelling up and down the length of the inner tentacles with the ciliary current, frequently causing obstruction of the mouth. This may also have played a part in reducing the length of the feeding episodes. Where attempts to feed by individual lophophores were persistently thwarted, this frequently led to the complete retraction of all lophophores in the colony, for periods of up to several hours. The gut passage time and level of digestion also seemed to be affected by external cell concentration, since cells ingested at high levels of food supply frequently left the gut wholly or partially undigested. The gut was never seen to empty completely in active polypides.

Certain cheilostome species, such as Pasythea tulipifera (Winston 1978), have been found predominantly to use tentacle-flicking and other movements rather than cilia-generated currents. The significance of the increased level of tentacular activity observed in C. hyalina at high cell concentrations must remain speculative, especially the 'linking-arms' behaviour. Tentacle flicking at low cell

concentrations appeared to occur sporadically and was not triggered by contact with algal cells. Okamura (1987a) has suggested that tentacular feeding may be favoured over filtration by the lateral cilia at high flow velocities in certain species.

Feeding in C. hyalina is, therefore, carried out by the interaction of three active, cilia-generated, feeding currents, which are mediated by behavioural responses such as lophophore movements and gut-passage time. It should be noted that the number of cells drawn into the feeding crown always greatly exceeded the number of cells ingested, a factor which has frequently been ignored by previous workers (Bullivant 1968a, Strathmann 1973, Menon 1974). Possibly, clearance rate, calculated from the depletion rate of suspended algal cells, may grossly underestimate filtration capacity, and a clear distinction should always be made between clearance and ingestion rates.

C. hyalina colonies were able to grow and reproduce in all the cell concentrations tested, despite the fact that most of these were greatly in excess of natural densities (Novarino 1991, pers. comm.). Growth rates were slow at 10 cells.μl^{-1}, rising to a peak at around 100 cells.μl^{-1}, and being suppressed again at extremely high concentrations. Statistical analysis revealed the effect of cell concentration to be more important for somatic parameters, notably total autozooids and area. On the other hand, the colony effect was consistently more important for sexual parameters, sex ratio and reproductive output in particular, which were largely unaffected by cell concentration.

Relative investment into somatic and sexual function was not found to vary significantly across the range of cell concentrations used. However, an apparent 'trade-off' (Stearns 1989) did occur at 50 cells.μl^{-1}, with reduced somatic investment resulting in extensive frontal male and female production. The lowest level of food supply was associated with the highest level of somatic investment and with the lowest levels of frontal male and female investment. Mean level of basal male production was, by contrast, considerably greater than that found at any other level of food supply. In general, the production of basal males was inversely proportional to final colony size, whereas frontal male investment was greater where colony growth was extensive.

These results would correlate with previous findings, where colonies grown on diets of low nutritional adequacy have been found to foster an increased relative production of basal males (see chapter 1). It has already been noted that the simple form and derivation of basal male zooids may provide a means by which colonies can achieve reproductive success at a very low colony size, at relatively low cost, and with no restriction on potential colony growth. However, the lack of significant difference between the relative levels of investment provides further evidence that life-history strategy in C. hyalina is governed to a large extent by genetic constraints. The full significance of relative levels of investment is discussed at length elsewhere in this thesis (see chapter 3).

Interestingly, the highest proportion of ovicells containing embryos was found at the lowest level of food supply. This proportion was otherwise relatively constant, except at a concentration of 300 cells.μl^{-1}, where the number of embryos relative to ovicells was greatly depressed. The number of ovicells produced by laboratory-cultured colonies of C. hyalina has generally been found to exceed the number of embryos ever produced (see chapter 3), possibly as a result of the costs involved in the placental nourishment and maintenance of embryos. Results from the current study suggest that C. hyalina may be capable of great economy of resources where food supply is limiting, with a maximum number of larvae being produced per unit of investment. The elevated number of ovicells at more adequate cell concentrations may be an adaptation to facilitate outcrossing. However, the depressed number of embryos at 300 cells.μl^{-1} may reflect inefficient feeding at such high cell densities, when lophophores were frequently retracted for extended periods of time. Best and Thorpe (1983) found that a cell concentration of around 100 cells.μl^{-1} was sufficient to saturate the feeding capacity of F. hispida. In the present study however, very large numbers of particles were ingested initially by C. hyalina, but since these were frequently only partially digested, colony nutrition was probably retarded, depressing both growth rate and larval brooding.

Findings from the current study, therefore, provide further evidence that feeding in the Bryozoa is an active process (Best and Thorpe 1983, 1986a, b), but although C.

hyalina was able to respond behaviourally to increasing cell concentrations, this was not reflected in relative colonial composition. However, since ingestion rates in C. hyalina rose with increasing cell concentration, the efficiency of feeding may have been optimised (Best and Thorpe 1983, 1986a). This relationship between algal concentration and feeding rate provides further evidence that food supply in the marine environment may be of significant ecological importance, and a limiting resource under certain conditions (Buss 1979b, Buss and Jackson 1981, Best and Thorpe 1986a, b).

Table 2.1

Performance after a period of 7 weeks of colonies grown on a range of cell concentrations. 10 colonies were used per experimental treatment.

Cells μl^{-1}	$\bar{x}$ area(mm^2)	$\bar{x}$ total zooids	$\bar{x}$ total sexual zooids	% ovicells occupied
10	17.10±5.12	158.9±45.5	29.6±20.4	94.8
25	34.66±10.93	309.3±104.7	77.1±40.1	77.2
50	55.58±21.11	657.0±281.0	225.9±99.1	79.8
75	76.86±12.03	693.2±142.0	146.2±68.5	78.9
100	73.31±16.73	819.0±262.0	249.0±124.5	67.4
150	63.30±25.50	611.0±249.0	120.3±75.6	71.5
200	58.72±16.99	562.3±156.9	160.2±45.2	71.1
300	18.55±7.20	167.7±83.2	43.7±31.0	49.4

Table 2.2

Comparison of growth parameters between colonies when grown on 8 different levels of food supply. Data are presented as a 3-factor ANOVA, with the 3 experimental factors CONCENTRATION, COLONY (nested within CONCENTRATION), and TIME. Data were transformed to natural logs throughout.

Abbreviations for the sources of variation:

C : Concentration (n = 8)

Col : Colony (n = 10)

T : Time (n = 8)

a) Colony area.

Source	DF	SS	MS	F	P
C	7	144.505	20.644	11.72	0.000
Col(C)	72	126.873	1.762	33.52	0.000
T	7	1181.896	168.842	3211.46	0.000
C*T	49	31.014	0.633	12.04	0.000
Error	504	26.498	0.053		

b) Total autozooids.

Source	DF	SS	MS	F	P
C	7	137.045	19.578	11.95	0.000
Col(C)	72	117.999	1.639	27.63	0.000
T	7	1144.224	163.461	2756.05	0.000
C*T	49	32.512	0.664	11.19	0.000
Error	504	29.892	0.059		
Total	639	1461.672			

c) Basal males.

Source	DF	SS	MS	F	P
C	7	262.027	37.432	2.30	0.036
Col(C)	72	1174.167	16.308	7.77	0.000
T	7	3526.652	503.807	240.11	0.000
C*T	49	246.334	5.027	2.40	0.000
Error	504	1057.508	2.098		
Total	639	6266.688			

d) Frontal males.

Source	DF	SS	MS	F	P
C	7	517.253	73.893	4.97	0.000
Col(C)	72	1071.213	14.878	6.17	0.000
T	7	3981.106	568.729	235.98	0.000
C*T	49	462.570	9.440	3.92	0.000
Error	504	1214.692	2.410		
Total	639	7246.834			

e) Females.

Source	DF	SS	MS	F	P
C	7	358.041	51.149	3.78	0.002
Col(C)	72	975.229	13.545	6.36	0.000
T	7	4827.914	689.702	323.82	0.000
C*T	49	468.669	9.565	4.49	0.000
Error	504	1073.479	2.130		
Total	639	7703.333			

f) Embryos.

Source	DF	SS	MS	F	P
C	7	449.910	64.273	4.40	0.000
Col(C)	72	1051.573	14.605	5.33	0.000
T	7	5242.187	748.884	273.14	0.000
C*T	49	582.829	11.894	4.34	0.000
Error	504	1381.843	2.742		
Total	639	8708.342			

g) Total sexual zooids.

Source	DF	SS	MS	F	P
C	7	187.937	26.848	2.72	0.015
Col(C)	72	710.450	9.867	6.10	0.000
T	7	4858.654	694.093	429.30	0.000
C*T	49	274.650	5.605	3.47	0.000
Error	504	814.863	1.617		
Total	639	6846.553			

h) Total zooids.

Source	DF	SS	MS	F	P
C	7	133.512	19.073	11.49	0.000
Col(C)	72	119.518	1.660	28.95	0.000
T	7	1295.254	185.036	3226.94	0.000
C*T	49	34.978	0.714	12.45	0.000
Error	504	28.900	0.057		
Total	639	1612.161			

Table 2.3

Proportion of total sum-of-squares (see table 2.2), accounted for by each source of variation for colonies grown on 8 different levels of food supply. For each variable, values given are percentages of Total S.S., with T*Col(C) as the error term.

	VARIABLE						
SOURCE:	Area	Auto	Mb	Mf	Fem	Emb	Tot
C :	9.56	9.38	4.18	7.14	4.56	5.17	8.28
Col :	8.40	8.07	18.74	14.78	12.53	12.07	7.41
T :	78.23	78.28	56.28	54.94	62.64	60.20	80.35
C*T :	2.05	2.22	3.93	6.38	6.01	6.69	2.17
ERROR :	1.75	2.05	16.88	16.67	14.26	15.87	1.79

Table 2.4

Comparison of the perimeter:area ratio and the number of zooids per unit area for colonies grown on 8 levels of food supply. Data are presented as a 3-factor ANOVA. Data were transformed to natural logs.

Abbreviations for sources of variation:

C : Concentration (n = 8)

Col : Colony (n = 10)

T : Time (n = 8)

1) Perimeter:area ratio.

Source	DF	SS	MS	F	P
C	7	54.7218	7.8174	14.61	0.000
Col(C)	72	38.5264	0.5351	21.61	0.000
T	7	300.6414	42.9488	1734.77	0.000
C*T	49	13.5652	0.2768	11.18	0.000
Error	504	12.4778	0.0248		
Total	639	419.9326			

2) Zooids per unit area.

Source	DF	SS	MS	F	P
C	7	3.37756	0.48251	2.64	0.017
Col(C)	72	13.13643	0.18245	10.94	0.000
T	7	0.96733	0.13819	8.29	0.000
C*T	49	5.94837	0.12140	7.28	0.000
Error	504	8.40347	0.01667		
Total	639	31.83316			

Table 2.4 b)

Partitioning of the total variation (see above).

Abbreviations: PAR-perimeter:area ratio, ZPU-zooids per unit area

Source:	PAR	ZPU
C :	13.03	10.61
Col :	9.17	41.27
T :	71.59	3.04
C*T :	3.32	18.69
ERROR :	2.97	26.40

Table 2.5

Comparison of sex ratio and reproductive output for colonies grown on 8 levels of food supply. Data are presented as a 3-factor ANOVA. Data were transformed to natural logs.

Abbreviations for sources of variation:

C : Concentration (n = 8)

Col : Colony (n = 10)

T : Time (n = 8)

a) Sex ratio.

Source	DF	SS	MS	F	P
C	7	241.627	34.518	1.75	0.111
Col(C)	72	1420.993	19.736	4.90	0.000
T	7	625.112	89.302	22.16	0.000
C*T	49	459.759	9.383	2.33	0.000
Error	504	2031.138	4.030		
Total	639	4778.628			

b) Total reproductive output.

Source	DF	SS	MS	F	P
C	7	129.889	18.556	1.65	0.136
Col(C)	72	810.508	11.257	5.73	0.000
T	7	1720.434	245.776	125.21	0.000
C*T	49	257.948	5.264	2.68	0.000
Error	504	989.290	1.963		
Total	639	3908.069			

c) Male reproductive output.

Source	DF	SS	MS	F	P
C	7	146.086	20.869	1.73	0.114
Col(C)	72	866.103	12.029	6.29	0.000
T	7	1168.151	166.879	87.24	0.000
C*T	49	239.590	4.890	2.56	0.000
Error	504	964.051	1.913		
Total	639	3383.982			

d) Female reproductive output.

Source	DF	SS	MS	F	P
C	7	222.707	31.815	2.47	0.025
Col(C)	72	926.444	12.867	5.04	0.000
T	7	2131.868	304.553	119.37	0.000
C*T	49	419.082	8.553	3.35	0.000
Error	504	1285.891	2.551		
Total	639	4985.993			

Table 2.5 b)

2) Partitioning of the variation (see above).

Abbreviations: SR-sex ratio, TRO-total reproductive output, MRO-male reproductive output,FRO-female reproductive output.

	VARIABLE			
SOURCE:	SR	TRO	MRO	FRO
C :	5.06	3.32	4.32	4.47
Col :	29.74	20.74	25.59	18.58
T :	13.08	44.02	34.52	42.76
C*T :	9.62	6.60	7.08	8.41
ERROR :	42.50	25.31	28.49	25.79

Table 2.6

Colony composition after 7 weeks growth, given as percentage autozooids, basal males, frontal males and females.

Abbreviations: Auto-total autozooids, conc-concentration, Fem-females, Mb-basal males, Mf-frontal males.

Conc.		Auto	Mb	Mf	Fem
10	:	81.4	11.0	2.7	4.8
25	:	75.1	6.4	2.9	15.5
50	:	58.5	3.8	19.8	17.8
75	:	78.9	2.6	7.1	11.4
100	:	69.6	1.5	9.1	19.8
150	:	77.1	5.4	3.3	14.0
200	:	71.5	5.5	6.9	16.1
300	:	73.9	6.3	5.1	14.7
$\bar{x}$		73.3	5.3	7.1	14.3

Table 2.7

Cell ingestion rates and length of feeding episodes, measured by direct observation of C. hyalina colonies after starvation for 24h.

Conc.	:	$\bar{x}$ feeding episode (s)	$\bar{x}$ cells ingested/min
10	:	67.81±12.45	20.63±5.03
25	:	64.81±14.81	36.77±5.56
50	:	53.75±12.63	36.75±5.73
75	:	31.65±10.47	59.81±14.98
100	:	56.44±10.53	43.43±7.94
150	:	40.86±10.42	65.53±11.47
200	:	54.94±14.49	59.78±9.40
300	:	22.60±4.68	86.03±7.86

Figure 2.1

Diagrammatic representation of the feeding mechanism in C. hyalina.

(a) Principle feeding currents.

i) The main feeding current, generated by the lateral cilia, bringing food particles up into the lophophore.

ii) Ciliary reversal brings cells over the mouth.

iii) The pharyngeal current, responsible for cell ingestion.

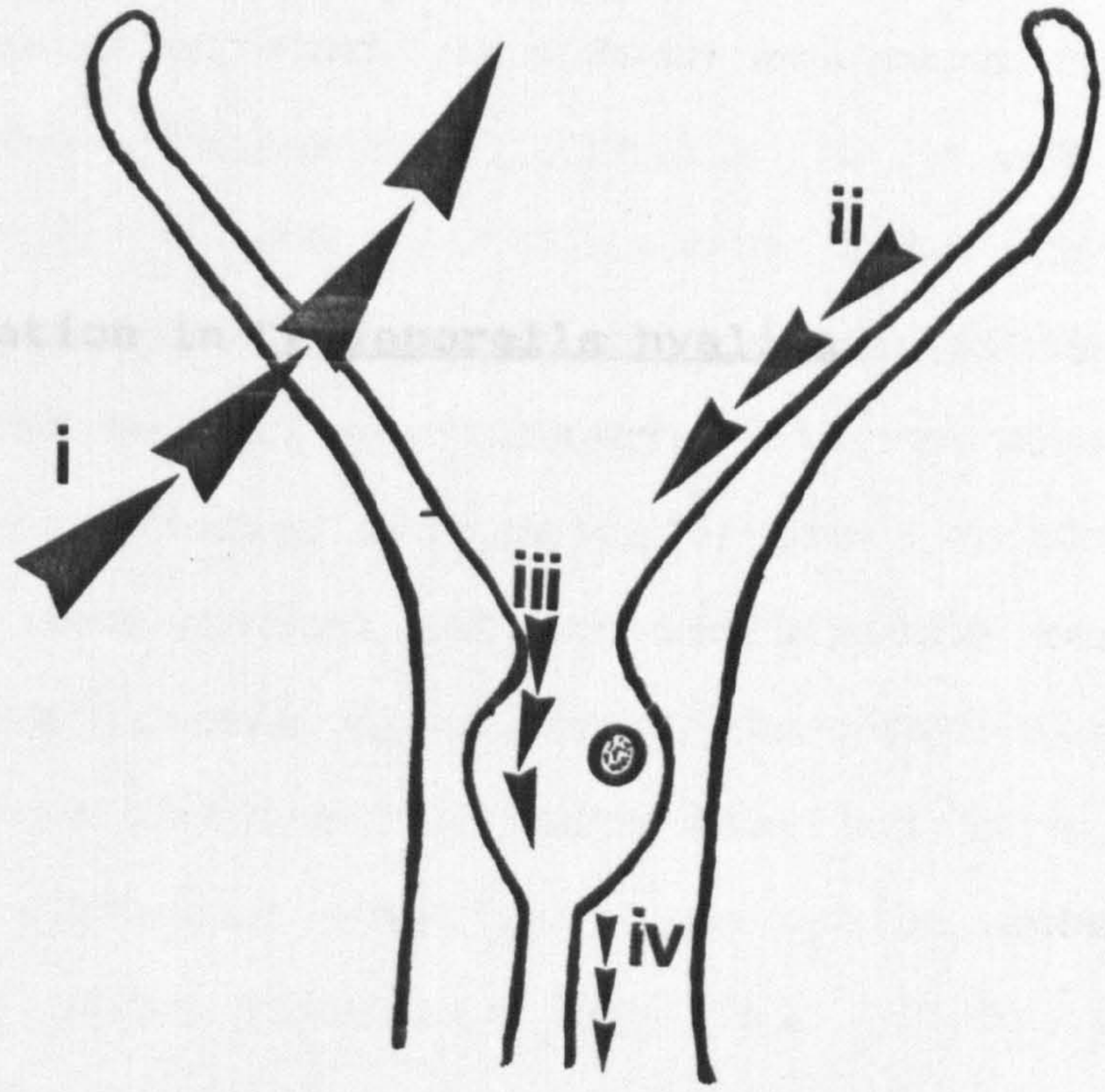
iv) Peristaltic contraction takes particles down into the caecum.

(b) Feeding in high cell concentrations.

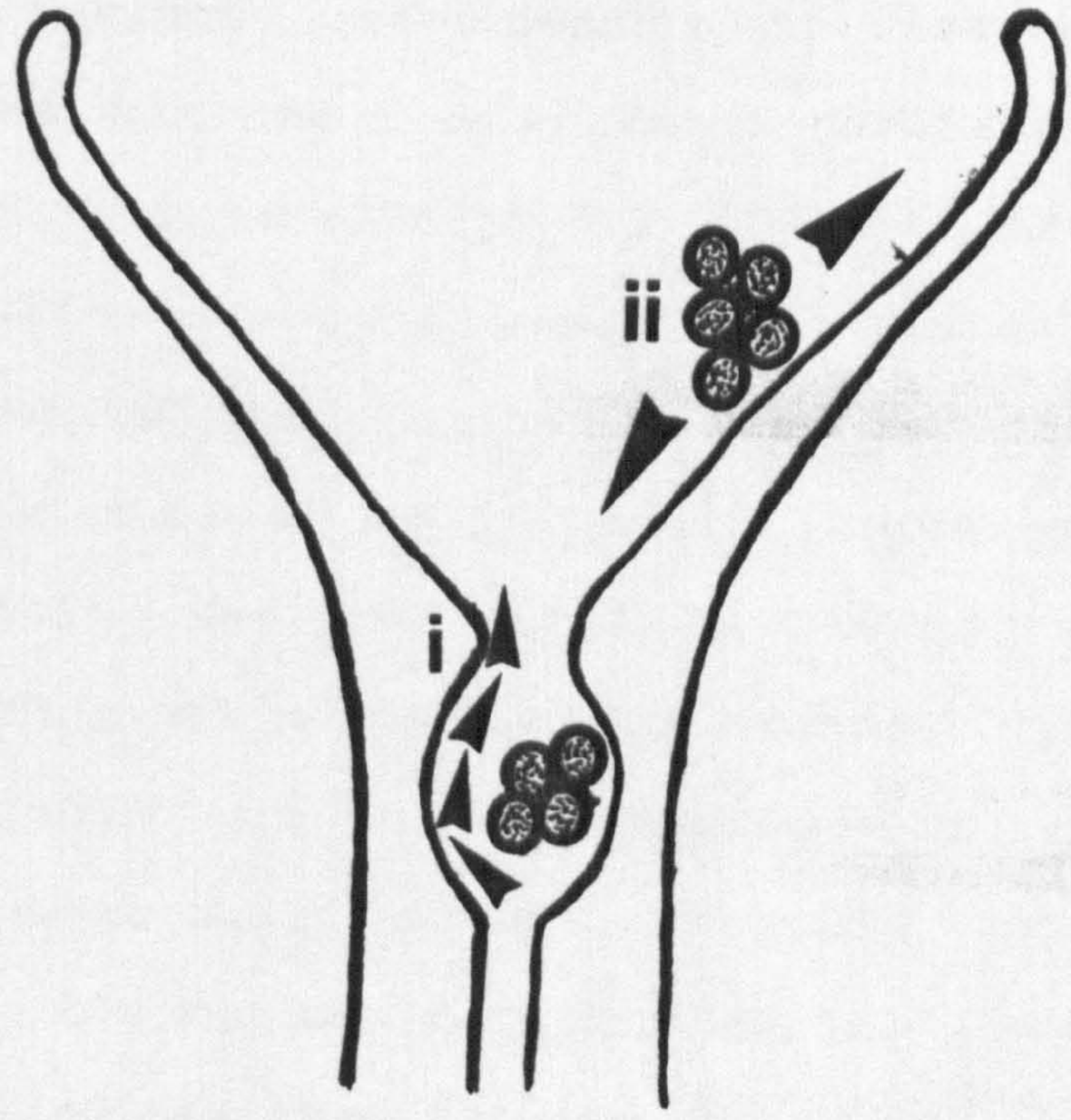
i) Reversal of the pharyngeal current removes large bolus (> 4 cells.

ii) The bolus may then become trapped in the lophophore, travelling up and down the frontal cilia.

A



B



Chapter 3:

Clonal variation in *Celleporella hyalina*.

3.1 Introduction

The first detailed study of growth and reproduction in the marine bryozoan Celleporella hyalina (L.), was carried out by Cancino and Hughes (1987), with water flow over colonies settled on glass Petri dishes being experimentally manipulated in the natural environment. This was achieved by the use of plastic funnels, with colonies grown in water flow that was either unrestricted, or was increasingly restricted by short and long funnels fitted over the Petri dishes. It was assumed that a reduced flow rate resulted in a reduced food supply, a virtually untestable assumption under field conditions. The main findings from this study were as follows. Restricted water flow enhanced colony growth during the winter months, but resulted in a reduced growth rate during the rest of the year. Those colonies experiencing greater water flow had a higher proportion of sexually active zooids, reproductive allocation being at its highest level in June, and lowest in winter, irrespective of treatment. By transferring colonies from one flow regime to another, it was further noted that the experimentally induced trends in growth and reproduction could be reversed. Colonial budding rate was reduced when colonies were grown in close proximity to conspecifics, as opposed to growth in isolation, but reproductive allocation was unaffected by the presence of conspecifics. Sex ratio varied considerably between different individuals, but with no clear relationship to the experimental flow regimes. This gave the first indication that relative sexual allocation in C. hyalina was genetically controlled. These findings were interpreted with respect to

the expected lifespan of naturally occurring colonies growing on the kelp Laminaria saccharina.

Using the same experimental methods, D.J. Hughes (1989), extended this work, splitting colonies in order to examine clonal response, focusing on the genotypic and environmental components of sex ratio and reproductive allocation in C. hyalina. Significant variation in growth rate, sex ratio and relative sexual allocation was found between clones growing in the autumn and winter, and water flow was again found to affect growth rate, but not sex ratio and reproductive allocation. A trade off between sexual and somatic functions was detected, with those clones displaying a reduced sexual investment eventually reaching a larger colony size. As with Cancino and Hughes' (1987) findings, the effect of ambient flow rate was reduced and inconsistent during the summer months, but with the exception of reproductive allocation, all characters were found to exhibit significant genetically based variation.

In a subsequent reanalysis of this work (D.J.Hughes 1991, in press), clonal performance, and thus clonal fitness, was found to vary considerably between experimental treatments, and although fitness ranking between environments showed a mainly positive correlation, many clones showed large shifts in rank order between environments. This can be taken as evidence of fine-scale niche partitioning between clones of C. hyalina.

The interaction of genotype with environment is critical in providing vital evidence towards answering one of the primary questions in modern evolutionary biology, the

maintenance of sexual reproduction in spite of the well-documented 'costs of meiosis' (reviewed by Lewis 1987).

It has been noted that apomictic taxa tend to occupy a wider geographical range, and typically inhabit marginal and peripheral habitats with a greater range of environmental extremes than their sexual counterparts (Bell 1982, Bierzychudek 1989) This has given rise to the hypothesis that asexual genera may possess 'general purpose' genotypes, where performance of an individual will be relatively insensitive to changes in environmental conditions. General purpose genotypes have been demonstrated in certain plant species (Bierzychudek 1985, 1989), and apomicts in possession of such genotypes are theoretically less likely to become extinct when faced with environmental extremes, a finding at odds with the concurrent maintenance of sexual mechanisms within these and other species capable of asexual proliferation.

The primary benefit of sexual reproduction has generally been put forward as the production of genetically diverse progeny (Bell 1982). Because of genetic recombination, a broadly adapted sexual parent will not necessarily produce broadly adapted offspring (Templeton 1982), but it is generally assumed that sexually produced progeny will partition the environment more finely than asexual progeny (Bell 1982). Although numerous ideas have been put forward about the maintenance of sexual reproduction within populations (reviewed in Bell 1982), two principal theories have emerged as the most likely explanations of the maintenance of sexual diversity. Both are based on the

principle of partitioning of a heterogeneous environment by genetically diverse individuals (Bell 1987).

The first of these is the 'tangled bank' theory, developed by Bell (1982), from the model originally put forward by Ghiselin (1974), related to the sib-competition model of Williams (1975) and subsequently modified first by Maynard Smith (1976a) and then by Price and Waser (1982). Tangled Bank considers heterogeneity of the environment on a highly localised scale, with specific genotypes showing optimal performance at different sites. Since the number of individuals that can occupy any one niche is limited, the genetically uniform progeny of an asexual female will compete intensely for the same set of resources, whereas the genetically diverse progeny of a sexual female can exploit different niches, reducing competition, and therefore resulting in greater overall reproductive success.

The 'Red Queen' hypothesis (Van Valen 1973, Jaenicke 1978), by contrast, places an emphasis of the temporal aspect of heterogeneity within the environment, with a continual necessity of individuals to respond to an ever-changing array of predators, parasites and pathogens (Bell 1982). Any antagonist will eventually be able to counteradapt to a given genotype within the host or prey population. Sex therefore, is necessary to the host or prey organism in order to continually create resistance in the progeny by producing new combinations of genes, and is equally necessary for the counteradaptation of predators and parasites, in an indefinitely perpetuating cycle.

The response of a single genotype over a range of environmental conditions is the best way to gain an accurate evaluation of the genotypic and environmental components of life-history variation. The existence of genotype-environment interactions is an essential prerequisite for the operation of both the Tangled Bank and the Red Queen (Bell 1987, 1990a). Clonal organisms with modular construction generally are the only organisms on which such experiments can be carried out. Where the soma is divided into independent modular units (Hughes and Cancino 1985, Hughes 1989), a range of treatments can be applied simultaneously to the same genotype.

Studies of this type have widely been used to investigate life-history variation in plants, most notably in grasses, where cohorts of replicate individuals can be created by vegetative propagation (eg.Hickman 1975, Primack and Antonovics 1982, Marshall et al 1986). In general, genotype-by-environment interactions have been found in most pasture communities studied, demonstrating extremely fine-scale environmental partitioning (eg Turkington 1979, Turkington and Harper 1979, Evans and Turkington 1988, Turkington 1989a, 1989b). More recently, Bell (1990a, 1990b, 1991a, 1991b), has published a series of studies demonstrating extensive genotype-environment interaction within and between laboratory strains of the protist Chlamydomonas.

Another essential feature of the Tangled Bank and the Red Queen is that sexually produced offspring should compete less intensely for available resources on account of fine-

scale, environmental partitioning. Evidence of sib competition from the botanical literature has been less convincing (Willson et al 1987, Schmidt and Ehrhardt 1987, Kelley 1989a, 1989b, McCall et al 1989, Tonsor 1989), although Schmitt and Antonovics (1986) found that plants surrounded by sibs suffered far more severely from a fortuitous aphid infestation than did plants surrounded by unrelated individuals. Kelley et al (1988) have also reported reproductive rates over two years as being 1.43 times greater in sexually generated progeny of the grass Anthoxanthum odoratum when compared with asexually generated progeny.

The number of comparable animal studies however, is minimal. Modular benthic invertebrates, such as sponges, ascidians, corals and bryozoans show close analogies to plants in growth form and demography (Jackson et al 1985, Harper et al 1986). Somatic growth in such organisms, as in plants, occurs by modular iteration. The capacity for sexual reproduction is usually also present, with many species being simultaneous hermaphrodites (Ryland 1981). Genotype-environment interactions have generally been found where looked for in animals (Bell 1982), and evidence of fine-scale, environmental adaptation has been documented in sea anemones (Shick et al 1979, Ayre 1985), thrips (Karban 1989), and in asexual brine shrimp (Browne and Hoopes 1990). Transplantation experiments using clones of the sea anemone Actinia tenebrosa showed that asexual fecundity was significantly higher in 'native' anemones when compared with individuals transplanted to foreign sites (Ayre 1985). The

relative ecological success of clones may in some cases lead to the domination of local populations by one or a few clones of high fitness (Ayre 1985, Hunter 1985). Larval output of clones may be determined by relative differences in sexual reproductive traits, and thereby also contribute to their differential evolutionary success. Little information on interclonal variation within a species exists, (Richmond 1987, Cancino and Hughes 1987, Grosberg 1988, D.J.Hughes 1989, chapter 4, this thesis). This mainly reflects the difficulty in making the necessary detailed measurements of sex allocation and reproductive effort.

Studies on variability in reproductive strategy are made more difficult by the occurence, in most colonial invertebrates, of hermaphroditic 'ramets' (sensu Harper 1977). The cheilostome bryozoan Celleporella hyalina (L.), however, exhibits complete modular partitioning between feeding, male and female function. Celleporella colonies are predominantly comprised of an underlying layer of feeding autozooids, from which sexual zooids are budded frontally. Zooid morphs are distinct, so simple counts of each morph allow estimation of relative investment into somatic, male and female functions (Cancino and Hughes 1987). Division and transplantation of individual colonies to provide genetically identical replicates is seldom attempted (Wellington 1982, Palumbi 1984, Szmant-Froelich 1985), despite the desirability and feasibility of such operations (Cancino and Hughes 1987, Grosberg 1988, Harvell and Grosberg 1988, D.J.Hughes 1989). The ease with which Celleporella can be cultured lends itself to this type of operation.

In the present study, division and transplantation of laboratory-grown Celleporella clones were used under carefully controlled environmental regimes in order to evaluate the relative importance of genotypic and environmental variation in the determination of the colonial life-cycle, the occurence and extent of genotype-environment interaction, and the relative performance of clones in a range of environmental conditions. This study is meant to complement the data of D.J.Hughes (1989, 1991, in press), by removing the environmental variation inevitably associated with experiments conducted under natural conditions.

3.2 Materials and methods

3.2.1 Experimental method

Larvae of Celleporella hyalina were released from colonies growing on Fucus serratus and induced to settle on glass microscope slides (7.6 * 3.9cm) following the method described by Cancino and Hughes (1987). Glass Slides bearing newly settled larvae were transferred to tanks containing 0.2 μm filtered, U.V.-irradiated seawater, to which the alga Rhodomonas baltica had been added as a food source, and were allowed to metamorphose at 18°C. After 24 hours, excess ancestrulae (metamorphosed larvae) were removed, leaving three well-spaced individuals, which were then returned to their tanks and the young colonies allowed to develop.

Once the colonies had reached an area of approximately 10-15mm^2, they were divided into four equal pieces using a hand-held, diamond-pointed cutter, such that the planes of fracture passed through the bryozoan colony. Fragments bearing the quartered colonies were then glued onto a second microscope slide (7.6 * 3.9cm) using silicone sealant, with two genotypes per slide. Colonies were then returned to the growing tanks and allowed to redevelop until the new colonies had grown down onto the second glass surface. The glass chip bearing the original colony fragment was then scraped away along with any remaining silicone sealant, and each colony reduced to less than eight feeding zooids, all sexual zooids being carefully removed. In this way, the starting procedure was standardised, leaving a small cluster of autozooids on a flat, uniform surface.

Unlike manipulation of water flow in the natural environment (D.J.Hughes 1986), laboratory culture of Celleporella allows carefully controlled conditions to be maintained throughout a colonial lifetime. It was decided that temperature and food supply would be the two most easily manipulable environmental variables, food quality and quantity having already been shown to have a significant effect on colonial growth (see chapters one and two). Since the number of divisions that could be taken from a single colony was restricted by the time available for propagation, it was decided to split colonies into four pieces. In this way, two extremes in temperature and food supply could be tested against each other for their relative effects on growth and sex allocation. The four possible combinations of temperature and food supply will be referred to as "macroenvironments" (sensu D.J. Hughes 1991, in press). Because of inevitable losses among daughter colonies, very large numbers of clones had to be propagated initially in order to ensure that a sufficient quantity were each represented by four colonies. At this stage, colonies were ready to be placed under experimental conditions.

The newly formed colonies were placed in plastic histological staining racks, and were transferred to tanks containing either 10 or 100 cells.μl^{-1} of Rhodomonas baltica, at either 8 or 18°C. Since all colonies had previously been maintained at 18°C, those colonies destined for the lower temperature regimes were taken down to 8°C over a period of one week, in order to avoid temperature-shock reactions. It was assumed that fine-scale microenvironmental variation at

the level of the glass slide was insignificant, but as a precaution, slides were rotated in position after each feeding in order to negate any variation which might occur. Thus, the four macroenvironments were as follows:

Macroenvironment 1 : 8°C, 10 cells.μl^{-1}

Macroenvironment 2 : 8°C, 100 cells.μl^{-1}

Macroenvironment 3 : 18°C, 10 cells.μl^{-1}

Macroenvironment 4 : 18°C, 100 cells.μl^{-1}

After two trials using seven and eleven clones respectively, a set of 26 clones was finally used, each clone being derived from colonies settled in October 1989. The maximum feasible duration of the experiment was judged to be twelve weeks. After twelve weeks, it became impossible to accurately count the zooids of the largest colonies and, in any case, counting became immensely time-consuming. Moreover, deteriorating colonial cohesion threatened to further complicate the analysis .

Colonies were drawn weekly with a camera lucida, and counts were taken of feeding autozooids, degenerated autozooids, fully formed buds, basal males, frontal males, females and embryos. Colony area and perimeter were derived from camera lucida drawings, using a digitiser.

3.2.2 Genotype-Environment interactions

In order to make use of all the data collected over the 12 weeks of the experiment, the analysis undertaken to determine the principal sources of variation was a four-factor analysis of covariance (ANCOVA), with the four factors CLONE, representing the number of clones used per

experimental series, TEMP, the two temperatures used, CONC, representing the two levels of diet used, and TIME, the sequential number of observations made in each series. These four main effects are fully orthogonal (ie each level of each factor occurred with all levels of each of the other factors). Since successive measurements were made on colonies over an extended period, this represents a design with repeated measures on the TIME factor. CLONE was treated as a fixed factor (Underwood 1981), because although clones present in the experiments were, in effect, a random selection from a very large pool of clones existing in the Celleporella population, it was only those particular genotypes that were of interest. TEMP and CONC were also treated as fixed factors, with levels chosen to represent the range that could potentially have been used in the experiment. The sample size was as follows:-

CLONE=26, TEMP=2, CONC=2, TIME=13

Counts of all zooid types were transformed to natural logs to normalise the data and to correct for inequality of variances. Analysis was also carried out on sex ratio and reproductive allocation, both of which were partitioned into their respective male and female components.

A general linear-model, 4-factor ANCOVA of this type results in 6 first-order interactions (TEMP*CONC, TEMP*CLONE, TEMP*TIME, CONC*CLONE, CONC*TIME, CLONE*TIME), 4 second-order interactions (TEMP*CONC*CLONE, TEMP*CONC*TIME, TEMP*CLONE*TIME, CONC*CLONE*TIME) and one third-order interaction (TEMP*CONC*CLONE*TIME). Interaction occurs when the effects of one factor differ in the presence of different

levels of another factor. Thus the effects of the factors are not additive, and are not independent of one another. For example, a significant TEMP*CONC*TIME interaction means that the relative effects of the 4 treatments vary over time, such that there may be differences between treatment groups at some observations, but not at others. Interactions of this kind must be taken into account when considering the significance of tests for the main effects of clone, temperature, cell concentration and time.

Statistically significant F-ratios occurred for most interaction terms, notably for those involving time, since colonies started growing at a similar size and gradually diverged over time. However, a statistically significant F-ratio does not necessarily reflect the importance of an effect in its contribution to total variance. This could be assessed by partitioning the total variation as a percentage of the total sum-of-squares accounted for by each term (D.J.Hughes 1989).

ANCOVA tests dependent variables, in this case TEMP, CONC and CLONE, for homogeneity among a group of means, but before the means are tested, they are adjusted for the group differences in the independent variable, known as the covariate, in this case TIME. This adjustment is carried out by linear regression (Sokal and Rohlf 1981). By taking out TIME as a covariate, it was hoped to clarify the contribution of the other main factors.

Between clone and within clone effects were calculated using TEMP*CONC*CLONE and TIME*TEMP*CONC*CLONE as error terms respectively.

3.2.3 Clonal variation in performance

3.2.3.1 Terminology

The terminology and analytical methods adopted in the present study are adapted from those originally used by Bell (1990a), and subsequently modified by D.J. Hughes (1991, in press), so direct comparison of the results can be made with the latter.

The experimental treatments experienced by Celleporella in the current study represent four combinations of temperature and food supply, in an attempt to mimic large-scale, spatial and temporal environmental variation which colonies would experience naturally. As such, they are directly comparable to D.J. Hughes' (1991, in press), "macroenvironments". Clonal fitness is defined as the performance of a clone relative to itself and other clones in the four macroenvironments. Performance is a measure of fitness, and can be interpreted in a number of ways. D.J. Hughes (1991, in press), used zooid production after a fixed period as a measure of performance. However, the lifespan of a Celleporella colony is indeterminate, and so the experimental endpoint is essentially arbitrary, with no real biological meaning. Thus, in the present study, the growth rate "r" was calculated for each measured parameter, and this was used as a measure of performance, rather than the experimental endpoint. "r" was calculated from the regression coefficient of the log-transformed data, and provides an estimate of the exponential rate of increase. Since "r" takes into account colony growth over the entire experimental period, the results will not be biased by exceptionally fast

or slow growth at a particular point in time. Performance was measured not only in terms of total zooid production and total gonozooid production (D.J. Hughes 1991, in press), but also for autozooids, basal males, frontal males, total males and females, since these are all potentially competing items in terms of resource allocation. It is not known whether reproductive success in C. hyalina is dependent more on the number of males or females produced (D.J. Hughes 1989), but it was hoped that differentiation among the above measures of fitness might provide a greater understanding of relative clonal performance.

3.2.3.2 Analysis of clonal variation

A significant Genotype*Environment interaction in ANCOVA shows that clonal performance is affected by the environment in which it occurred. No information , however, can be obtained from this with regard to the performance of individual clones (Bell 1990a) and, therefore, on whether ranking of clonal performance is affected by macroenvironment. It has already been noted that there is no way of deciding the biological importance of differences in performance, and it should be assumed, therefore, that any difference is important (D.J. Hughes 1991, in press). The relative performance of Celleporella clones could be examined superficially by summing the final numbers of zooids across all four macroenvironments. This may demonstrate gross differences in clonal performance, but gives no information about changes in the ranking of clonal fitness between macroenvironments (Bell 1990a).

Spearman rank correlation coefficients were calculated for all measures of clonal performance. A significant correlation between two macroenvironments would indicate that the ranking of clonal performance was significantly similar, but could still conceal major changes in rank order. Theoretically, the correlation of clonal performance should be greater where the environments being compared are more similar (Bell 1990a). This was tested by plotting the rank correlation coefficients for the six possible pairs of macroenvironments as a function of the difference in mean growth rates between the paired macroenvironments (D.J. Hughes 1991, in press). Since the physical scale chosen by the experimenter may not itself be a limiting factor in clonal fitness (Bell 1987, 1990a), the use of clonal performance as an index of macroenvironmental value may have more biological significance (D.J. Hughes 1991, in press).

The performance of individual clones and changes in rank order between treatments was examined in detail using "genotypic norms of reaction" (Bell 1990a, D.J. Hughes 1991, in press). The performance of each clone (in this case "r"), is plotted against the mean score of all colonies in the same treatment. From this, major shifts in rank order can be seen, which might otherwise have been concealed by rank correlation, for example, where a significant correlation may be dependent on a minority of clones of exceptionally high or low fitness in both environments (D.J. Hughes 1991, in press).

The performance of each clone in each of the four macroenvironments was then plotted against its average score

across all four macroenvironments. This plot, termed "genetic regression" (Bell 1990a), shows the specific environmental effect on fitness distribution in the clonal population. The performance of genotypes in an environment with a regression slope of unity should vary in parallel with their average scores over all environments. Inferior genotypes should perform relatively well, and superior genotypes perform relatively poorly where the regression slope is <1. Conversely, where the regression slope is >1, inferior clones should perform even worse, while superior clones perform even better. Variation in the genetic regression may, therefore, play a part in identifying the environmental sources of the genotype-by-environment interaction (Bell 1990a).

3.3 Results

3.3.1 Genotype-Environment interactions

3.3.1.1 Somatic parameters

Since the measured growth parameters responded in a similar manner over all experimental treatments, they are treated here as a single group, for convenience.

The number of functional autozooids (figure 3.1 a), and degenerated autozooids (figure 3.1 b), increased linearly, the numbers produced being strongly temperature-dependent, with far fewer autozooids being produced at 8°C. Although the ratio of functional to degenerate autozooids varied considerably at small autozooid size, this eventually stabilised at around unity (figure 3.1 c). The number of fully formed buds present was more variable (figure 3.1 d), but as a general rule, larger colonies produced more buds, thus following a pattern similar to that of feeding and degenerated autozooids. Since similar trends were followed (above), these categories could be amalgamated without loss of information (figure 3.1 e). Highly significant effects for autozooids were found for TEMP, TIME and the TEMP*TIME, CONC*TIME, CLONE*TIME and TEMP*CONC*TIME interaction terms ($P<0.001$, table 3.1), with moderate significance of the TEMP*CONC interaction term ($P<0.01$), table 3.1). Partitioning of the total variation (table 2.12 a), revealed that TIME accounted for 74.2% of the total variability. Dominance of the TIME component was to be expected, since colonies started growing at roughly the same size, and gradually diverged as the experiment progressed. The other three main effects, TEMP, CONC and CLONE, accounted for less than 1% of the total variation between them placing an emphasis on the importance of the interaction terms and suggesting that

colony growth over time varied not only between temperature and food-supply treatments, but also interclonally.

The growth of frontal autozooids (figure 3.1 f), was considerably different from that of basal autozooids. Since this zooid morph had been found infrequently, it was previously considered unimportant, and remained uncounted in previous experiments. In the current study, although only small numbers were produced, their occurrence was strongly influenced by cell concentration ($P<0.001$, table 3.2). Indeed, in preliminary experiments (own unpublished results), frontal autozooids were only produced at 100 cells.μl^{-1}, a result mirrored here, where the number of frontal autozooids produced at 10 cells.μl^{-1} was minimal.

The effect of TEMP was not significant, but, with the exception of the TEMP*CONC , TEMP*CLONE, TEMP*TIME and CONC*CLONE interaction terms, which were non-significant, all other terms were highly significant ($P<0.001$). Partitioning of the variation (table 3.12 a), found the ERROR term to be accountable for the largest amount of the total variation. Although TIME was the next most important component, it was responsible for only 15% of the total variation, but the other main components were the interaction terms involving both TIME and CLONE.

Colony area (figure 3.2 a), followed a pattern identical to that found for total autozooids, as was expected. When the perimeter to area ratio (PAR), is plotted, however (figure 3.2 b), it was seen to be much higher at 8°C than 18°C, most notably at 100 cells.μl^{-1}. This indicates a more compact growth form in the higher temperature condition, especially

at 100 cells ul^{-1}. This indicates a more compact growth form in the higher temperature condition, especially at elevated levels of food supply. In this case, despite several highly significant interaction terms ($P<0.001$, table 3.3), partitioning of the total variation (table 3.2), suggested that the TIME, TEMP and CONC main effects were the principal components of variation respectively.

Another important finding from the current study was that the number of autozooids per unit area was strongly temperature-dependent (figure 3.2 c), more zooids per unit area being produced at 18°C. Only the TEMP*TIME and CLONE*TIME interaction terms were found to be weakly significant for zooids per unit area ($P<0.05$, table 3.4), but all main effects except CONC were significant. Partitioning of the variation found 39.63% of the total variation accounted for by the ERROR term, but the TEMP main effect was responsible for 17.84%, far in excess of the remaining factors.

3.2.1.2 Sexual parameters

Basal male zooids (figure 3.3 a) were invariably the first sexual zooids to appear, and in some cases occurred as early as the first week of growth. Cell concentration was the major influence, basal male production being maximal at 100 cells.μl^{-1}.

CONC and TIME were found to be highly significant, with moderate significance of the TEMP main effect, and no significance of CLONE. The TEMP*CONC, TEMP*TIME and TEMP*CONC*TIME interaction terms were highly significant ($P<0.001$), with moderate significance of the CONC*TIME interaction ($P<0.01$), and weak significance of the CONC*CLONE*TIME interaction. When the total

variation was examined, the CONC main effect accounted for 5.25%, a figure considerably greater than any previously found for a main effect on a growth parameter (table 3.12 b).

Frontal males started to appear shortly after basal males, usually from the third week of growth onwards (figure 3.3 b). The number of frontal male zooids was generally much less than the number of basal males. The effect of cell concentration on frontal male production was highly significant ($P<0.01$, table 3.6) but the CONC main effect accounted for only 0.28% of the total variation (table 3.12b). CLONE accounting for only 1.96% of the total variation, was significant ($P<0.05$), but TEMP was not. Only the CONC*TIME interaction was highly significant ($P<0.001$), but TEMP*CLONE, CLONE*TIME and TEMP*CLONE*TIME were weakly significant (table 3.7).

It was noted however, that the CLONE*TIME interaction was accountable for a sizable 9.4% of the total variation.

Since the number of frontal males produced was relatively small, when basal and frontal males were pooled (figure 3.3 c, table 3.8), the pattern was similar to that found for basal males.

Female zooids, produced from the third to fourth week onwards (figure 3.4 a), were only prevalent at 100 cells ul^{-1} and 18°C. Both CONC*TIME, as well as the TEMP*TIME, CONC*TIME, CLONE*TIME and TEMP*CONC*TIME interaction terms were found to be highly significant ($P<0.001$, table 3.8), with moderate significance of CLONE and TEMP*CONC ($P<0.01$), and weak significance of CONC and TEMP*CLONE*TIME ($P<0.05$). The TIME, ERROR and CLONE*TIME interaction term were the three most important components of the total variation (table 3.2).

The plot of embryo production is identical to that for female zooid production (figure 3.4 b), with the exception that the number of embryos produced was always considerably less than the total number of females. This was not due to individual clones producing barren ovicells, but was a consistent result, in that the number of ovicells was always in excess of the number of ovicells bearing embryos. A plot of the number of females against number of embryos (figure 3.4 c), revealed that females often outnumbered embryos by 4:1.

On pooling basal males, frontal males and females (figure 3.5), with the exception of

TEMP*CLONE, CONC*CLONE and TEMP*CLONE*TIME (ns), all interaction terms were found to be at least weakly significant ($P<0.05$, table 3.9). Partitioning the variation for total gonozooids, TIME accounted for 52.77% (table 3.12 a), compared with 74.26% for total autozooids, with the ERROR term being the other major component of variability. Possibly as a result of the relatively large basal male production, the CONC main effect was found to account for a relatively large percentage of the total variation, at 4.73%.

3.3.1.3 Sex ratio and reproductive allocation

The sex-ratio (females/total males) stabilised at around unity in both of the 18°C treatments (figure 3.6). The lower values for the 8°C treatments indicated a proportionally greater number of male zooids at reduced temperature, most notably in the 8°C 100 cells.μl^{-1} treatment.

The TEMP and CLONE main effects were found to be significant ($P<0.05$, $P<0.01$, table 3.10), but CONC was not. All interaction terms were at least weakly significant, with the exception of TEMP*CLONE and CONC*CLONE*TIME (ns).

Male reproductive allocation (total males/autozooids), also appeared to be temperature-dependent, with a greater level of proportional male investment in the lower temperature regimes (figure 3.7 a). The 18°C and 8°C regimes both reached asymptotes of around 0.1 and 0.3 respectively. Female reproductive allocation (figure 3.7 b) was greatest at 18°C, 100 cells.μl^{-1}, a result to be expected, since this was the only environmental condition in which a large number of female zooids was produced. When the results are pooled to give the total reproductive allocation (total gonozooids/autozooids), the pattern was very similar to that for male allocation (figure 3.7 c). All main effects were found to be highly significant ($P<0.001$), apart from CLONE (n.s.). With the exception of the TEMP*CLONE, TEMP*TIME and CONC*CLONE interactions (N.S.), all other interactions were at least weakly significant. The TIME and ERROR terms were again the main components of the total variation (table 3.12 c), but in this case, the TEMP*CONC interaction was accountable for 11.02%, and the CONC main effect for a considerable 9.1% of the total variability.

3.3.2 Clonal variation in performance

3.3.2.1 Relative performance across series

When the final numbers of zooids from the four macroenvironments were pooled, there were obvious differences in gross total autozooid and gonozooid production between clones (figure 3.8a). Total zooid production varied from 156 to 2076 zooids, with total gonozooid production ranging from 11 to 448 zooids. When gonozooid production was split into basal males, frontal males and females, the range in overall production was similarly large (figure 3.8b).

3.3.2.2 Rank correlation of clonal fitness

When Spearman rank correlation coefficients were calculated for the growth rate "r" of all measured parameters, the level of correlation in all cases was unexpectedly low, and frequently negative (table 3.13). With the exception of weak correlations between macroenvironments 1 and 2 for autozooidal growth rate, macroenvironments 3 and 4 for frontal male production, and macroenvironments 1 and 3 for female production ($P<0.05$), no other significant correlations were detected. The unexpectedly low levels of rank correlation provide very strong evidence that clonal performance was environmentally determined, and that clones which performed well in one environment did not necessarily perform well in other environments.

Environmental disparity in clonal ranking was further emphasised when the clonal fitness correlations were plotted as a function of the differences in mean performance of the macroenvironments being compared (figure 3.9 a-g). Fitness

correlation actually appeared to increase as the environments became more divergent in the case of frontal males and total gonozooids. However, in all cases, the calculated y intercepts were much lower than the theoretical intercept of unity, where identical macroenvironments are being compared.

Significant negative correlations suggest that reversal in fitness ranking may occur, such that the best-performing clones in one macroenvironment may sometimes become the worst in other macroenvironments.

3.3.2.3 Genotypic norms of reaction

The reaction norms for individual clones (figure 3.10 a-g) demonstrate very clearly that genotypic fitness was environmentally determined, and that the rank order of fitness had little consistency between treatments. Many clones showed dramatic changes in rank order between macroenvironments, with no apparent relation to experimental treatment. There were some clones, however, which showed a certain amount of consistency in performance between some of the environments. For example, when examining the rank order of clonal performance in terms of total zooid production (figure 3.10 a), the growth rate of clone 11 was found to be ranked 25 out of 26 in macroenvironments 2 and 4, and 26 in macroenvironments 1 and 3. Clone 11 was consistently low-ranking, irrespective of the measure of fitness, but this was not true of most clones. For example, for the same measure of fitness, the top-ranking clone in macroenvironment 4, clone 25, was also the lowest-ranking clone in macroenvironment 1.

Reaction norms based on total zooid production (figure 3.10 a), were essentially similar to those based on total gonozooid production (figure 3.10 b), though far from identical. But when individual zooid types were taken as separate measures of fitness (figure 3.10 c-g), changes in clonal rank order within treatments were considerable, giving further evidence that individual zooid types react independently to the same environmental variables, according to genotype.

Most clones were found ranked above the mean clonal growth rate for most of the measures of fitness. This was because the mean growth rate tended to be depressed by a few exceptionally poorly performing clones. In the case of frontal male (figure 3.10 e), and female zooids (figure 3.10 g), many clones failed to produce these gonozooids in the least productive macroenvironments, with the result that all clones below the line of mean performance had a growth rate of zero.

3.3.2.4 Genetic regression

When genetic regressions were plotted, it was found that, with the exception of female zooid production, macroenvironments 1 and 2 consistently produced regression slopes of >1, whereas macroenvironments 3 and 4 produced slopes of <1 (figure 3.11 a-g). This suggests that clones of high relative performance were favoured at lower temperatures, whereas clones with a low average performance were at an advantage under conditions of elevated temperature. This effect was least marked for total gonozooid

production in macroenvironment 1 ($P<0.05$, figure 3.11 b), with a slope of 1.03, which was close enough to unity to suggest that clones in this treatment were performing close to their average values in terms of reproductive output.

In the case of female production (figure 11 g), slopes of 1.09 ($P<0.001$) and 1.31 ($P<0.001$) were found for macroenvironments 1 and 3, and slopes of 0.926 ($P<0.001$) and 0.664 ($P<0.01$) in macroenvironments 2 and 4. This suggests that female performance in low-ranking genotypes was increased where food supply was elevated, whereas the female performance of high-ranking individuals was depressed where food supply was reduced. It should be noted however, that female zooid production was prevalent only in macroenvironment 4.

3.4 Discussion

The results from this study compliment and extend those obtained by Cancino and Hughes (1987), and D.J.Hughes (1989, 1991 in press). Colonies reared in the laboratory were cleaner and therefore easier to examine quantitatively than in the previous studies. Moreover, they were maintained under constantly controlled conditions, increasing experimental resolution. Experiments were terminated after a maximum of 12 weeks. At this stage, the laboratory-grown clones were much smaller than D.J.Hughes' sea-grown colonies after a similar time period. Thus, colonies in the best-performing group had a mean size of approximately only 500 autozooids, whereas sea-grown colonies would have contained more than 1000 autozooids by that time (D.J.Hughes 1989). Gonozooid production was also considerably less than that found in the equivalent sea-grown colonies. However, although some of these differences may have resulted as an artifact of laboratory culture, this difference in gonozooid production was mainly attributable to starting the experiment at a much-reduced colony size (<8 autozooids, compared with 64±4 active autozooids). Also, rather than proportional estimates (D.J.Hughes 1989), the data presented here are total counts, and as such can be deemed highly accurate.

3.4.1 Variation of zooid size with temperature

An incidental, but important, point to emerge, was the very clear variation in autozooid size with both temperature and, to a lesser extent, with genotype. The size of bryozoan zooids has been used both in a taxonomic context and as a

morphological character to indicate evolutionary change on a geological time scale (Coates and Jackson 1985, Okamura 1987b, Jackson and Cheetham 1990), but it is becoming apparent that consideration of spatial and temporal measures of zooid size must also be taken into account (Okamura 1987b). Indeed, previous studies of C. hyalina have assumed constant autozooid size (D.J.Hughes and Hughes 1986a, Cancino and Hughes 1987,1988, D.J.Hughes 1989). Ryland (1963) first provided evidence of a latitudinal gradient in zooid size in the genus Haplopoma, which was subsequently corroborated by evidence for the species H. sciaphilum (Silen and Harmelin 1976). It has also been noted that fossil Hippothoa colonies from northern regions have larger zooids (Morris 1980). Experimental culture of the anascans Electra pilosa and Conopeum reticulum has demonstrated the production of smaller zooids at higher temperatures (Menon 1972, own unpublished results). Okamura (1987b) moreover, has reported temporal variation in the mean zooid size in naturally occurring colonies of E. pilosa, with smaller zooids being produced during the summer months.

It has been suggested that the two most likely environmental influences that may explain seasonal trends in zooid size are temperature and food supply (Okamura 1987b). Sebens (1979), suggested that changes in body size among colonial suspension feeders may reflect changes in food availability. A decrease in prey size, or a high level of food supply should make it advantageous for a single polyp, or zooid, to divide into two smaller units with the concomitant increase in surface area for prey intake, based

on the relative scaling of surface area to volume. However, results from the current study clearly demonstrate that the mean zooid size in C. hyalina decreases at higher temperatures, irrespective of the level of food supply. This corroborates Okamura's (1987b) observation that larger zooids were produced by E. pilosa in the Menai Straits at the time of peak primary productivity, with mean zooid size falling as temperatures increased over the summer months. Menon (1972) also found reduced zooid size at higher temperatures. Unlike the current study however, the possibility of an energetic response could not be discarded, since equal amounts of food were provided in each case, and higher metabolic costs at elevated temperature may have reduced zooid growth.

Variation of body size with temperature and latitude appears to be a fundamental trait of both endotherms and ectotherms (eg Kinne 1970, Mayr 1970, Vermeij 1978, Nevo et al 1986), although the mechanisms by which temperature-mediated changes in body size take place remain speculative (Okamura 1987b). Postulated mechanisms range from simple, volume-to-surface-area relationships (Mayr 1963), to the observed attainment of larger cell sizes at lower temperatures, resulting in larger body size (Vermeij 1978). Sebens (1982), proposed that for ectotherms, the increased metabolic rate at higher temperatures would result in reduced growth if energy intake was constant. It would appear, however, that in C. hyalina autozooid size is reduced at higher temperatures independently of energy intake. Considerable temporal and genetic variation in autozooid size may, therefore, occur in this species, and so care should be

taken where autozooidal parameters are used for taxonomic and physiological purposes.

3.4.2 Genotype-Environment interactions

In general, the highly significant interaction terms, for both somatic and sexual characters, indicated that colony growth showed considerable variation, with treatment, genotype, and with time. This was also clear from partitioning of the variation, where, with the exception of TIME, which was always a major component of the total variability, there was a very low contribution from the other three main factors, TEMP, CONC and CLONE. The TIME component did appear to be more dominant for somatic, rather than sexual parameters. This was to be expected, since autozooidal production was more continuous over time, giving constant divergence, whereas sexual zooids were not produced for the first few weeks, and their production was somewhat more erratic. Otherwise, the only other notable contribution from a main effect contribution was from CONC in the case of basal males, suggesting that basal male production may be influenced by external levels of food supply more than other zooid morphs. CONC was of greater consequence statistically for frontal autozooid and frontal male production, whereas TEMP had a greater statistical significance in the case of female production. The presence of a statistically significant CLONE effect for several parameters provides further evidence for D.J.Hughes' (1989) finding of genetically based variation in life-history parameters in C. hyalina.

The low PAR in the optimal 18°C, 100 cells.μl^{-1} treatment suggests that although autozooid production may be genetically limited, the way in which zooids become arranged in two-dimensional space may be influenced by the prevailing environmental conditions. This is consistent with findings from other encrusting bryozoans (Winston 1976), where colonies of Conopeum seurati were found to adopt less compact, 'runner'- type morphologies under conditions of low nutritional adequacy. Previous studies have found that C. hyalina colonies recruited during the summer rapidly attained a compact, circular form, whereas colonies remained elongated or fan-shaped during the winter (Cancino and Hughes 1988). The present results suggest that this was almost certainly due to reduced levels of food supply during the winter. Winter-growing colonies were therefore unable to expand concentrically, unlike summer-growing colonies, or colonies in macroenvironment 4 (current study), where internal nutrient levels were high enough in some colonies to maintain an active meristem (Harper 1977) round the entire colony edge.

Basal males were effectively the only type of sexual zooid occurring in the basal layer, and were the zooids showing the greatest environmental response. Cancino (1983) reported that basal male zooids are formed when two distal buds fail to fuse into a normal feeding autozooid. Basal male production therefore may be an adaptation, whereby a colony can rapidly increase its reproductive success at a very early stage and at a relatively low cost without sacrificing potential colony size. The adaptive nature of this zooid type

is illustrated by the production of relatively large numbers of basal males under poor and deteriorating conditions. Where energy resources are restricted either internally or externally ,therefore, it may be better to achieve reproductive success as a male and avoid the burden of egg and larval production.

The number of female and frontal males is, by contrast, potentially limited by the extent, hence feeding capacity, of the underlying basal layer. A higher level of genetic control may therefore have been expected from those zooid types. However, frontal males and females were produced in substantial numbers only at higher levels of temperature and food supply. Numbers of frontal males in particular, were greatly reduced in comparison with sea-grown colonies (D.J.Hughes pers. comm.), but this was possibly a result of the relatively small colony size in laboratory reared colonies.

Previous studies have inferred a trade-off between reproductive output and colony size (Hughes and Cancino 1987), resources being allocated at a level maintaining the highest possible ratio of non-feeding gonozooids to feeding autozooids. The number of females produced in the current study was always greatly in excess of the number of ovicells bearing embryos. Although the costs of reproduction in Celleporella can be seen as increasing from the production of energetically cheap 'incidental' basal males, through purpose-built frontal males and females, it would appear that the most important costs in this species are entailed in the placental nourishment and maintenance of embryos. The high

ratio of available ovicells to embryos at any one time, suggests that the number of embryos that a colony of a given size can produce and maintain, is limited. Excess females may be an adaptation enhancing the colony's chance of outcrossing, so boosting larval fitness. However, the genetic component (clone effect) places a limit on female production, so when additional resources become available, these may be channelled into frontal male and frontal autozooid production. In this way, reproductive success is boosted, both as a male, where the chance of fertilising other colonies is enhanced, and as a female, where the energy-gathering power of the colony is enhanced, so boosting larval output. The effect of spatial and temporal variation on relative reproductive success is unknown in C. hyalina, but heritable variability in sex ratio and reproductive allocation suggest that reproductive success to some extent, may be environmentally determined. Increasing female investment under favourable conditions might reduce overall reproductive success, since the number of embryos which can successfully be produced per unit area is strictly limited. Detailed measurements of sex-specific reproductive success would be required to settle this matter, but would be difficult to achieve. Some progress possibly could be made using electrophoretically detected allelic markers to identify paternal origins in laboratory-reared populations (D.J.Hughes 1989), or using in situ mark-recapture technique complemented by histocompatibility assays (Grosberg 1991).

The optimal levels of investment detected at higher temperature and food supply in the present study can be

compared with a postulated seasonal adjustment in sexual allocation, maintaining an optimal ratio of gonozooids to feeding autozooids (D.J.Hughes 1989). This seasonal variation for sex allocation in Celleporella has previously been related to the expected lifespan of colonies settling on natural substrata (Cancino and Hughes 1987). In particular, Celleporella frequently forms dense, monospecific stands on the kelp Laminaria saccharina. On this kelp, colony lifespan is inversely related to the turnover rate of the algal tissue, which is generated from a basal meristem and lost at the distal end of the frond. A colony settling in March/April may have an expected lifespan of as little as 20 to 30 days, when algal growth rate is at its highest, and as much as 200 days during August/September, assuming that larvae preferentially select the youngest areas of the frond for settlement (Cancino and Hughes 1987). With a longer expected lifespan, it may be to the advantage of colonies settling in the autumn to delay maximal investment in gamete production over the winter, in favour of somatic investment. A switch to sexual investment during the spring may then allow colonies ultimately to produce more larvae than if they had invested maximally in reproduction from the start.

Celleporella is of course by no means restricted to Laminaria, a point often overlooked, and may occur on substrata as diverse as Mytilus valves and discarded plastics (pers. obs.). As a poor competitor, however (Cancino and Hughes 1987), it would not be a good strategy for Celleporella to postpone sexual investment during conditions of intense biological activity. Such a strategy would be

disadvantageous, moreover, if any size-independent mortality were to occur, such as the removal of Laminaria plants during the winter (D.J.Hughes 1989). It would appear, therefore, that sexual reproduction plays an essential part in the life history of C. hyalina, notwithstanding the 'costs of meiosis', on account of potentially restricted longevity of substratum. Polymorphism in reproductive strategy would seem then, to be regulated by a sensitivity to environmental cues, such as changes in food supply and temperature, indirectly predictive of clonal longevity.

Correlation between resource limitation and reduced sexual investment conforms with the idea that maintenance of the soma takes precedence where clonal organisms are faced with temporary sublethal stress (Hughes and Cancino 1985). Stress with the probable outcome of death, on the other hand, should stimulate an increased sexual investment, since dispersive propagules form a means by which a sessile animal can colonise a more favourable habitat. Such a response has been demonstrated in clonal hydroids on exposure to toxic metals and osmotic stress (Stebbing 1980). This type of response may also explain the relatively large numbers of males produced at low temperature and food supply, and also when colonies are grown on unfavourable diets (see chapter 1).

3.4.3 Relative performance of clones

The extensive clonal variation for life-history traits in Celleporella implies heritable variation for these characters, and confirms previous findings from field studies

(Cancino and Hughes 1987, D.J.Hughes 1989). The substantial Genotype-Environment interactions suggested that clonal performance in C. hyalina was environmentally determined, but the level of environmental specificity found when clonal reaction norms were examined were much greater than expected, since previous work had suggested that extensive changes in clonal rank order of clonal performance does not occur (D.J.Hughes 1991, in press). Ranking of clonal performance in the current study was found to vary not only between experimental macroenvironments, but also according to the measure of performance employed. It seemed, therefore, that not only was clonal performance environment-specific, but that genes coding for different parameters were responding independently to the same environmental variables. If this is the case, then reproductive success in C. hyalina may be affected by spatial and temporal environmental variation acting upon modular flexibility. The artificial environmental combinations used in the current study however, may have been more extreme than anything colonies would experience in nature. This calls into question the validity of the experimental design (Bierzychudek 1987). Certainly, like D.J.Hughes' studies (1989, 1991 in press), colonies used in the current experiment were drawn from a natural population, and therefore could have potentially coexisted in nature. Initially, colonies were selected purely on the basis of having settled in a convenient position on a glass slide. There may have been some inadvertent selection for fast-growing colonies, through using products of rapid regeneration of the original clonal divisions. This, however,

proved to be minimal, since many of the original clones failed to regenerate in all four replicates. Allowance therefore, had to be made in waiting for slower-growing clones.

The relevance of the experimental macroenvironments, was however, more debatable. Easily controlled environmental factors were chosen out of necessity, and it was also desirable to examine environmental extremes within a realistic range. Food supply is known to play an important role in bryozoan ecology (see chapters 1 and 2, this thesis), and naturally occurring colonies would certainly not rely on a single food source. However, a monospecific diet of Rhodomonas, although not representative of any natural situation, was found to be the best diet for rearing colonies in the laboratory (chapter 1, this thesis). Similarly, the upper food level employed in the current study was possibly much greater than anything that colonies would encounter in the natural environment. Nevertheless, the two levels used were found to cause considerable disparity in growth under laboratory conditions (chapter 2, this thesis).

The levels of temperature used were chosen to approximate natural extremes. 18°C slightly exceeds the average maximum sea temperature of about 16°C reached in the Irish Sea during the summer, and 8°C similarly exceeds the average minimum of 4°C reached during the winter (Anon 1955), but colonies exposed during low tide will invariably experience greater extremes in temperature than this.

Like previous studies of life history variation in C. hyalina (Cancino and Hughes 1987, D.J.Hughes 1991, in press),

the present experiment was carried out in 'mixed culture'. It was assumed that no inter-genotypic interactions occurred (Bell 1990a,b,1991a,b) and therefore played no part in influencing the Genotype-Environment interactions. Although significant depletion of food could have occurred in the low-food-supply macroenvironments, severe competition would have been unlikely, since colonies were generally spaced by at least 3cm, and inter colonial interactions resulting from feeding currents were therefore unlikely, due to the small polypide size in C. hyalina (Best and Thorpe 1986b, Hughes and Cancino 1988).

Unfortunately, only one clonal replicate could be used per macroenvironment (see methods). Comparisons of clonal fitness between environments must assume identical genetic identity among clone-mates. It has been found in a previous study, however, that a considerable degree of inconsistency could occur between clonal replicates within a single macroenvironment (D.J.Hughes 1991, in press). Since this was most unlikely to have come about as a result of microenvironmental variation (D.J.Hughes 1991, in press), it must be assumed that clone-mates may not always be genetically identical, but may vary as a result of somatic mutation (Slatkin 1984). Intra-clonal variation is examined in detail in the following chapter. If, however, genotypic performance within a clone cannot be assumed to be invariant, then somatic mutation could have been responsible for some of the dramatic changes in the rank order of clonal performance observed in the current study.

Genetic variance contributed by genes that are uniformly successful or unsuccessful over a wide range of environments will tend to be removed by selection, whereas genes that enhance fitness in some environments, but not in others, will tend to be eliminated from the population more slowly, or may be conserved indefinitely, as a result of dilution of directional selection (Bell 1991a). Genotype-environment interactions arising as a result of disruptive selection among environments will be a property of evolved populations. This is the essence of the Tangled Bank theory.

Unlike D.J.Hughes[7] (1991, in press) study, the seemingly least productive environments caused a greater depression of performance in relatively high-ranking genotypes for all measures of fitness except female production, compared with genotypes of low average fitness. The occurence and unexpected magnitude of genotype-environment interactions, and the frequent inversion of clonal rank order between environments demonstrates forcibly that overall 'fitness' of C. hyalina genotypes may vary dramatically according to local conditions. Although the two environmental variables used in the current study were fixed at levels intended to mimic natural variation, it is impossible to distinguish between the spatial and temporal aspects of this design. Temperature and food supply may not be the main basis of 'niche partitioning' among genotypes within this species (Bierzychudek 1987). This study therefore, rather than corroborating or contradicting either the 'Tangled Bank' or the 'Red Queen' theories, provides further evidence that sexual reproduction, in C. hyalina at least, is an essential

mechanism not only allowing dispersal from ephemeral substrata, but also for generating a diverse array of genotypes that effectively exploit the wide range of microhabitats in a widely varying environment.

	Auto	Af	PAR	ZPU	Mb	Mf	Mt	Fem	Sex	TSR	TRA
Between clone											
TEMP	***	ns	***	***	**	ns	**	***	**	*	***
CONC	ns	***	***	ns	***	**	***	*	***	ns	***
CLONE	ns	*	ns	**	ns	*	ns	**	ns	**	ns
TEMP*CONC	**	ns	***	ns	***	ns	***	**	**	*	***
TEMP*CLONE	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
CONC*CLONE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Within clone											
TIME	***	***	***	***	***	***	***	***	***	***	***
TIME*TEMP	***	ns	*	*	***	ns	***	***	***	***	ns
TIME*CONC	***	***	***	ns	**	***	**	***	**	*	***
TIME*CLONE	***	***	ns	*	ns	*	ns	***	*	***	**
TIME*TEMP*CLONE	ns	***	ns	ns	ns	ns	ns	*	ns	**	*
TIME*CONC*CLONE	ns	***	ns	ns	*	ns	ns	ns	*	ns	**
TIME*TEMP*CONC	***	**	***	ns	***	*	***	***	***	***	***

Table 3.1 Autozooids

Source	F	P
Between clone		
TEMP	28.478	***
CONC	3.452	ns
CLONE	0.767	ns
TEMP*CONC	7.835	**
TEMP*CLONE	0.708	ns
CONC*CLONE	1.102	ns
Within clone		
TIME	3352.087	***
TIME*TEMP	312.132	***
TIME*CONC	115.914	***
TIME*CLONE	8.071	***
TIME*TEMP*CLONE	1.768	ns
TIME*CONC*CLONE	1.251	ns
TIME*TEMP*CONC	49.058	***

Tables 3.1-3.11

Analysis of covariance (ANCOVA), of the performance of 26 clones, grown in four different macroenvironments, over 12 weeks. The 4 experimental factors were TEMP, CONC, CLONE and TIME. Data were transformed to natural logs.

Sources of variation: TEMP : temperature (n = 2)

CONC : food supply (n = 2)

CLONE : clone (n = 26)

TIME : time (n = 13)

The between clone and within clone effects are summarised on the facing page.

Table 3.1

ANCOVA of total autozooid production

Source	DF	Adj SS	Adj MS	F	P
temp	1	6.721	6.721	87.05	0.000
conc	1	0.836	0.836	10.83	0.001
clone	25	4.536	0.181	2.35	0.000
time	1	1042.499	1042.499	1.4E+04	0.000
temp*conc	1	1.849	1.849	23.95	0.000
temp*clone	25	4.168	0.167	2.16	0.001
temp*time	1	97.073	97.073	1257.35	0.000
conc*clone	25	6.502	0.260	3.37	0.000
conc*time	1	36.071	36.071	467.22	0.000
clone*time	25	62.746	2.510	32.51	0.000
temp*conc*clone	25	5.891	0.236	3.05	0.000
temp*conc*time	1	15.257	15.257	197.62	0.000
temp*clone*time	25	13.746	0.550	7.12	0.000
conc*clone*time	25	9.735	0.389	5.04	0.000
temp*conc*clone*time	25	7.778	0.311	4.03	0.000
Error	1144	88.322	0.077		
Total	1351				

Table 3.2 Frontal autozooids

Source	F	P
Between clone		
TEMP	0.307	ns
CONC	26.889	***
CLONE	1.930	*
TEMP*CONC	0.010	ns
TEMP*CLONE	0.984	ns
CONC*CLONE	0.766	ns
Within clone		
TIME	279.929	***
TIME*TEMP	0.425	ns
TIME*CONC	175.110	***
TIME*CLONE	11.052	***
TIME*TEMP*CLONE	6.036	***
TIME*CONC*CLONE	5.460	***
TIME*TEMP*CONC	10.651	**

Table 3.3 PAR

Source	F	P
Between clone		
TEMP	690.227	***
CONC	659.964	***
CLONE	1.616	ns
TEMP*CONC	749.128	***
TEMP*CLONE	1.042	ns
CONC*CLONE	1.458	ns
Within clone		
TIME	628.297	***
TIME*TEMP	5.797	*
TIME*CONC	216.148	***
TIME*CLONE	1.837	ns
TIME*TEMP*CLONE	0.739	ns
TIME*CONC*CLONE	0.640	ns
TIME*TEMP*CONC	163.110	***

Table 3.2

ANCOVA of frontal autozooid production

Source	DF	Adj SS	Adj MS	F	P
temp	1	0.447	0.447	0.78	0.377
conc	1	39.123	39.123	68.30	0.000
clone	25	70.205	2.808	4.90	0.000
time	1	407.297	407.297	711.08	0.000
temp*conc	1	0.015	0.015	0.03	0.870
temp*clone	25	35.793	1.432	2.50	0.000
temp*time	1	0.618	0.618	1.08	0.299
conc*clone	25	27.885	1.115	1.95	0.004
conc*time	1	254.785	254.785	444.82	0.000
clone*time	25	402.010	16.080	28.07	0.000
temp*conc*clone	25	36.373	1.455	2.54	0.000
temp*conc*time	1	6.103	6.103	10.65	0.001
temp*clone*time	25	219.570	8.783	15.33	0.000
conc*clone*time	25	198.596	7.944	13.87	0.000
temp*conc*clone*time	25	223.604	8.944	15.62	0.000
Error	1144	655.268	0.573		
Total	1351				

Table 3.3

ANCOVA of PAR (perimeter:area ratio)

Source	DF	Adj SS	Adj MS	F	P
temp	1	49.6273	49.6273	3374.41	0.000
conc	1	47.3795	47.3795	3221.57	0.000
clone	25	2.9059	0.1162	7.90	0.000
time	1	113.9731	113.9731	7749.60	0.000
temp*conc	1	53.8623	53.8623	3662.37	0.000
temp*clone	25	1.8723	0.0749	5.09	0.000
temp*time	1	1.0516	1.0516	71.50	0.000
conc*clone	25	2.6210	0.1048	7.13	0.000
conc*time	1	39.2093	39.2093	2666.03	0.000
clone*time	25	8.3337	0.3333	22.67	0.000
temp*conc*clone	25	1.7963	0.0719	4.89	0.000
temp*conc*time	1	29.5889	29.5889	2011.90	0.000
temp*clone*time	25	3.3521	0.1341	9.12	0.000
conc*clone*time	25	2.9306	0.1172	7.97	0.000
temp*conc*clone*time	25	4.5346	0.1814	12.33	0.000
Error	1144	16.8248	0.0147		
Total	1351				

Table 3.4 ZPU

Source	F	P
Between clone		
TEMP	143.120	***
CONC	0.194	ns
CLONE	3.080	**
TEMP*CONC	3.139	ns
TEMP*CLONE	0.685	ns
CONC*CLONE	0.755	ns
Within clone		
TIME	92.882	***
TIME*TEMP	7.028	*
TIME*CONC	2.736	ns
TIME*CLONE	2.309	*
TIME*TEMP*CLONE	1.280	ns
TIME*CONC*CLONE	0.810	ns
TIME*TEMP*CONC	0.060	ns

Table 3.5 Basal males

Source	F	P
Between clone		
TEMP	10.669	**
CONC	112.545	***
CLONE	1.151	ns
TEMP*CONC	147.660	***
TEMP*CLONE	0.718	ns
CONC*CLONE	0.862	ns
Within clone		
TIME	1593.628	***
TIME*TEMP	45.821	***
TIME*CONC	12.984	**
TIME*CLONE	1.387	ns
TIME*TEMP*CLONE	0.784	ns
TIME*CONC*CLONE	2.145	*
TIME*TEMP*CONC	85.182	***

Table 3.4

ANCOVA of ZPU (zooids per unit area)

Source	DF	Adj SS	Adj MS	F	P
temp	1	6.69227	6.69227	504.56	0.000
conc	1	0.04273	0.04273	3.22	0.073
clone	25	3.60088	0.14404	10.86	0.000
time	1	3.82582	3.82582	288.45	0.000
temp*conc	1	0.14680	0.14680	11.07	0.001
temp*clone	25	0.80079	0.03203	2.42	0.000
temp*time	1	0.28949	0.28949	21.83	0.000
conc*clone	25	0.88220	0.03529	2.66	0.000
conc*time	1	0.11269	0.11269	8.50	0.004
clone*time	25	2.37741	0.09510	7.17	0.000
temp*conc*clone	25	1.16896	0.04676	3.53	0.000
temp*conc*time	1	0.00247	0.00247	0.19	0.666
temp*clone*time	25	1.31821	0.05273	3.98	0.000
conc*clone*time	25	0.83370	0.03335	2.51	0.000
temp*conc*clone*time	25	1.02964	0.04119	3.11	0.000
Error	1144	15.17350	0.01326		
Total	1351				

Table 3.5

ANCOVA of basal male production

Source	DF	Adj SS	Adj MS	F	P
temp	1	43.112	43.112	22.60	0.000
conc	1	454.796	454.796	238.38	0.000
clone	25	116.383	4.655	2.44	0.000
time	1	4274.110	4274.110	2240.26	0.000
temp*conc	1	596.694	596.694	312.76	0.000
temp*clone	25	72.507	2.900	1.52	0.049
temp*time	1	122.893	122.893	64.41	0.000
conc*clone	25	87.118	3.485	1.83	0.008
conc*time	1	34.823	34.823	18.25	0.000
clone*time	25	92.981	3.719	1.95	0.004
temp*conc*clone	25	101.034	4.041	2.12	0.001
temp*conc*time	1	228.457	228.457	119.75	0.000
temp*clone*time	25	52.546	2.102	1.10	0.332
conc*clone*time	25	143.808	5.752	3.02	0.000
temp*conc*clone*time	25	67.050	2.682	1.41	0.089
Error	1144	2182.595	1.908		
Total	1351				

Table 3.6 Frontal males

Source	F	P
Between clone		
TEMP	2.264	ns
CONC	8.409	**
CLONE	2.312	*
TEMP*CONC	1.481	ns
TEMP*CLONE	2.146	*
CONC*CLONE	0.991	ns
Within clone		
TIME	247.529	***
TIME*TEMP	0.221	ns
TIME*CONC	43.758	***
TIME*CLONE	2.484	*
TIME*TEMP*CLONE	1.838	ns
TIME*CONC*CLONE	1.128	ns
TIME*TEMP*CONC	6.482	*

Table 3.7 Total males

Source	F	P
Between clone		
TEMP	8.196	**
CONC	122.438	***
CLONE	1.223	ns
TEMP*CONC	147.458	***
TEMP*CLONE	0.808	ns
CONC*CLONE	0.888	ns
Within clone		
TIME	1641.316	***
TIME*TEMP	37.207	***
TIME*CONC	13.117	**
TIME*CLONE	1.211	ns
TIME*TEMP*CLONE	1.086	ns
TIME*CONC*CLONE	1.802	ns
TIME*TEMP*CONC	93.398	***

Table 3.6

ANCOVA of frontal male production

Source	DF	Adj SS	Adj MS	F	P
temp	1	5.498	5.498	4.15	0.042
conc	1	17.616	17.616	13.31	0.000
clone	25	121.099	4.844	3.66	0.000
time	1	2328.755	2328.755	1759.48	0.000
temp*conc	1	3.103	3.103	2.34	0.126
temp*clone	25	87.401	3.496	2.64	0.000
temp*time	1	2.077	2.077	1.57	0.211
conc*clone	25	62.027	2.481	1.87	0.006
conc*time	1	411.673	411.673	311.04	0.000
clone*time	25	581.616	23.265	17.58	0.000
temp*conc*clone	25	52.375	2.095	1.58	0.035
temp*conc*time	1	60.979	60.979	46.07	0.000
temp*clone*time	25	432.367	17.295	13.07	0.000
conc*clone*time	25	265.263	10.611	8.02	0.000
temp*conc*clone*time	25	235.192	9.408	7.11	0.000
Error	1144	1514.134	1.324		
Total	1351				

Table 3.7

ANCOVA of total male production (basal + frontal males)

Source	DF	Adj SS	Adj MS	F	P
temp	1	32.933	32.933	16.90	0.000
conc	1	491.954	491.954	252.48	0.000
clone	25	122.852	4.914	2.52	0.000
time	1	4825.470	4825.470	2476.51	0.000
temp*conc	1	592.485	592.485	304.07	0.000
temp*clone	25	81.163	3.247	1.67	0.021
temp*time	1	109.388	109.388	56.14	0.000
conc*clone	25	89.225	3.569	1.83	0.008
conc*time	1	38.564	38.564	19.79	0.000
clone*time	25	88.976	3.559	1.83	0.008
temp*conc*clone	25	100.454	4.018	2.06	0.002
temp*conc*time	1	274.591	274.591	140.92	0.000
temp*clone*time	25	79.817	3.193	1.64	0.025
conc*clone*time	25	132.447	5.298	2.72	0.000
temp*conc*clone*time	25	73.509	2.940	1.51	0.052
Error	1144	2229.078	1.948		
Total	1351				

Table 3.8 Females

Source	F	P
Between clone		
TEMP	16.061	***
CONC	5.689	*
CLONE	2.881	**
TEMP*CONC	9.163	**
TEMP*CLONE	1.606	ns
CONC*CLONE	1.532	ns
Within clone		
TIME	251.981	***
TIME*TEMP	72.903	***
TIME*CONC	23.075	***
TIME*CLONE	4.675	***
TIME*TEMP*CLONE	2.071	*
TIME*CONC*CLONE	1.053	ns
TIME*TEMP*CONC	27.881	***

Table 3.9 Total gonozooids

Source	F	P
Between clone		
TEMP	10.607	**
CONC	135.881	***
CLONE	1.538	ns
TEMP*CONC	174.956	**
TEMP*CLONE	0.970	ns
CONC*CLONE	1.095	ns
Within clone		
TIME	2110.417	***
TIME*TEMP	65.579	***
TIME*CONC	11.751	**
TIME*CLONE	2.244	*
TIME*TEMP*CLONE	1.752	ns
TIME*CONC*CLONE	2.372	*
TIME*TEMP*CONC	126.283	***

Table 3.8

ANCOVA of female production

Source	DF	Adj SS	Adj MS	F	P
temp	1	27.192	27.192	21.92	0.000
conc	1	9.631	9.631	7.76	0.005
clone	25	121.930	4.877	3.93	0.000
time	1	1914.801	1914.801	1543.61	0.000
temp*conc	1	15.513	15.513	12.51	0.000
temp*clone	25	67.972	2.719	2.19	0.001
temp*time	1	553.990	553.990	446.60	0.000
conc*clone	25	64.853	2.594	2.09	0.001
conc*time	1	175.346	175.346	141.35	0.000
clone*time	25	888.195	35.528	28.64	0.000
temp*conc*clone	25	42.331	1.693	1.36	0.109
temp*conc*time	1	211.871	211.871	170.80	0.000
temp*clone*time	25	393.395	15.736	12.69	0.000
conc*clone*time	25	200.029	8.001	6.45	0.000
temp*conc*clone*time	25	189.979	7.599	6.13	0.000
Error	1144	1419.100	1.240		
Total	1351				

Table 3.9

ANCOVA of total gonozooid production

Source	DF	Adj SS	Adj MS	F	P
temp	1	37.495	37.495	18.91	0.000
conc	1	480.339	480.339	242.22	0.000
clone	25	135.962	5.438	2.74	0.000
time	1	5360.459	5360.459	2703.06	0.000
temp*conc	1	618.468	618.468	311.87	0.000
temp*clone	25	85.749	3.430	1.73	0.015
temp*time	1	166.570	166.570	83.99	0.000
conc*clone	25	96.808	3.872	1.95	0.004
conc*time	1	29.847	29.847	15.05	0.000
clone*time	25	142.531	5.701	2.87	0.000
temp*conc*clone	25	88.386	3.535	1.78	0.011
temp*conc*time	1	320.759	320.759	161.75	0.000
temp*clone*time	25	111.236	4.449	2.24	0.000
conc*clone*time	25	150.656	6.026	3.04	0.000
temp*conc*clone*time	25	63.495	2.540	1.28	0.162
Error	1144	2268.673	1.983		
Total	1351				

Table 3.10 Total sex ratio

Source	F	P
Between clone		
TEMP	5.585	*
CONC	0.236	ns
CLONE	2.686	**
TEMP*CONC	4.699	*
TEMP*CLONE	1.687	ns
CONC*CLONE	1.795	ns
Within clone		
TIME	233.101	***
TIME*TEMP	67.555	***
TIME*CONC	7.277	*
TIME*CLONE	6.126	***
TIME*TEMP*CLONE	2.702	**
TIME*CONC*CLONE	1.315	ns
TIME*TEMP*CONC	20.997	***

Table 3.11 Total reproductive allocation

Source	F	P
Between clone		
TEMP	33.770	***
CONC	226.288	***
CLONE	1.488	ns
TEMP*CONC	273.924	***
TEMP*CLONE	1.307	ns
CONC*CLONE	1.555	ns
Within clone		
TIME	1039.431	***
TIME*TEMP	0.117	ns
TIME*CONC	60.901	***
TIME*CLONE	2.644	**
TIME*TEMP*CLONE	2.285	*
TIME*CONC*CLONE	3.166	**
TIME*TEMP*CONC	128.111	***

Table 3.10

ANCOVA of total sex ratio (females/total males)

Source	DF	Adj SS	Adj MS	F	P
temp	1	4.440	4.440	6.79	0.009
conc	1	0.188	0.188	0.29	0.592
clone	25	53.378	2.135	3.27	0.000
time	1	635.900	635.900	972.89	0.000
temp*conc	1	3.736	3.736	5.72	0.017
temp*clone	25	33.531	1.341	2.05	0.002
temp*time	1	184.289	184.289	281.95	0.000
conc*clone	25	35.663	1.427	2.18	0.001
conc*time	1	19.852	19.852	30.37	0.000
clone*time	25	417.768	16.711	25.57	0.000
temp*conc*clone	25	19.879	0.795	1.22	0.213
temp*conc*time	1	57.281	57.281	87.64	0.000
temp*clone*time	25	184.252	7.370	11.28	0.000
conc*clone*time	25	89.705	3.588	5.49	0.000
temp*conc*clone*time	25	68.207	2.728	4.17	0.000
Error	1144	747.738	0.654		
Total	1351				

Table 3.11

ANCOVA of total reproductive allocation (total gonozooids/autozooids)

Source	DF	Adj SS	Adj MS	F	P
temp	1	32.463	32.463	51.78	0.000
conc	1	217.915	217.915	347.61	0.000
clone	25	35.833	1.433	2.29	0.000
time	1	737.996	737.996	1177.24	0.000
temp*conc	1	263.789	263.789	420.79	0.000
temp*clone	25	31.470	1.259	2.01	0.002
temp*time	1	0.083	0.083	0.13	0.715
conc*clone	25	37.422	1.497	2.39	0.000
conc*time	1	43.240	43.240	68.98	0.000
clone*time	25	46.913	1.877	2.99	0.000
temp*conc*clone	25	24.074	0.963	1.54	0.045
temp*conc*time	1	90.959	90.959	145.10	0.000
temp*clone*time	25	40.555	1.622	2.59	0.000
conc*clone*time	25	56.201	2.248	3.59	0.000
temp*conc*clone*time	25	17.759	0.710	1.13	0.296
Error	1144	717.159	0.627		
Total	1351				

Table 3.12

Proportion of sum-of-squares (see tables 3.1-3.11) accounted for by each source of variation for 26 clones grown in 4 different macroenvironments over a 12 week period. For each variable, the values given are percentages of the adjusted S.S.

Abbreviations: Af - frontal autozooids, Auto - autozooids, Fem - females, Mb - basal males, Mf - frontal males, Mt - total males, PAR - perimeter/area ratio, TRA - total reproductive allocation, TS - total gonozooids, TSR - total sex ratio, ZPU - zooids per unit area

a) Somatic parameters.

	VARIABLE			
SOURCE	Auto	Af	PAR	ZPU
TEMP	0.48	0.02	13.06	17.48
CONC	0.06	1.52	12.47	0.11
CLONE	0.32	2.72	0.76	9.40
TIME	74.26	15.80	30.00	9.99
TEMP*CONC	0.13	0.00	14.18	0.38
TEMP*CLONE	0.30	1.39	0.49	2.09
TEMP*TIME	6.93	0.02	0.28	0.75
CONC*CLONE	0.46	1.08	0.69	2.30
CONC*TIME	2.57	9.88	10.32	0.29
CLONE*TIME	4.47	15.60	2.19	6.21
TEMP*CONC*CLONE	0.42	1.41	0.47	3.05
TEMP*CONC*TIME	1.09	0.24	7.79	0.01
TEMP*CLONE*TIME	0.98	8.52	0.88	3.44
CONC*CLONE*TIME	0.69	7.70	0.77	2.18
TEMP*CONC*CLONE*TIME	0.55	8.67	1.19	2.69
ERROR	6.28	25.42	4.43	39.63

b) Sexual parameters.

	VARIABLE			
SOURCE	Mb	Mf	Mt	Fem
TEMP	0.50	0.09	0.35	0.43
CONC	5.25	0.28	5.25	0.15
CLONE	1.34	1.96	1.31	1.94
TIME	49.29	37.67	51.54	30.41
TEMP*CONC	6.88	0.05	6.33	0.25
TEMP*CLONE	0.84	1.41	0.87	1.08
TEMP*TIME	1.42	0.03	1.17	8.80
CONC*CLONE	1.00	1.00	0.95	1.03
CONC*TIME	0.40	6.66	0.41	2.78
CLONE*TIME	1.07	9.41	0.95	14.11
TEMP*CONC*CLONE	1.17	0.85	1.07	0.67
TEMP*CONC*TIME	2.63	0.99	2.93	3.37
TEMP*CLONE*TIME	0.61	6.99	0.85	6.25
CONC*CLONE*TIME	1.66	4.29	1.41	3.18
TEMP*CONC*CLONE*TIME	0.77	3.80	0.79	3.02
ERROR	25.17	24.50	23.81	22.54

c) Sex ratio and reproductive allocation

	VARIABLE		
SOURCE	TS	TSR	TRA
TEMP	0.37	0.17	1.36
CONC	4.73	0.01	9.10
CLONE	1.34	2.09	1.50
TIME	52.77	24.88	30.83
TEMP*CONC	6.09	0.15	11.02
TEMP*CLONE	0.84	1.31	1.31
TEMP*TIME	1.64	7.21	0.00
CONC*CLONE	0.95	1.40	1.56
CONC*TIME	0.29	0.78	1.81
CLONE*TIME	1.40	16.35	1.96
TEMP*CONC*CLONE	0.87	0.78	1.01
TEMP*CONC*TIME	3.16	2.24	3.80
TEMP*CLONE*TIME	1.10	7.21	1.69
CONC*CLONE*TIME	1.48	3.51	2.35
TEMP*CONC*CLONE*TIME	0.63	2.67	0.74
ERROR	22.34	29.26	29.96

Table 3.13

Spearman rank correlation coefficients, calculated by comparing the growth rate "r", of 26 clones, grown over a 12 week period in 4 different macroenvironments.

1 : 8°C, 10 cells μl^{-1}

2 : 8°C, 100 cells μl^{-1}

3 : 18°c, 10 cells μl^{-1}

4 : 18°C, 100 cells μl^{-1}

ns: non-significant

*: significant at P<0.05

**: significant at P<0.01

***: significant at P<0.001

a) Total zooids

	1	2	3
2	0.38ns		
3	0.103ns	0.081ns	
4	-0.212ns	-0.106ns	0.132ns

b) Total gonozooids

	1	2	3
2	0.260ns		
3	0.352ns	-0.038ns	
4	-0.269ns	0.109ns	0.144ns

c) Autozooids

	1	2	3
2	0.488*		
3	0.063ns	0.105ns	
4	-0.208ns	-0.159ns	0.038ns

Table 3.13 (cont.)

d) Basal males

	1	2	3
2	0.162ns		
3	0.207ns	-0.334ns	
4	-0.428ns	0.157ns	-0.105ns

e) Frontal males

	1	2	3
2	0.296ns		
3	0.235ns	0.017ns	
4	0.038ns	-0.099ns	0.414*

f) Total males

	1	2	3
2	0.324ns		
3	0.224ns	-0.185ns	
4	-0.274ns	0.278ns	0.042ns

g) Females

	1	2	3
2	0.352ns		
3	0.396*	0.310ns	
4	0.109ns	-0.012ns	0.378ns

Figure 3.1

Somatic growth of 26 clones grown in four different macroenvironments, over 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

Circle : 10 cells μl^{-1}

a) Feeding autozooids

b) Degenerated autozooids (brown bodies)

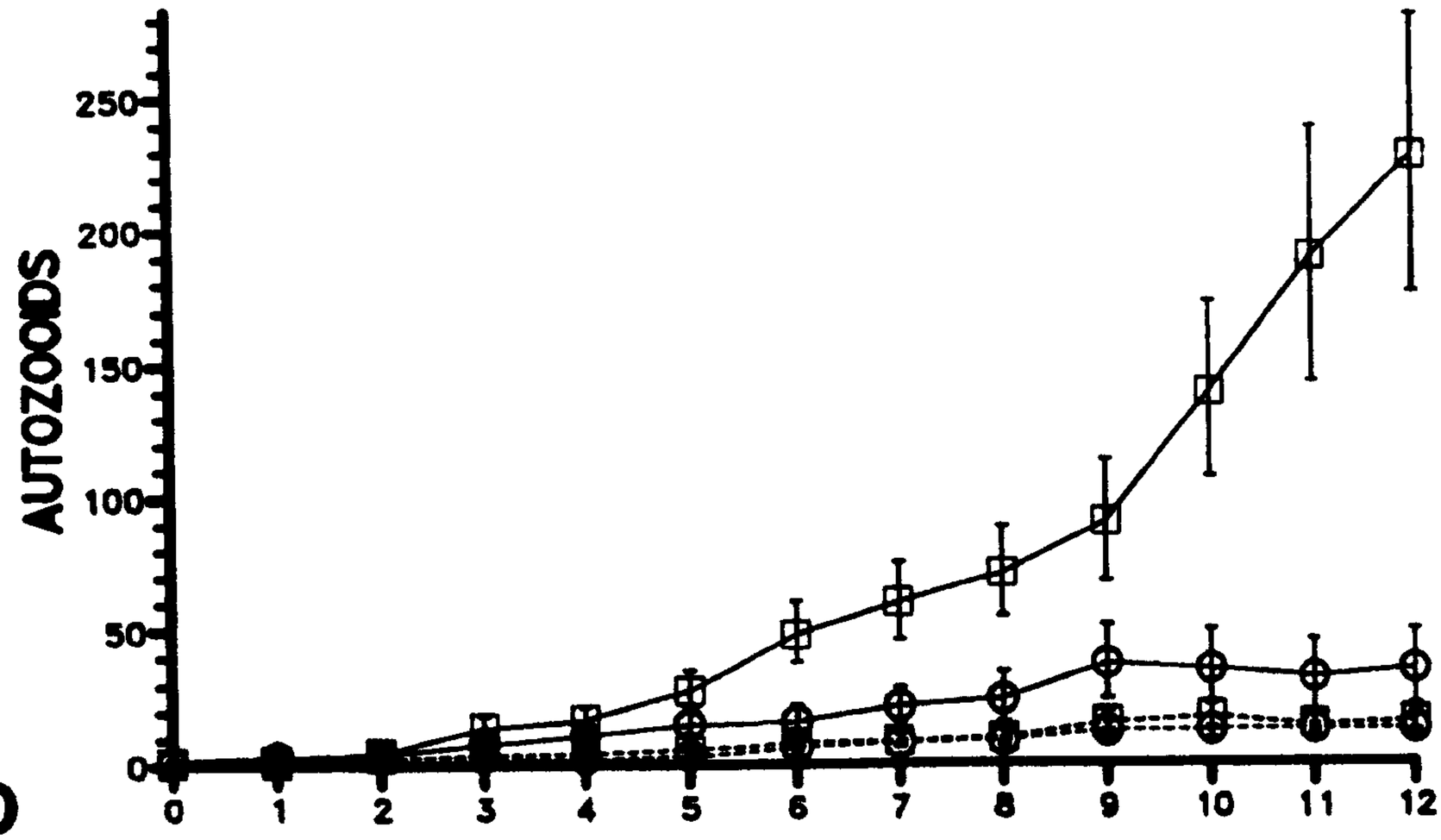
c) Feeding autozooids/brown bodies

d) Fully formed buds

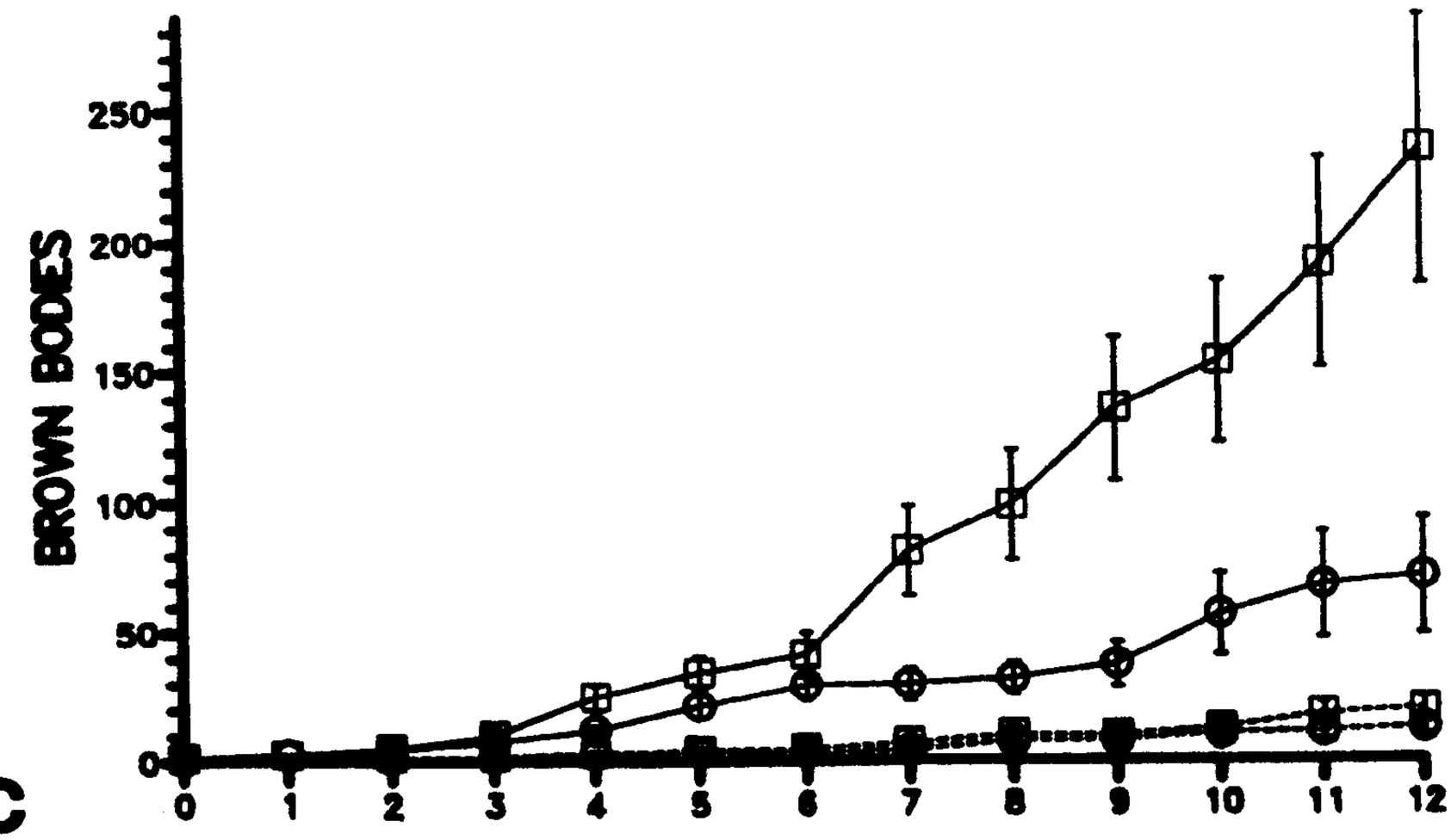
e) Total autozooids (feeding autozooids, brown bodies and buds)

f) Frontal autozooids

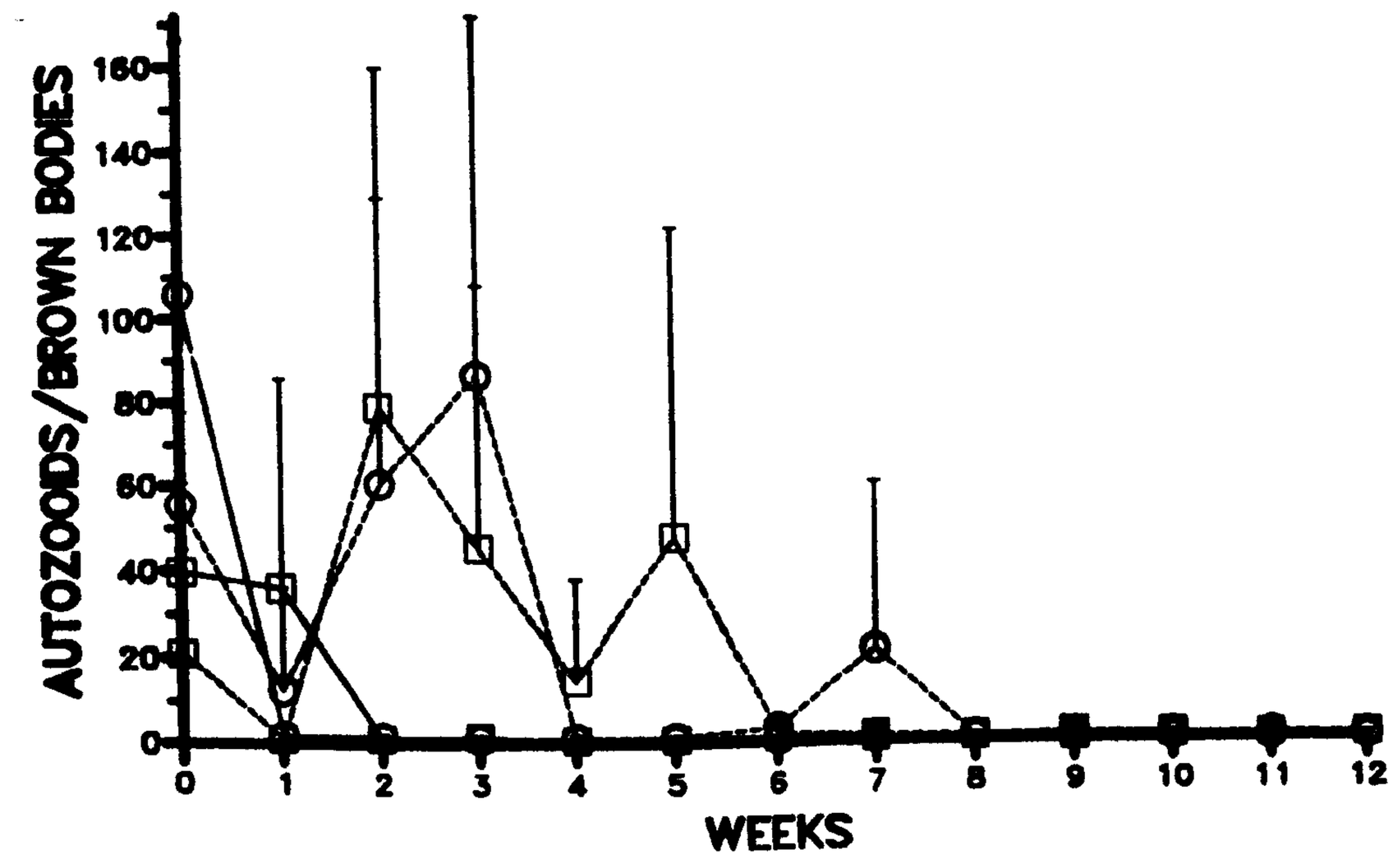
a


AUTOZOOIDS
0
50
100
150
200
250
0 1 2 3 4 5 6 7 8 9 10 11 12

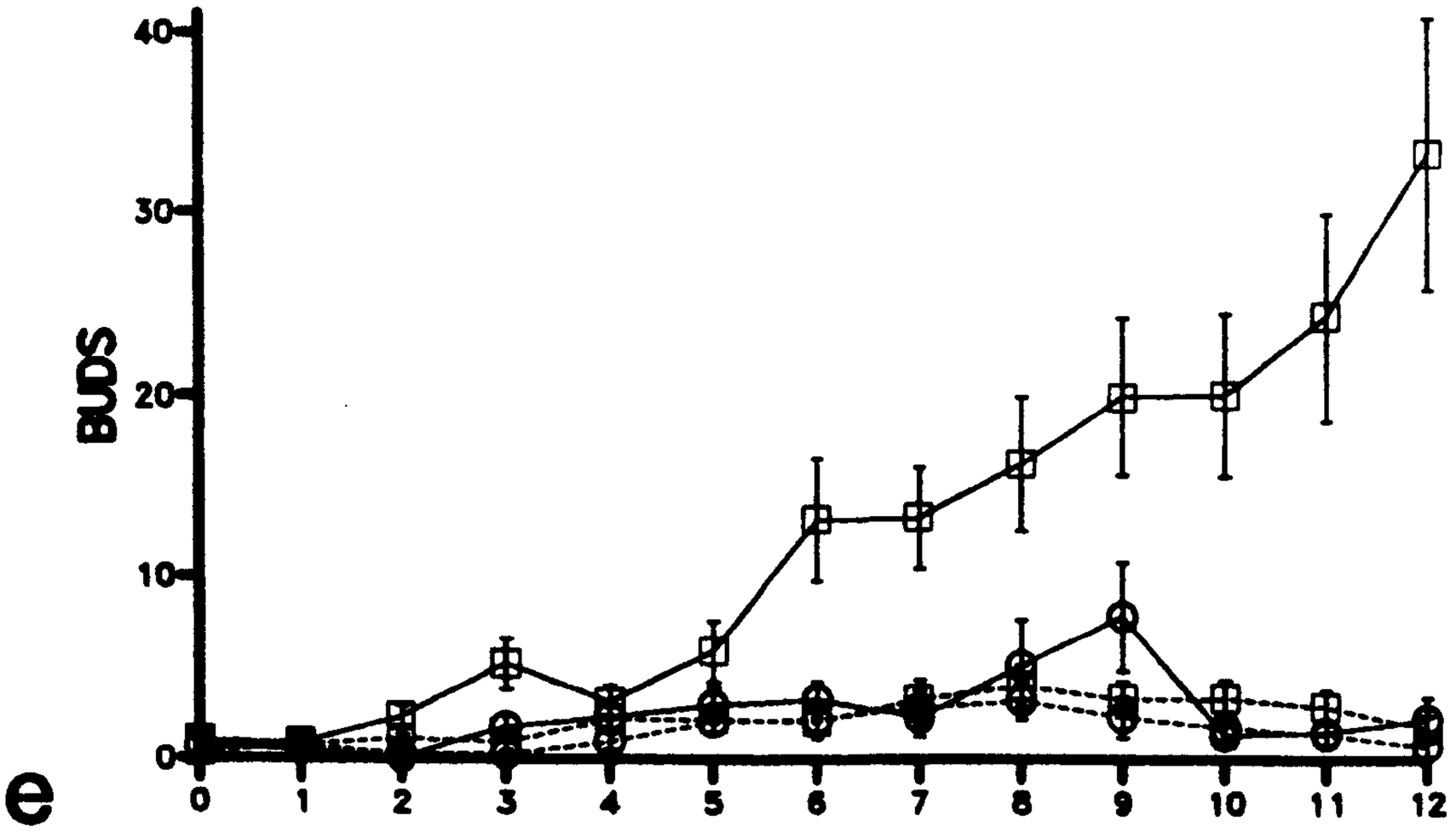
b


BROWN BODIES
0
50
100
150
200
250
0 1 2 3 4 5 6 7 8 9 10 11 12

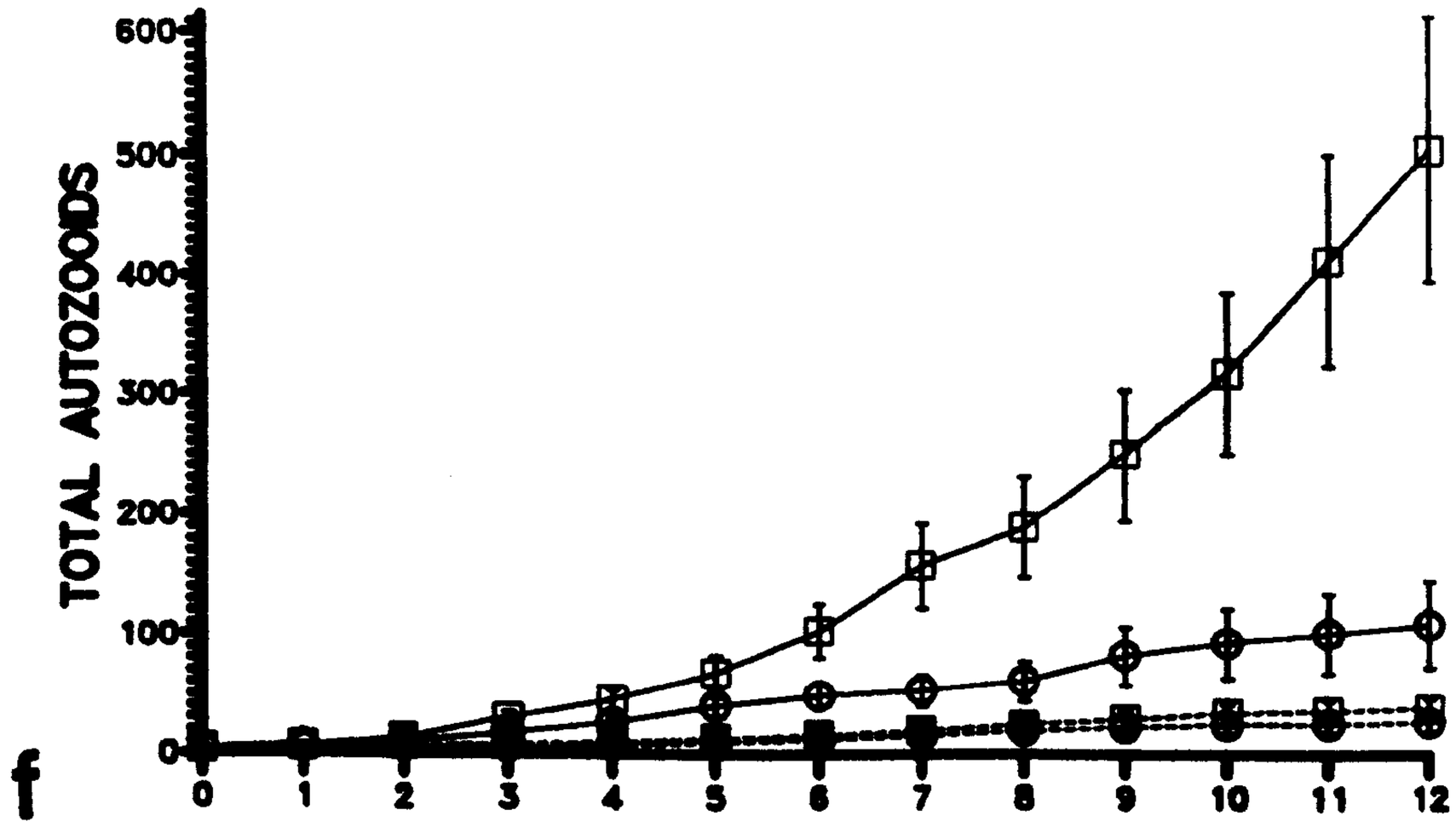
c


AUTOZOOIDS/BROWN BODIES
0
20
40
60
80
100
120
140
160
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

d


BUDS
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12

e


TOTAL AUTOZOOIDS
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12

f

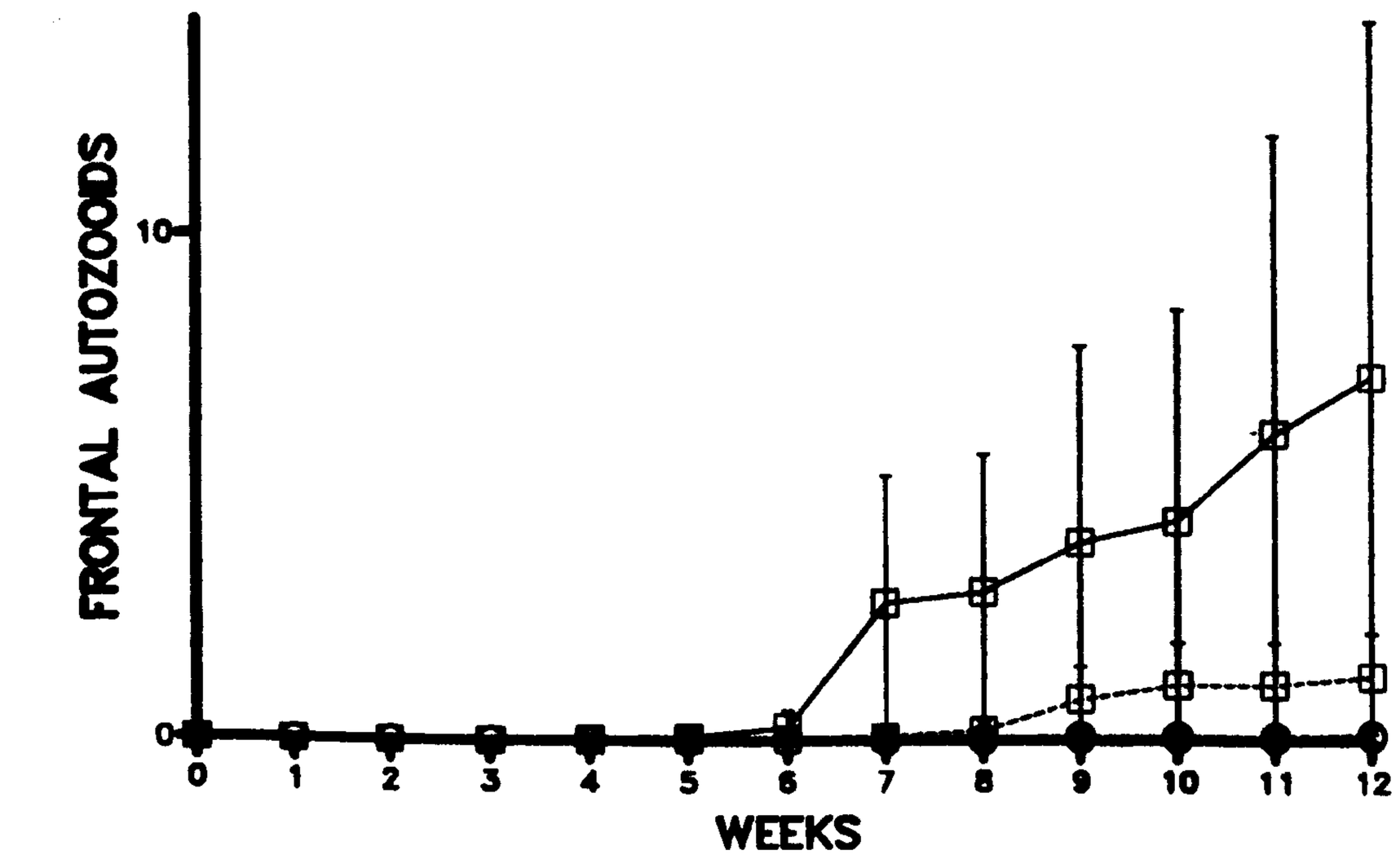

FRONTAL AUTOZOOIDS
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

Figure 3.2

Colony size parameters of 26 clones, grown in 4 different macroenvironments, over 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

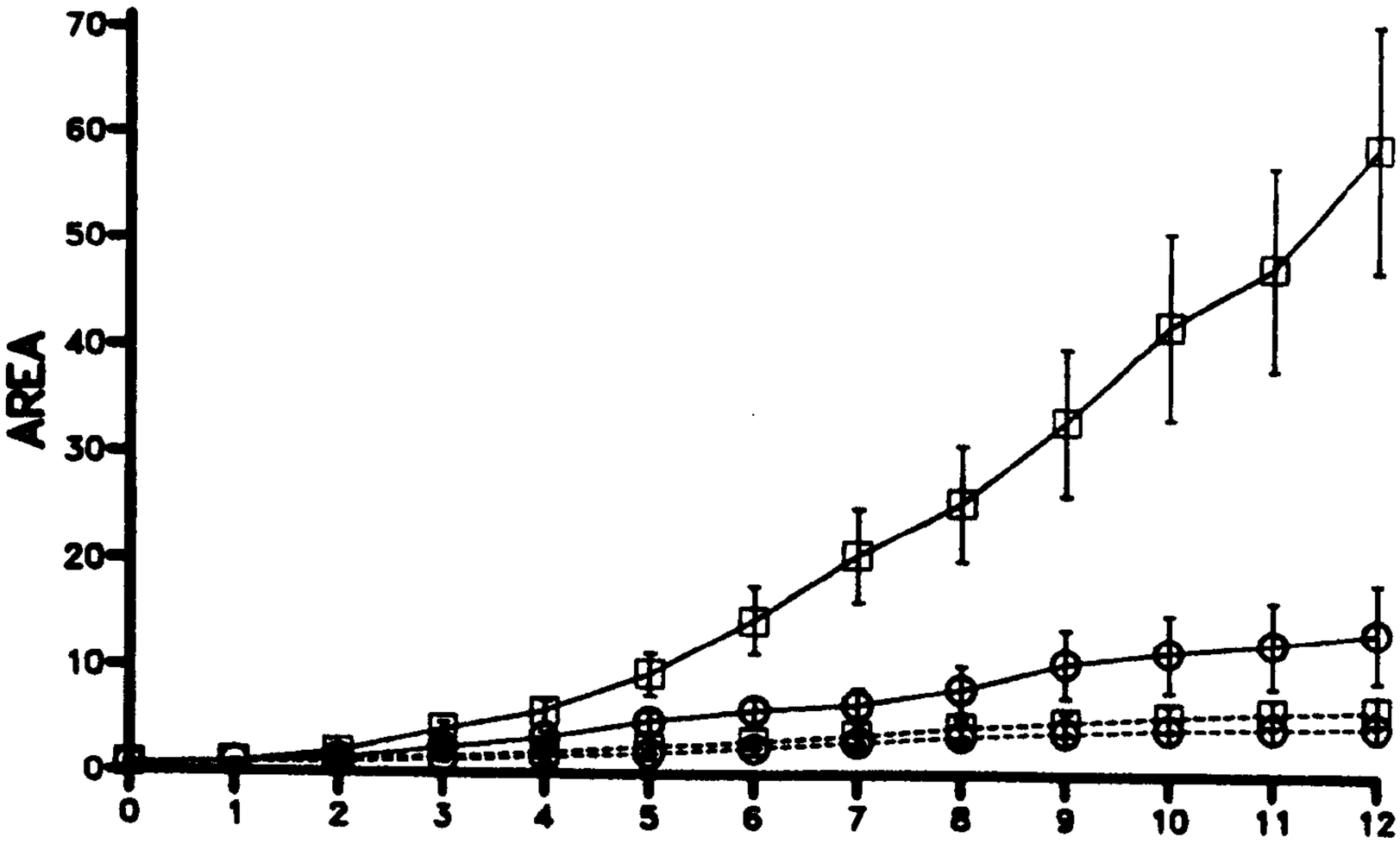
Circle : 10 cells μl^{-1}

a) Colony area (mm^2)

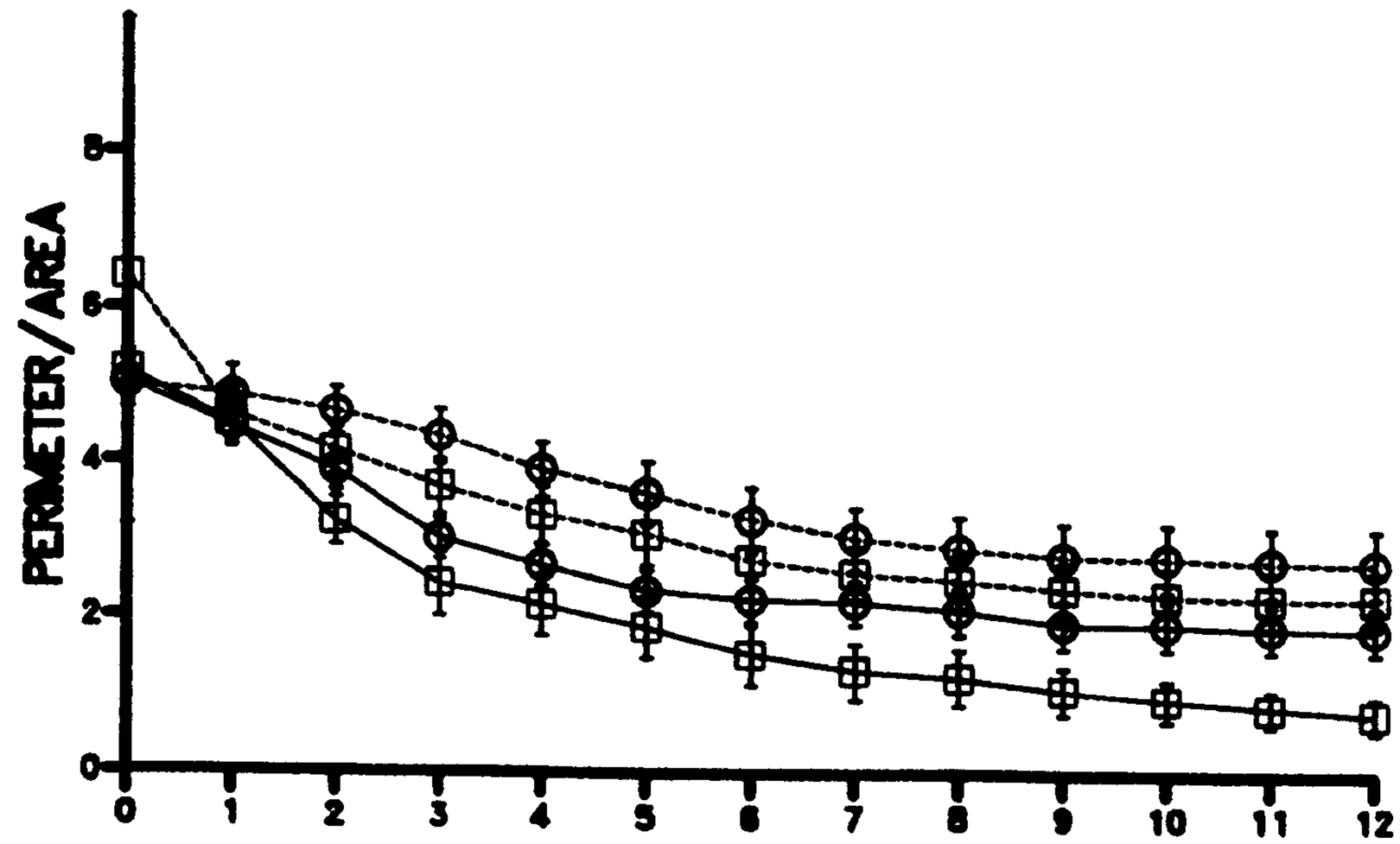
b) PAR (perimeter/area ratio)

c) Zooids per unit area (area/total autozooids)

a


AREA
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12

b


PERIMETER/AREA
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12

c

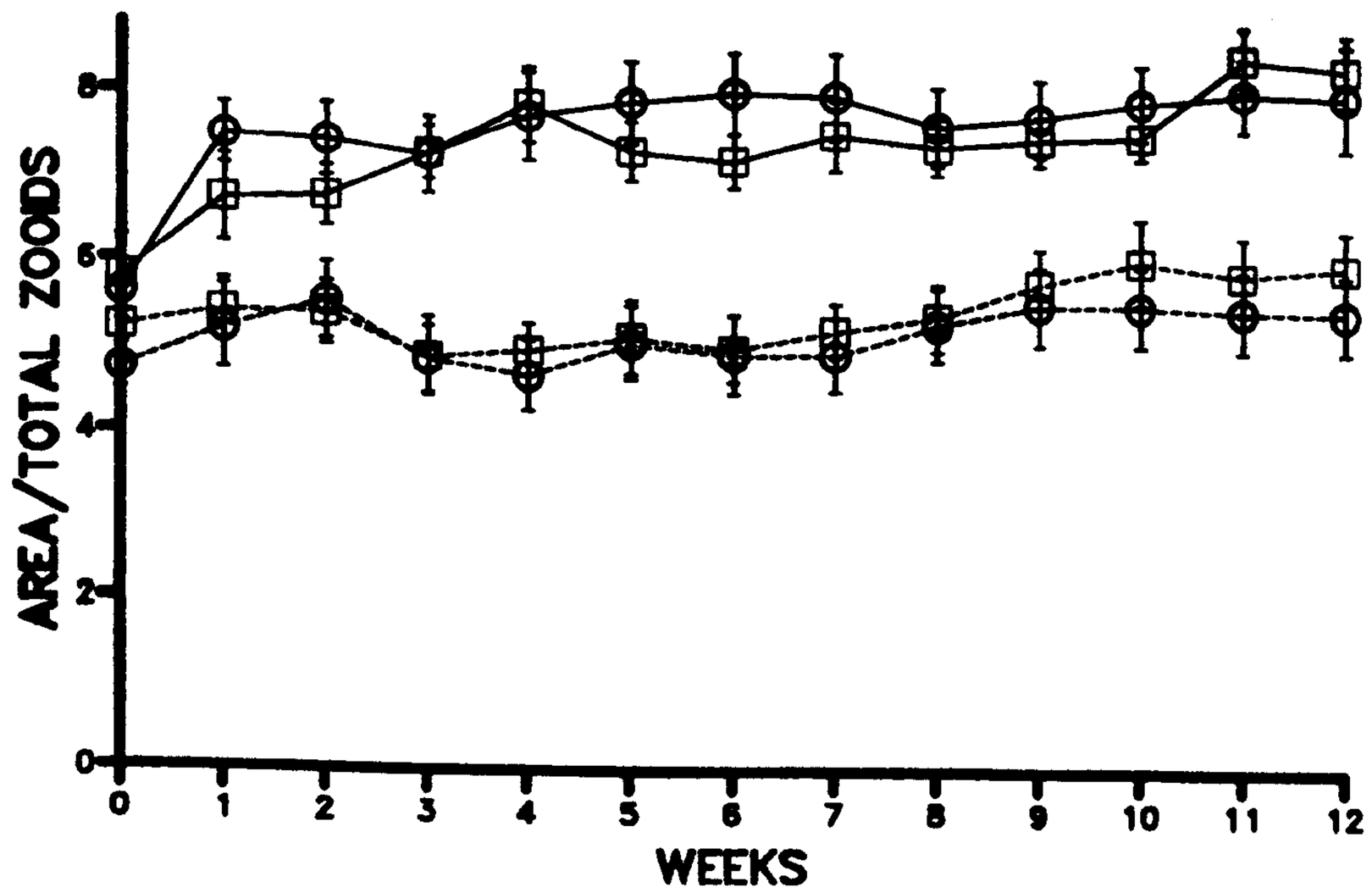

AREA/TOTAL ZOOIDS
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

Figure 3.3

Male zooids production in 26 clones, grown in 4 different macroenvironments, over a period of 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

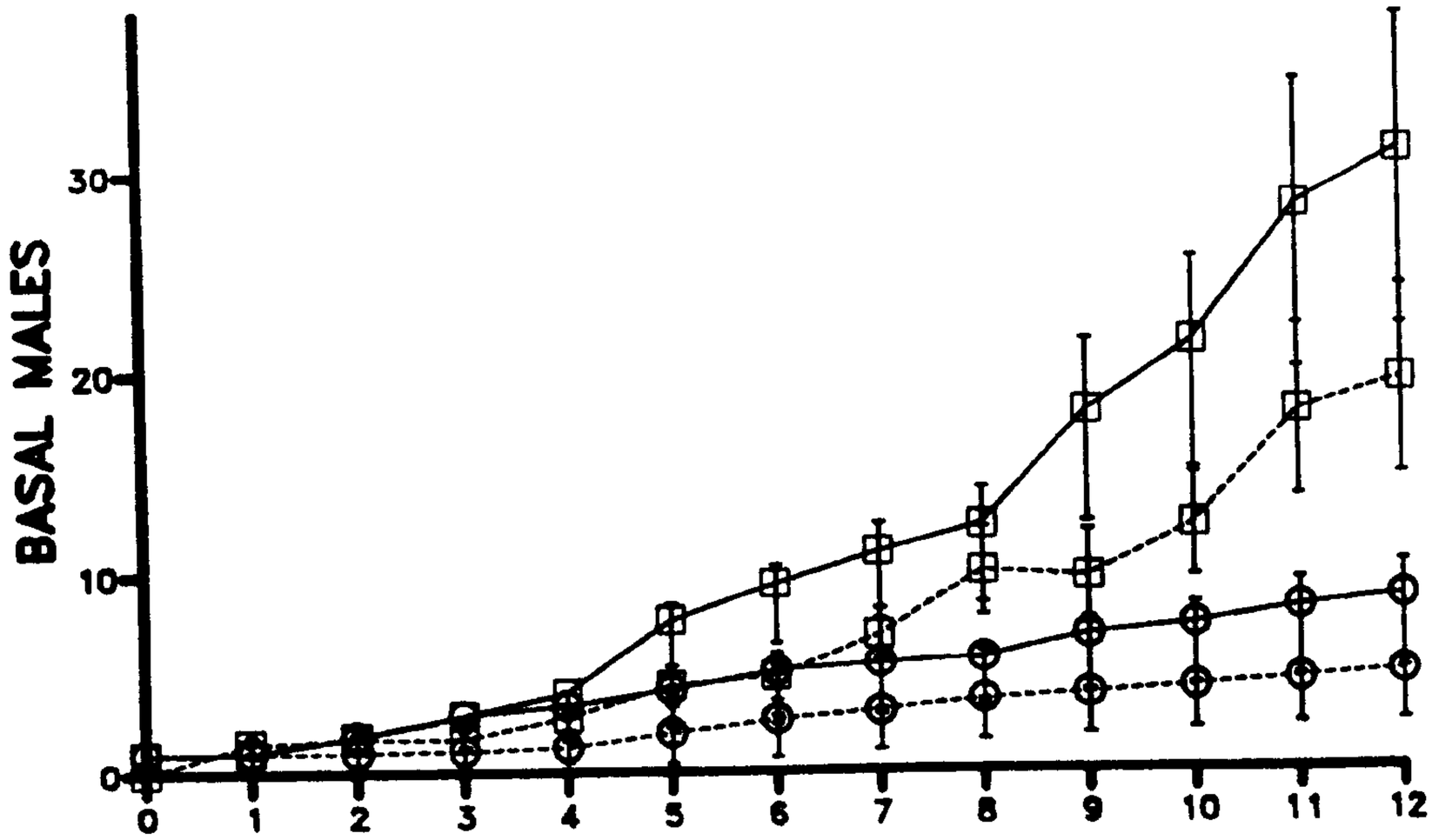
Circle : 10 cells μl^{-1}

a) Basal male zooids

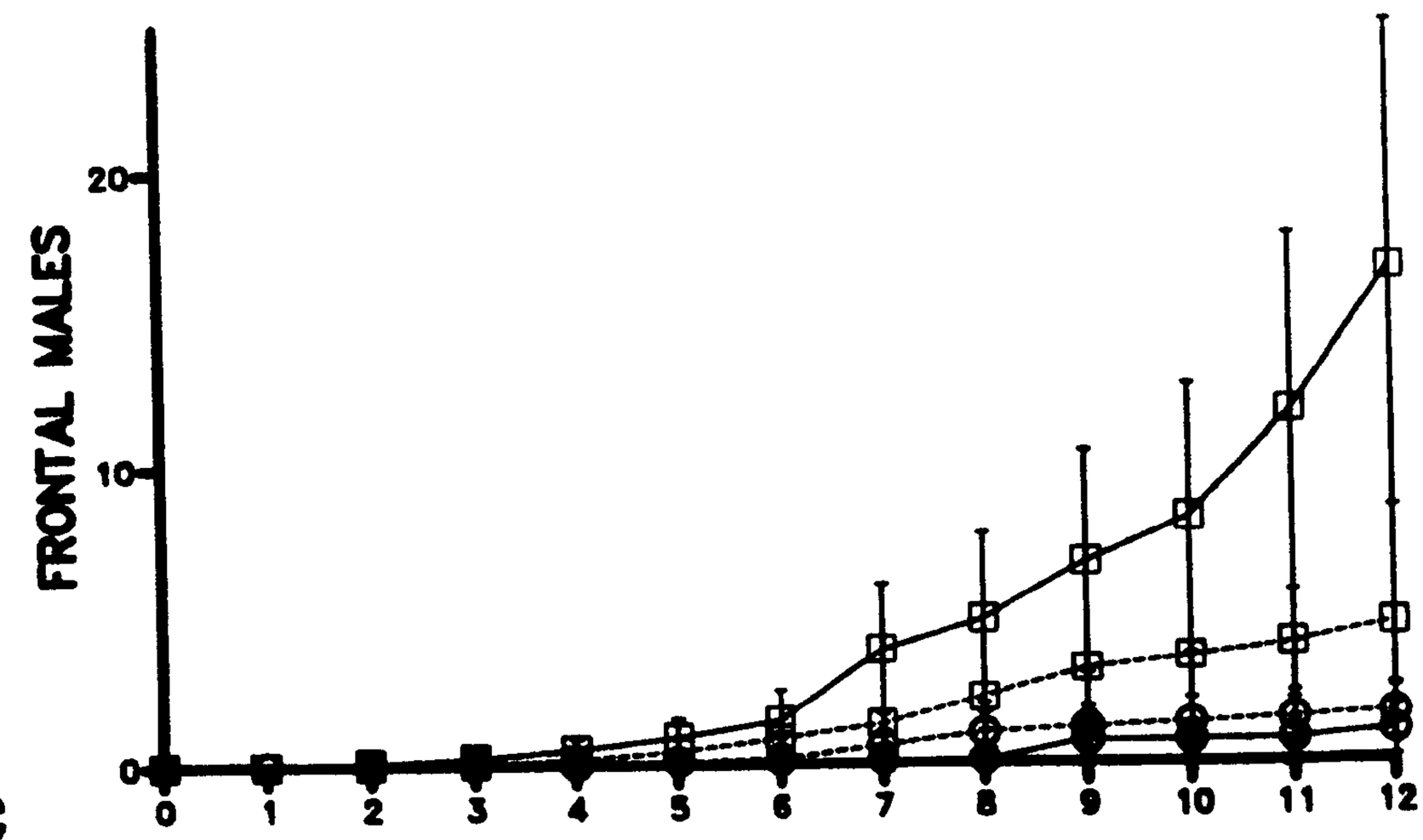
b) Frontal male zooids

c) Total male zooids (basal + frontal males)

a


BASAL MALES
0
10
30
0 1 2 3 4 5 6 7 8 9 10 11 12

b


FRONTAL MALES
0
10
20
0 1 2 3 4 5 6 7 8 9 10 11 12

c

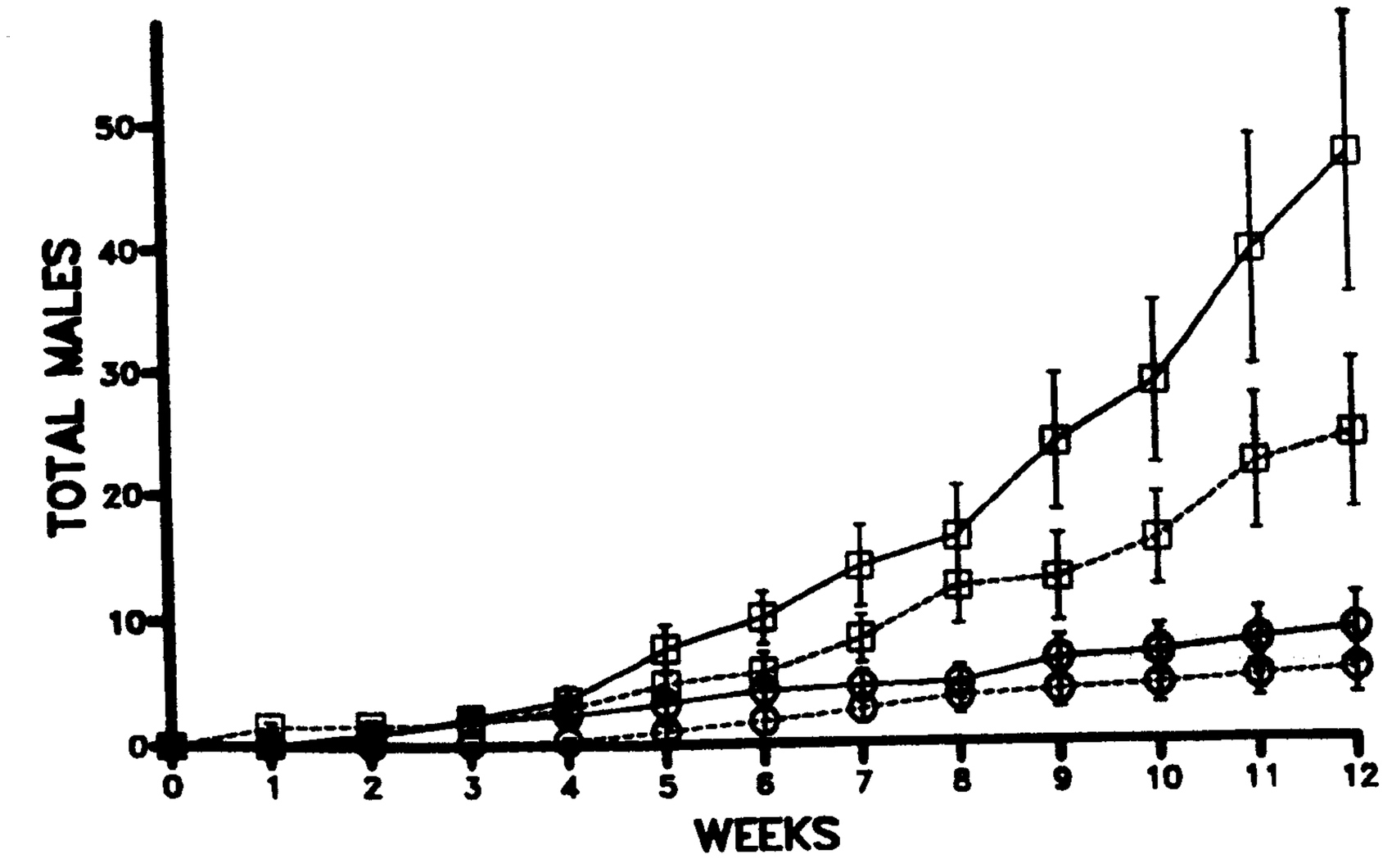

TOTAL MALES
0
10
20
30
40
50
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

Figure 3.4

Female and embryo production in 26 clones grown in four different macroenvironments, over a period of 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

Circle : 10 cells μl^{-1}

a) Female zooids

b) Embryos

c) Female zooids/embryos

a

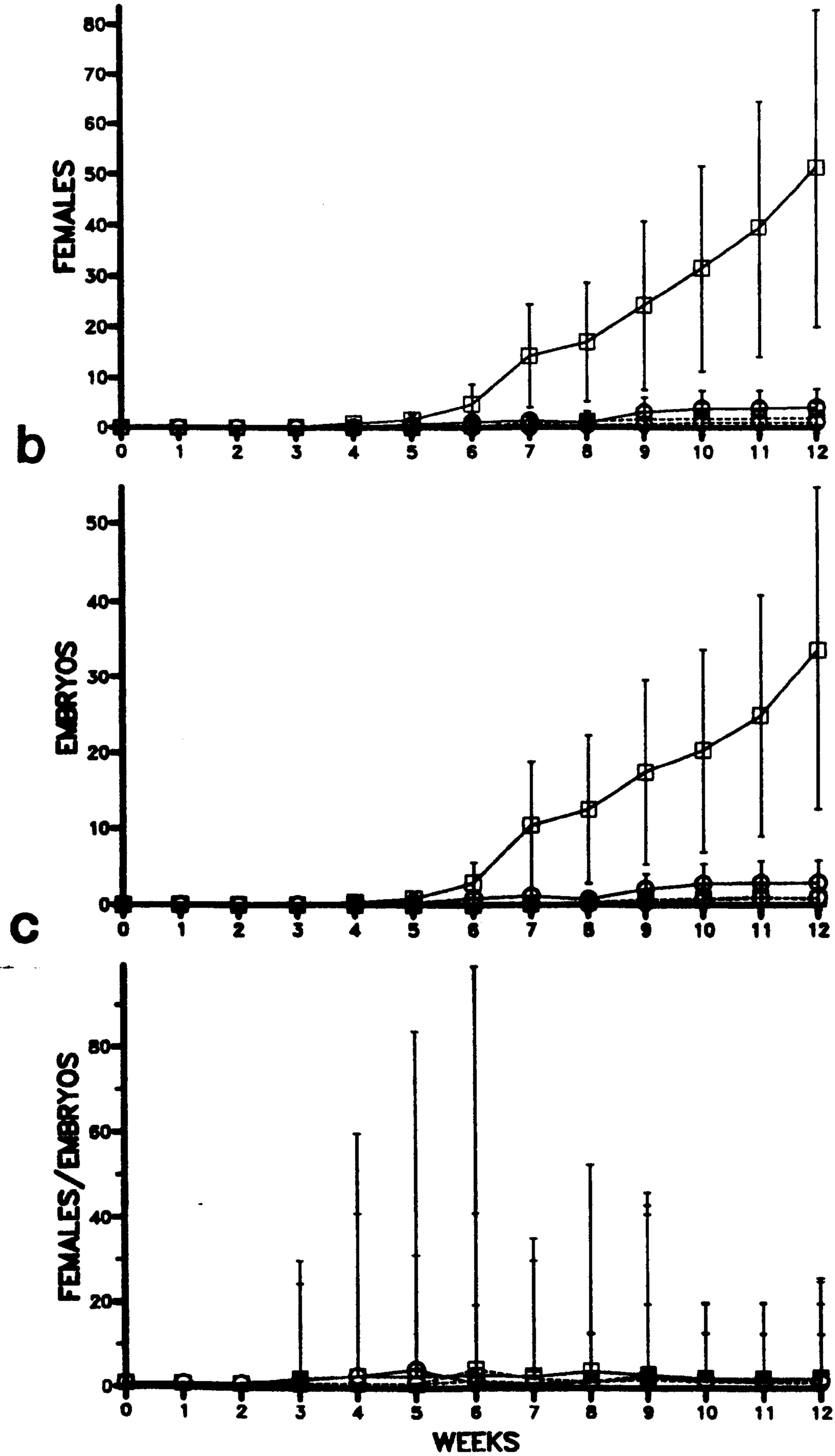

FEMALES
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
b
EMBRYOS
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
C
FEMALES/EMBRYOS
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

Figure 3.5

Total gonozooid production (basal males + frontal males + females) in 26 clones grown in four different macroenvironments over a period of 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

Circle : 10 cells μl^{-1}

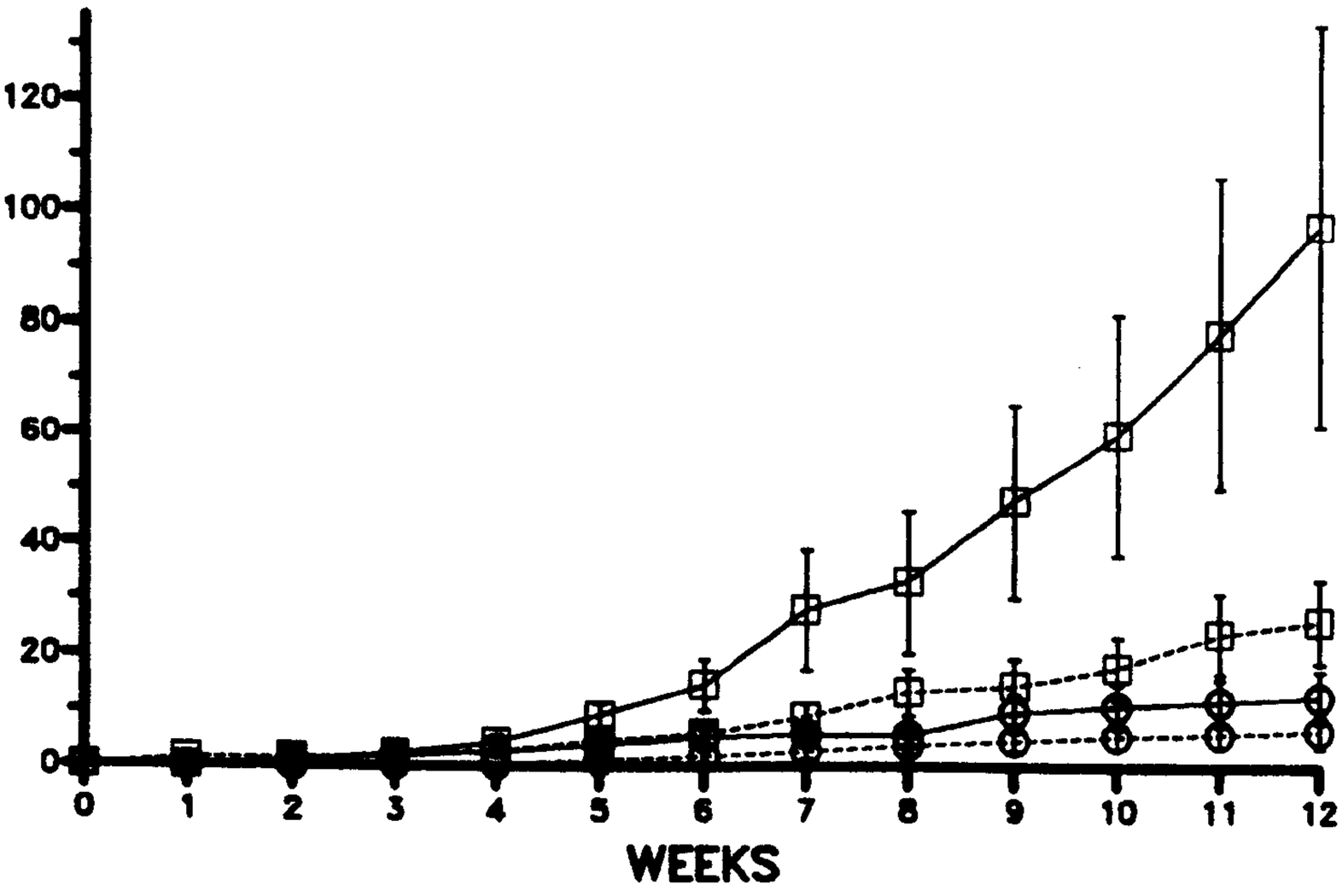

GONOZOOIDS
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
11
12
WEEKS

Figure 3.6

Sex ratio (females/total males) in 26 clones grown in four different macroenvironments over a period of 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

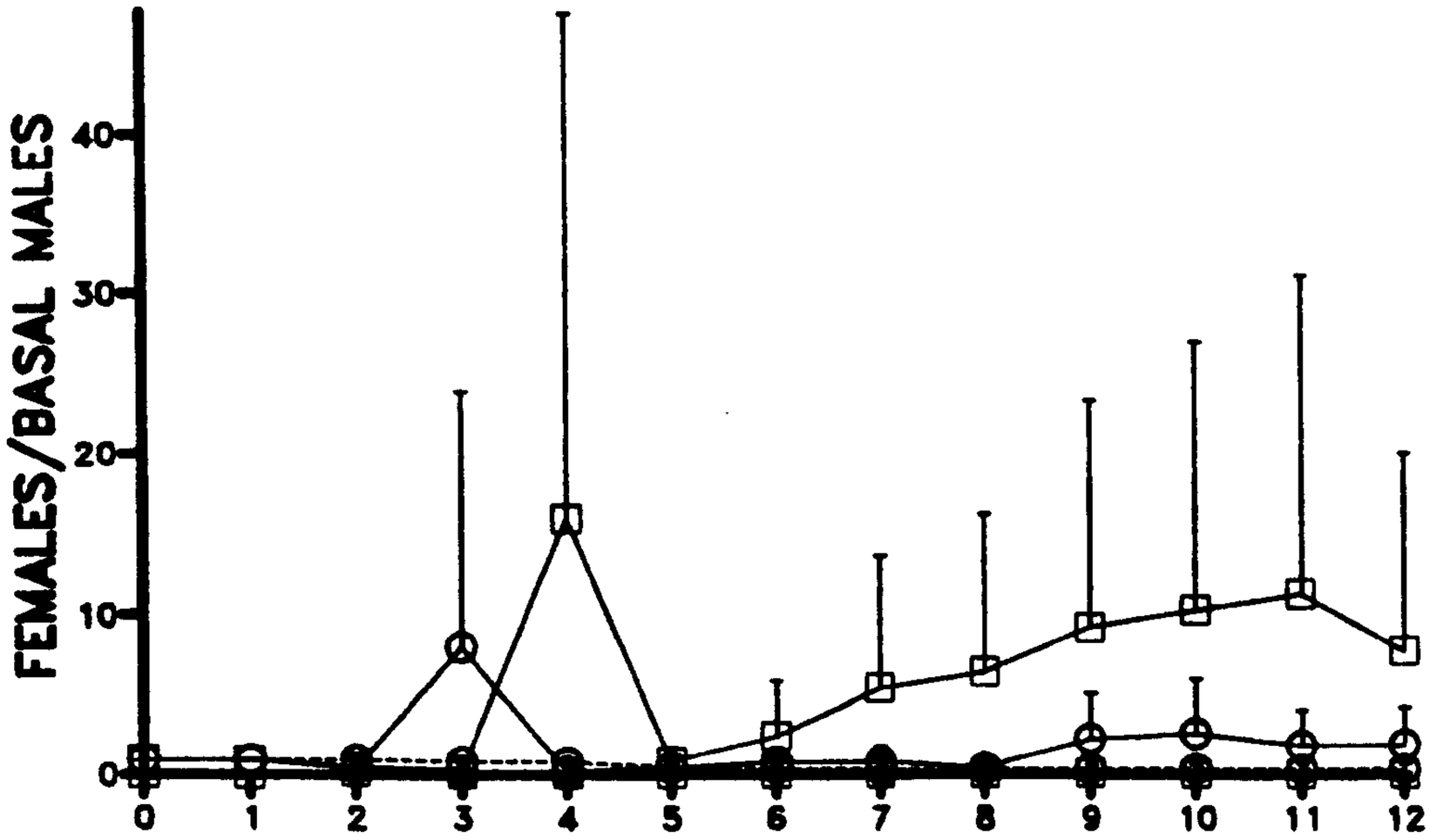
Circle : 10 cells μl^{-1}

a) Basal male sex ratio (females/basal males)

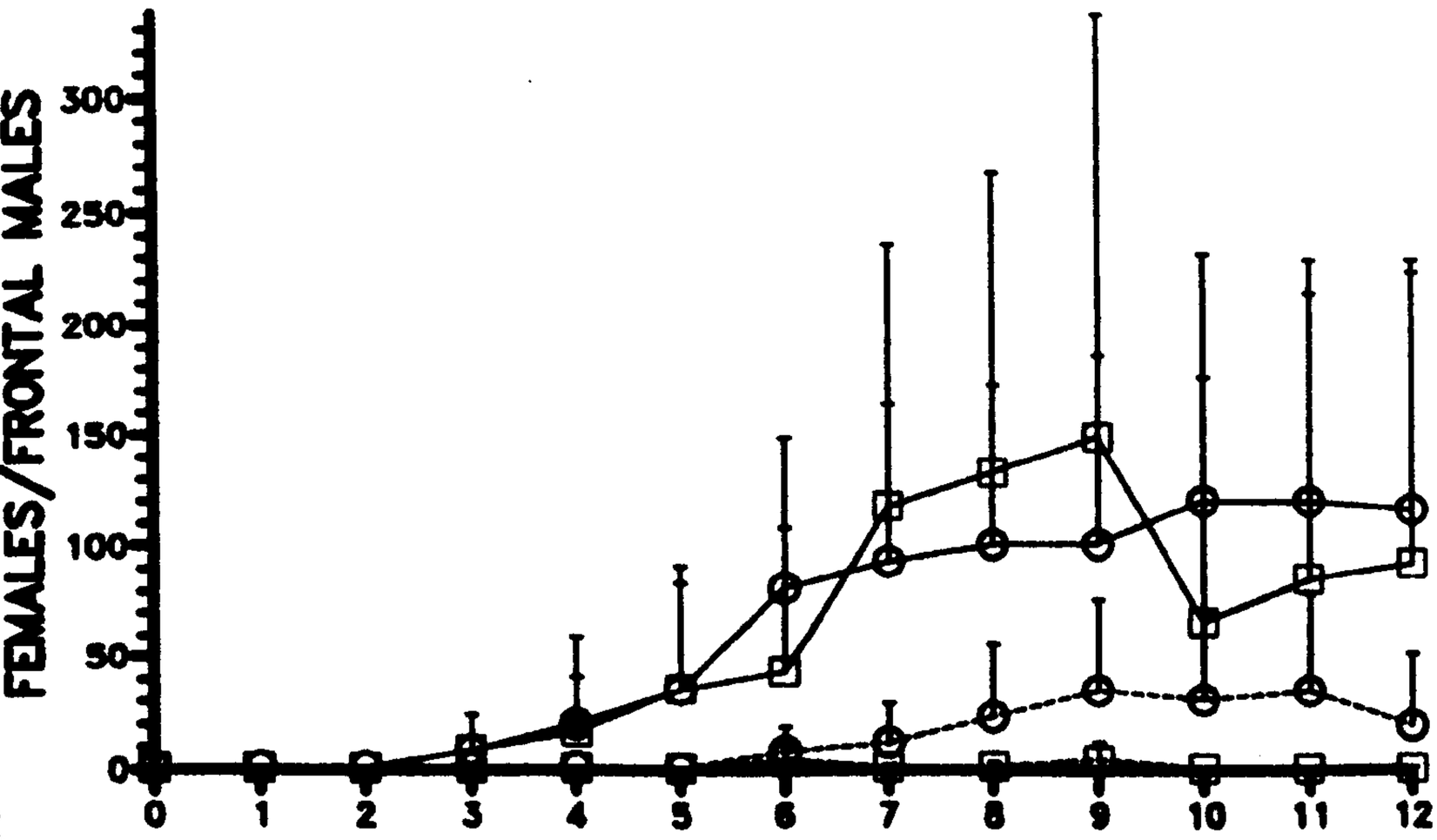
b) Frontal male sex ratio (females/frontal males)

c) Total sex ratio (females/basal + frontal males)

a


FEMALES/BASAL MALES
0
10
20
30
0 1 2 3 4 5 6 7 8 9 10 11 12

b


FEMALES/FRONTAL MALES
0
50
100
150
200
250
300
0 1 2 3 4 5 6 7 8 9 10 11 12

c

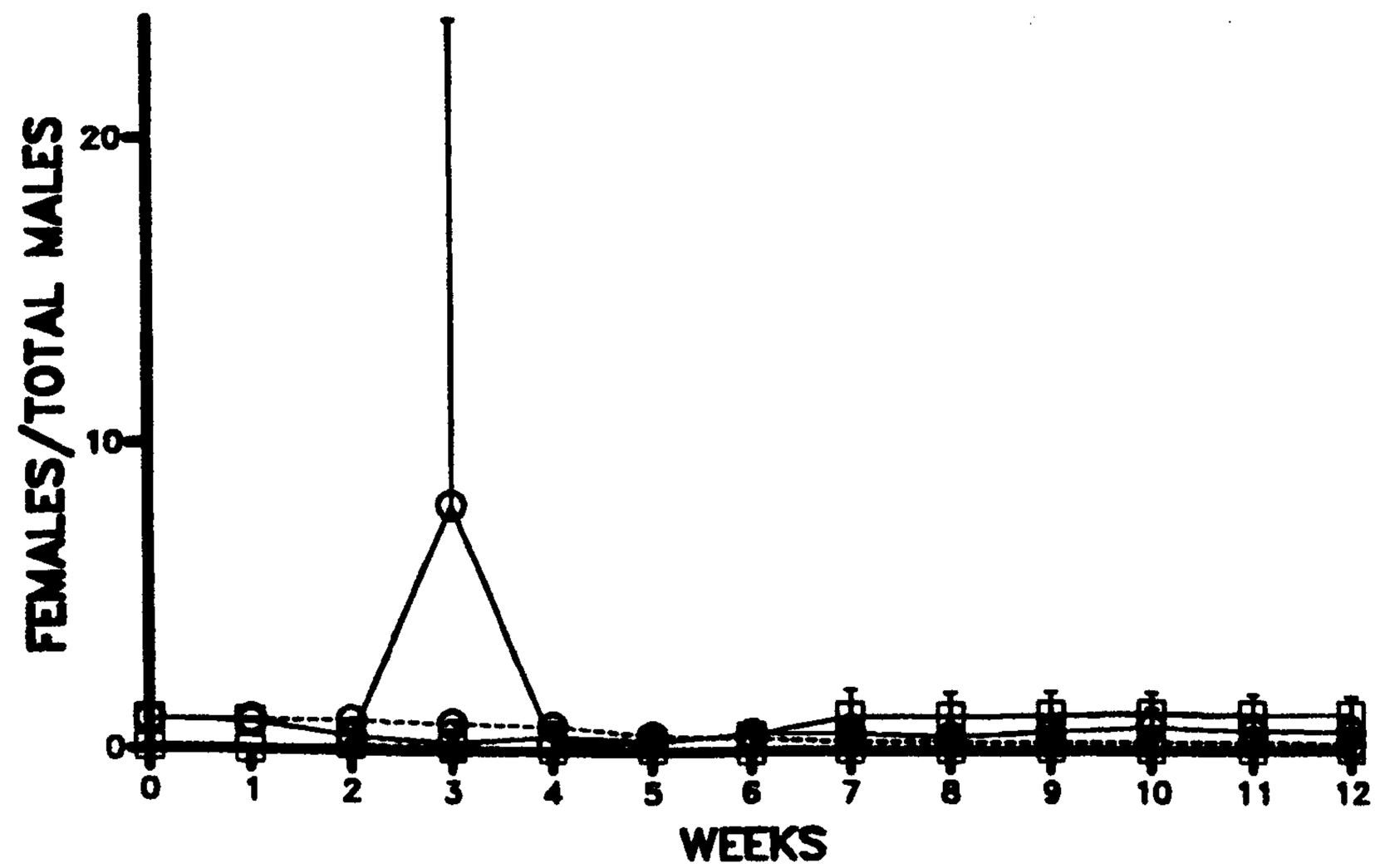

FEMALES/TOTAL MALES
0
10
20
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

Figure 3.7

Reproductive allocation in 26 clones grown in four different macroenvironments over a period of 12 weeks. Data points are mean values for each treatment group, ±95% confidence limits.

Solid lines : 18°C

Broken lines : 8°C

Box : 100 cells μl^{-1}

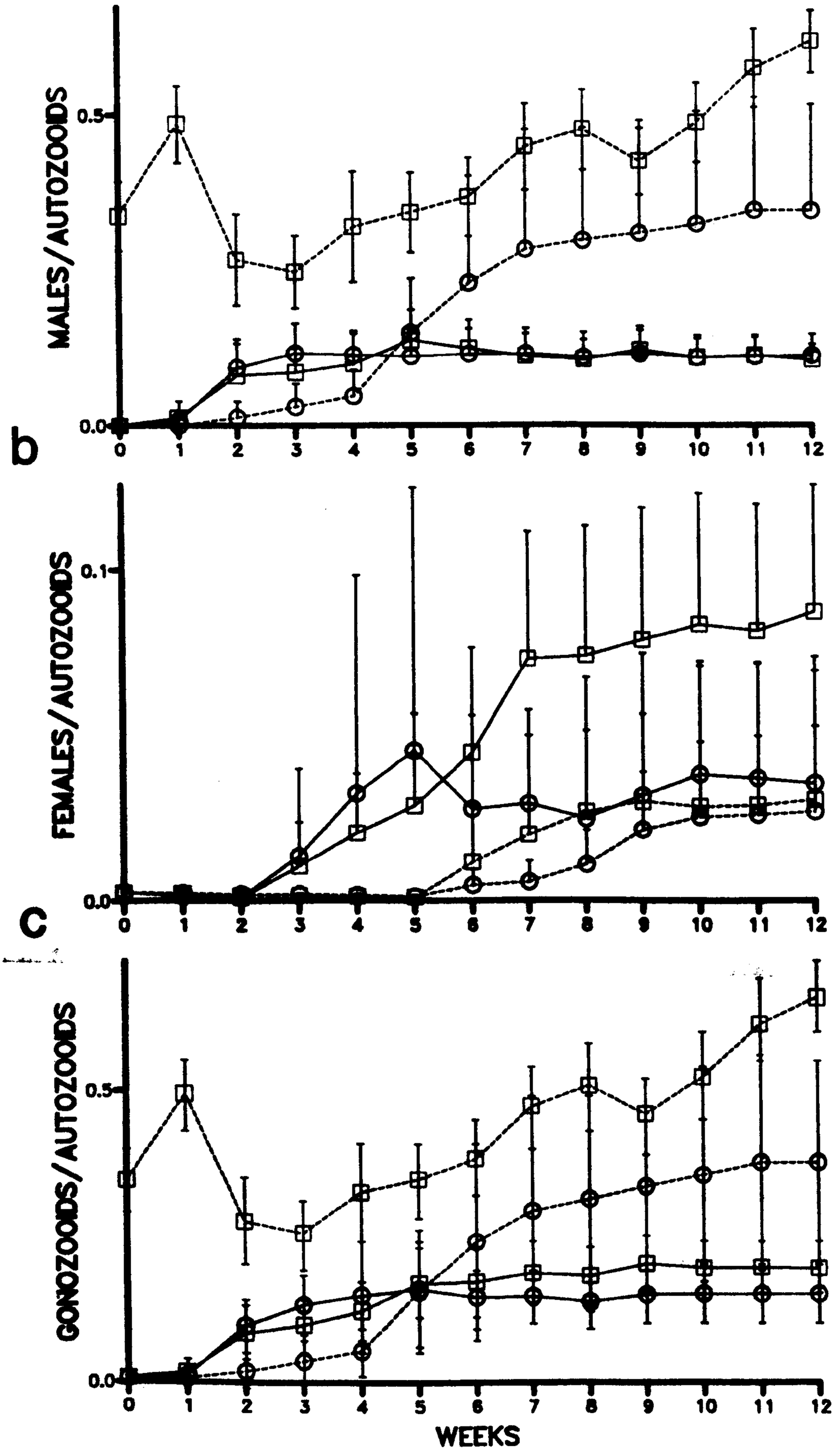
Circle : 10 cells μl^{-1}

a) Male reproductive allocation (basal + frontal males/ autozooids)

b) Female reproductive allocation (females/autozooids)

c) Total reproductive allocation (total gonozooids/ autozooids)

a


MALES/AUTOZOOIDS
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12

b

FEMALES/AUTOZOOIDS
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12

c

GONOZOOIDS/AUTOZOOIDS
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12
WEEKS

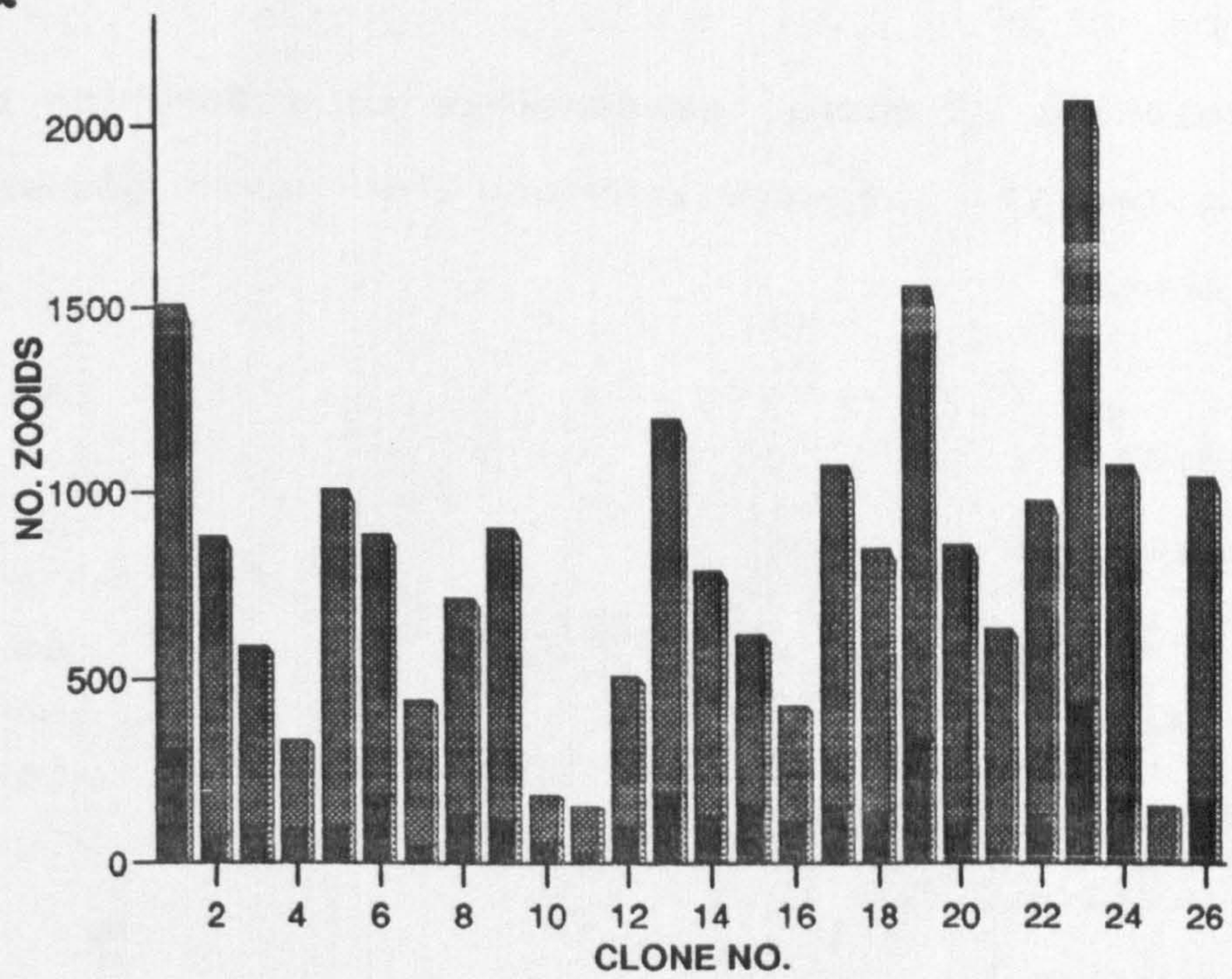
Figure 3.8

Performance of 26 C. hyalina clones in terms of total zooid production after 12 weeks, summed over the four experimental macroenvironments.

a) Total zooids (grey), and total gonozooids (black)

b) Basal males (black), frontal males (grey) and females (light grey)

a


NO. ZOOIDS
2000
1500
1000
500
0
2 4 6 8 10 12 14 16 18 20 22 24 26
CLONE NO.

b

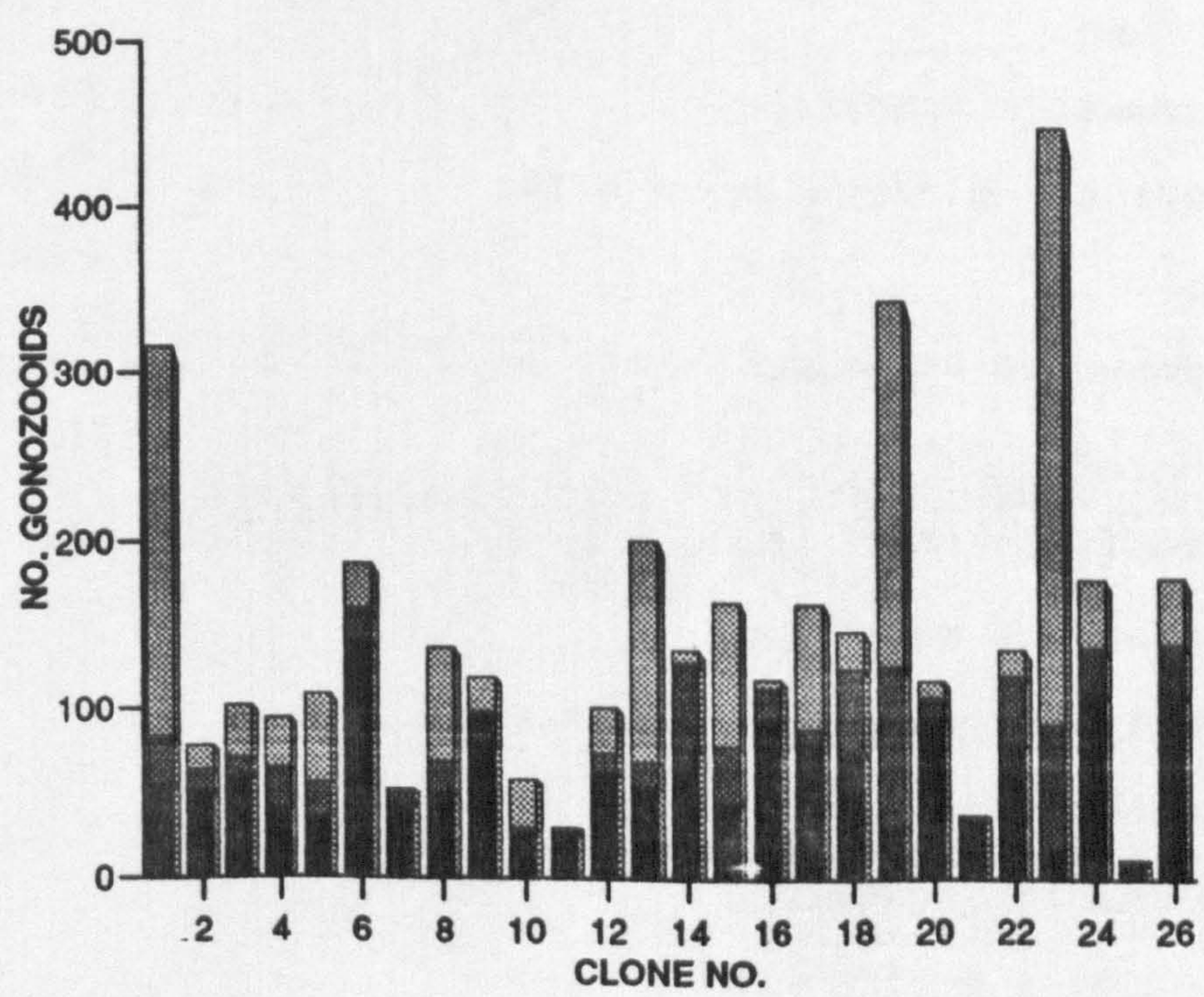

NO. GONOZOOIDS
500
400
300
200
100
0
2 4 6 8 10 12 14 16 18 20 22 24 26
CLONE NO.

Figure 3.9

Rank correlation of clonal performance as a function of the difference between macroenvironments for seven measures of clonal fitness.

a) Autozooids

Linear regression equation:

$y = 0.3 - 0.931x \quad r^2 = 25.79\%$

b) Basal males

Linear regression equation:

$y = 0.041 - 0.371x \quad r^2 = 4.7\%$

c) Frontal males

Linear regression equation:

$y = -0.019 + 0.0583x \quad r^2 = 20.9\%$

d) Total males

Linear regression equation:

$y = 0.084 - 0.057x \quad r^2 = 0.1\%$

e) Females

Linear regression equation:

$y = 0.198 - 0.541x \quad r^2 = 66.7\%$

f) Total zooids

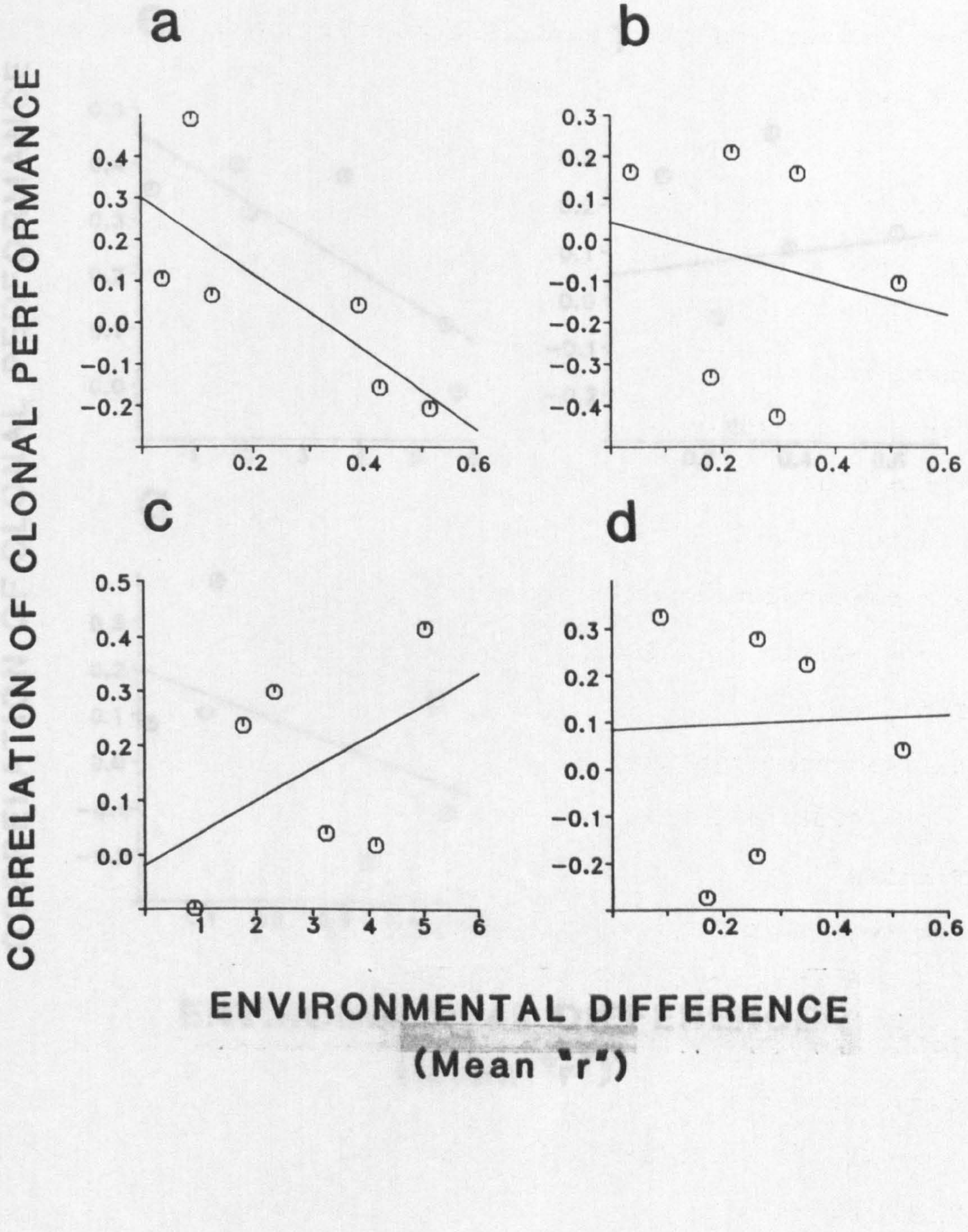
Linear regression equation:

$y = 0.198 - 0.541x \quad r^2 = 25.4\%$

g) Total gonozooids

Linear regression equation:

$y = 0.051 + 0.130x \quad r^2 = 1.0\%$


a
b
c
d
CORRELATION OF CLONAL PERFORMANCE
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
0.2
0.4
0.6
0.5
1
2
3
4
5
6
−0.3
−0.4
ENVIRONMENTAL DIFFERENCE
(Mean "r")

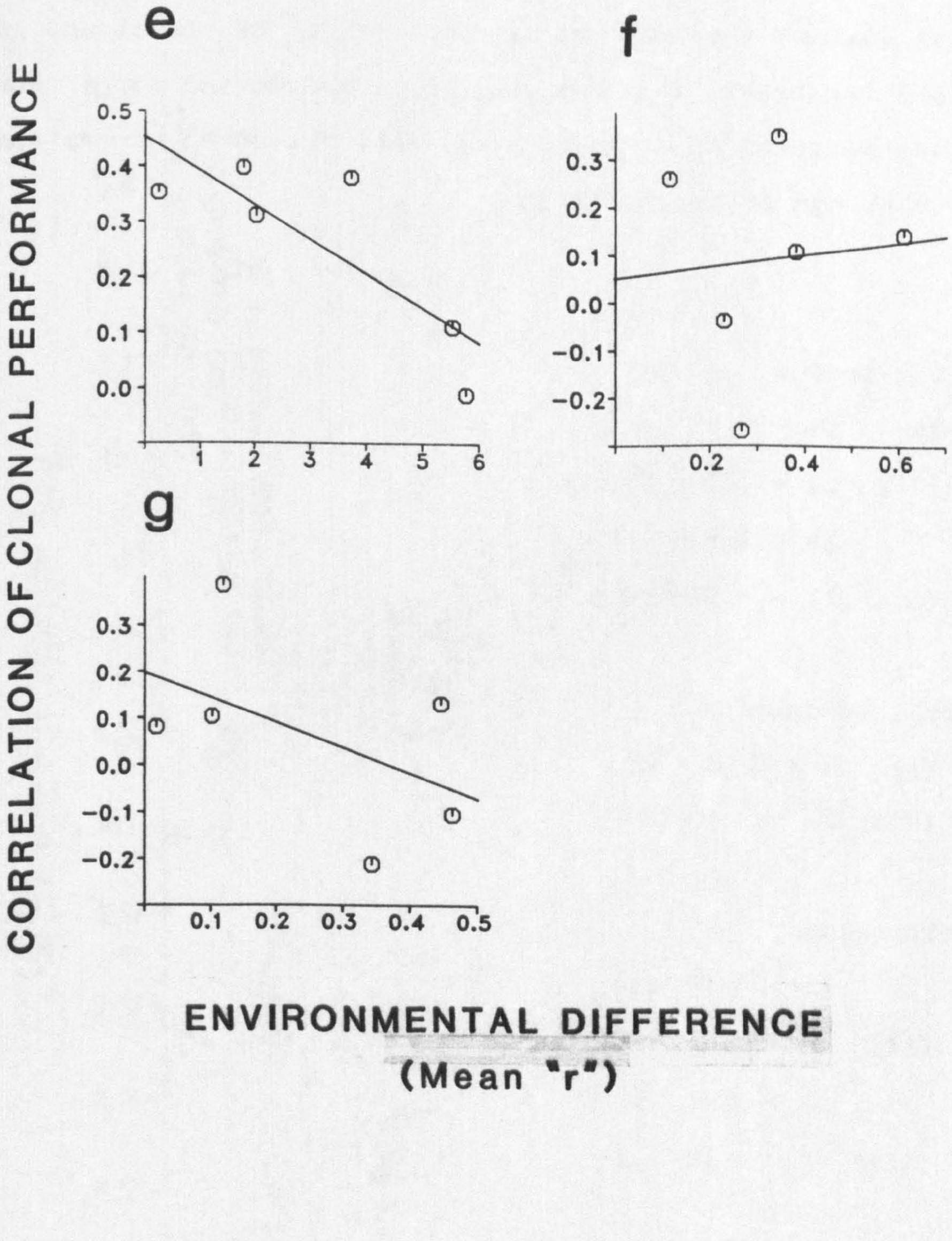

e
f
g
CORRELATION OF CLONAL PERFORMANCE
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
0.3
0.2
0.1
0.0
−0.1
−0.2
0.2
0.4
0.6
0.3
0.2
0.1
0.0
−0.1
−0.2
0.1
0.2
0.3
0.4
0.5
ENVIRONMENTAL DIFFERENCE
(Mean "r")

Figure 3.10

Reaction norms for clonal performance in 26 clones grown in four different macroenvironments. The growth rate "r" is plotted against the mean growth rate (*100) of all clones in the same treatment. The closed circles and dotted lines show the mean performance in each treatment. Unplotted clones and their rankings are listed below.

a) Total zooids

(i) 11 = 4.83 (26)

(ii) 11 = 4.47 (25)

15 = 2.92 (26)

(iii) 11 = 5.83 (26)

b) Total gonozooids

(i) 1 = 0.00 (26)

(iii) 20 = 3.65 (26)

c) Autozooids

(i) 11 = 2.07 (26)

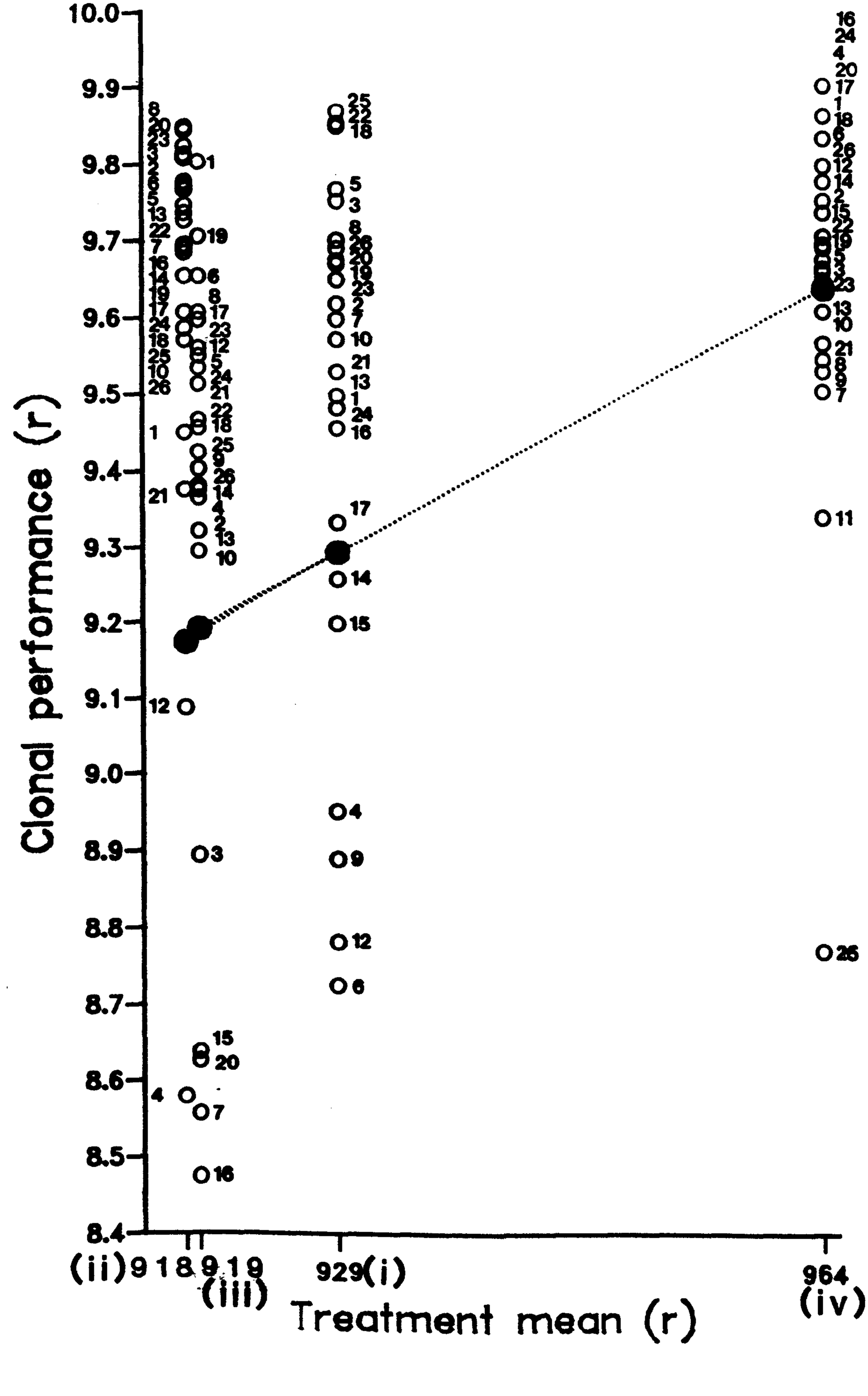
(ii) 11 = 4.47 (25)

15 = 3.01 (26)

(iii) 11 = 7.16 (26)

a

Total zooids



d) Basal males

(i) 20 = 4.63 (25)

1 = 0.00 (26)

(ii) 5 = 5.97 (23)

4 = 4.09 (24)

11 = 3.36 (25)

15 = 2.09 (26)

(iii) 20 = 3.65 (26)

(iv) 25 = 0.00 (26)

e) Frontal males

(i) 1-3,6,10-12,14-17,21,24 & 26 = 0.00 (26)

(ii) 1-3,11,15,21 & 25 = 0.00 (26)

(iii) 2,3,5-17,20,21,25 & 26 = 0.00 (26)

(iv) 7,10,16 & 25 = 0.00 (26)

f) Total males

(i) 1 = 0.00 (26)

(ii) 11 = 3.36 (25)

15 = 2.09 (26)

(iii) 20 = 3.65 (26)

(iv) 25 = 0.00 (26)

g) Females

(i) 1-7,9,11,12,14-16,20,21,23-26 = 0.00 (26)

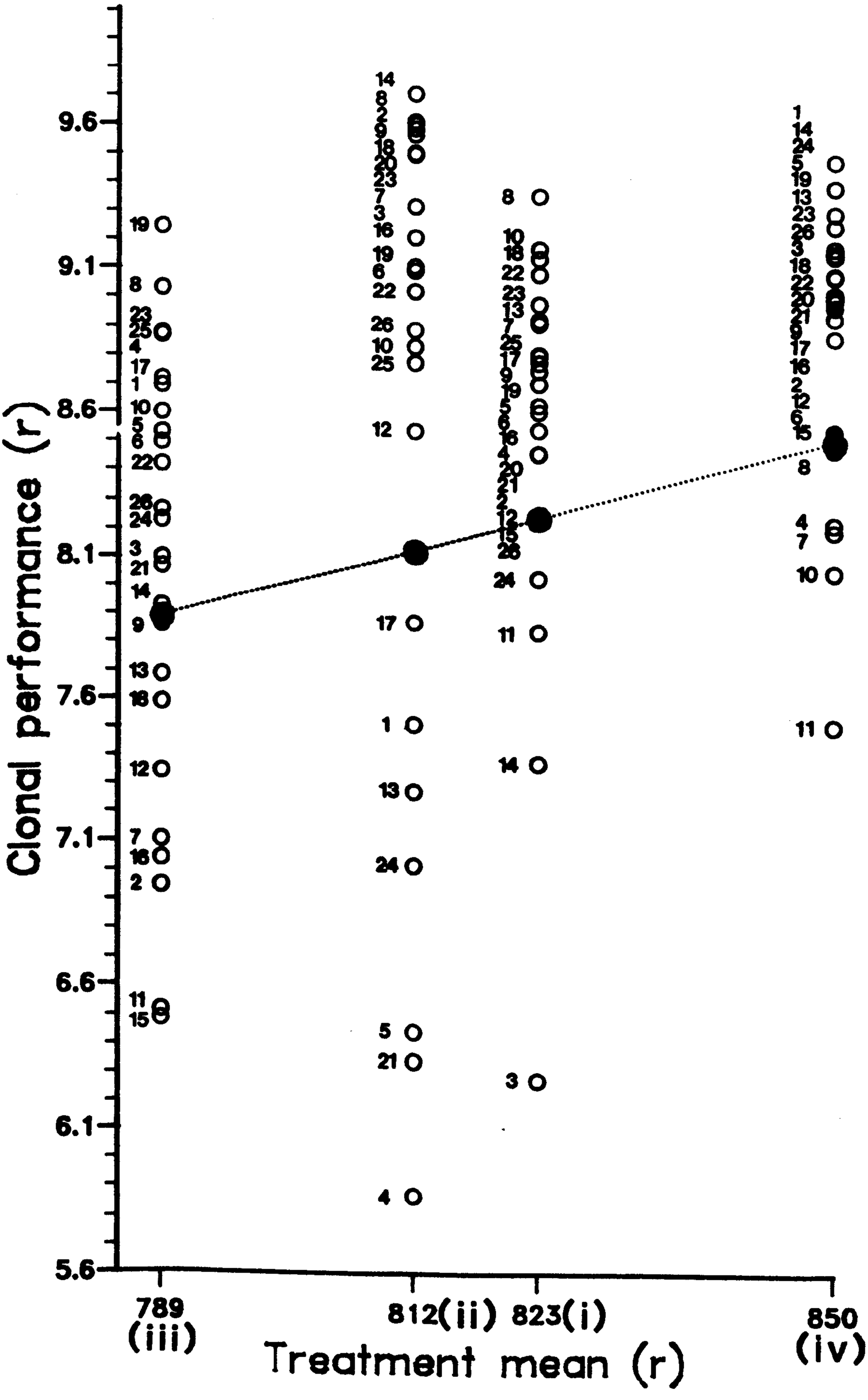
(ii) 1-7,9-13,15,18-21,25 & 26 = 0.00 (26)

(iii) 2,4,12,14-16,18,20,21,25 & 26 = 0.00 (26)

(iv) 11,21 & 25 = 0.00 (26)

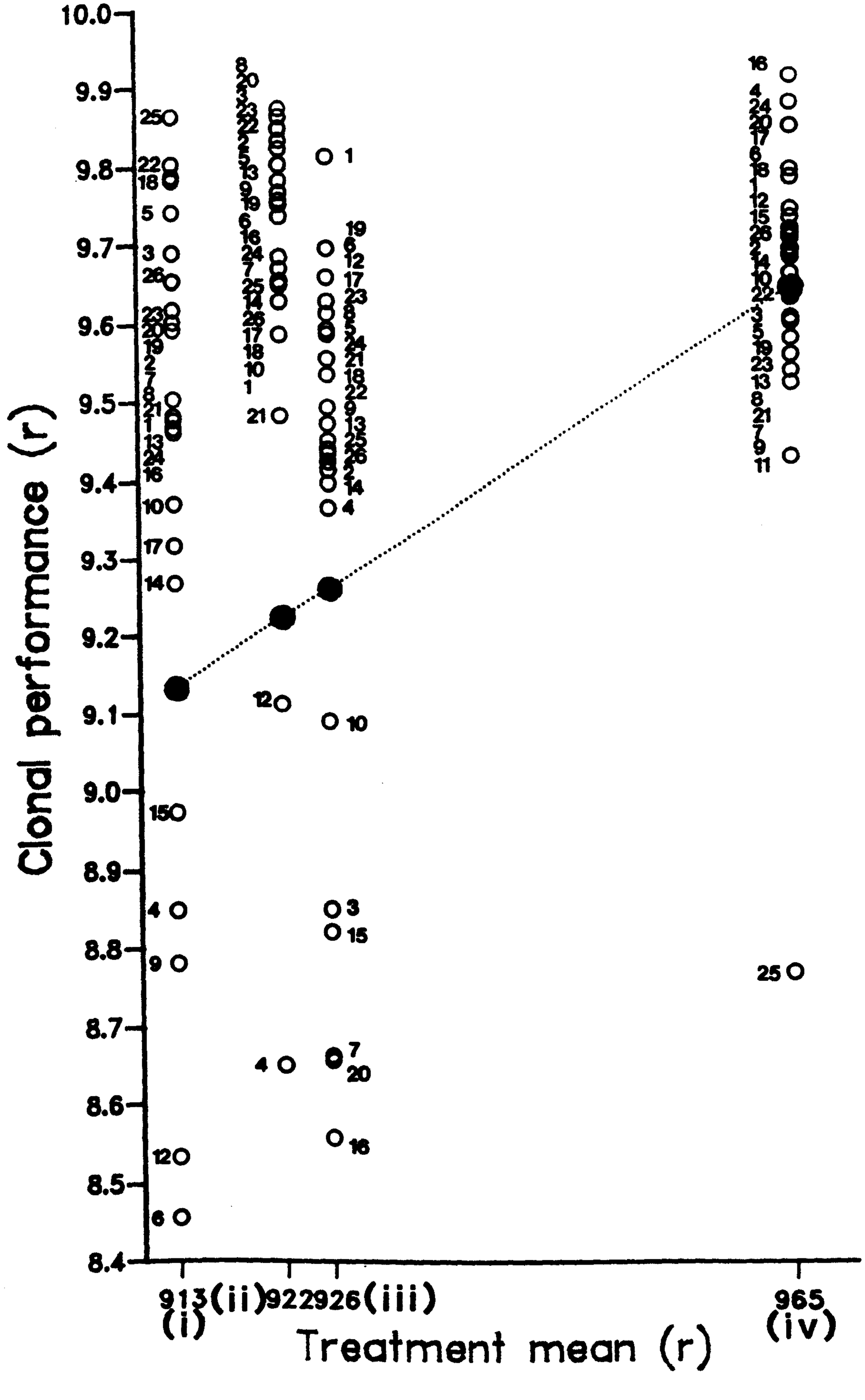
b

Total gonozooids



C

Autozooids



d

Basal males

Clonal performance (r)

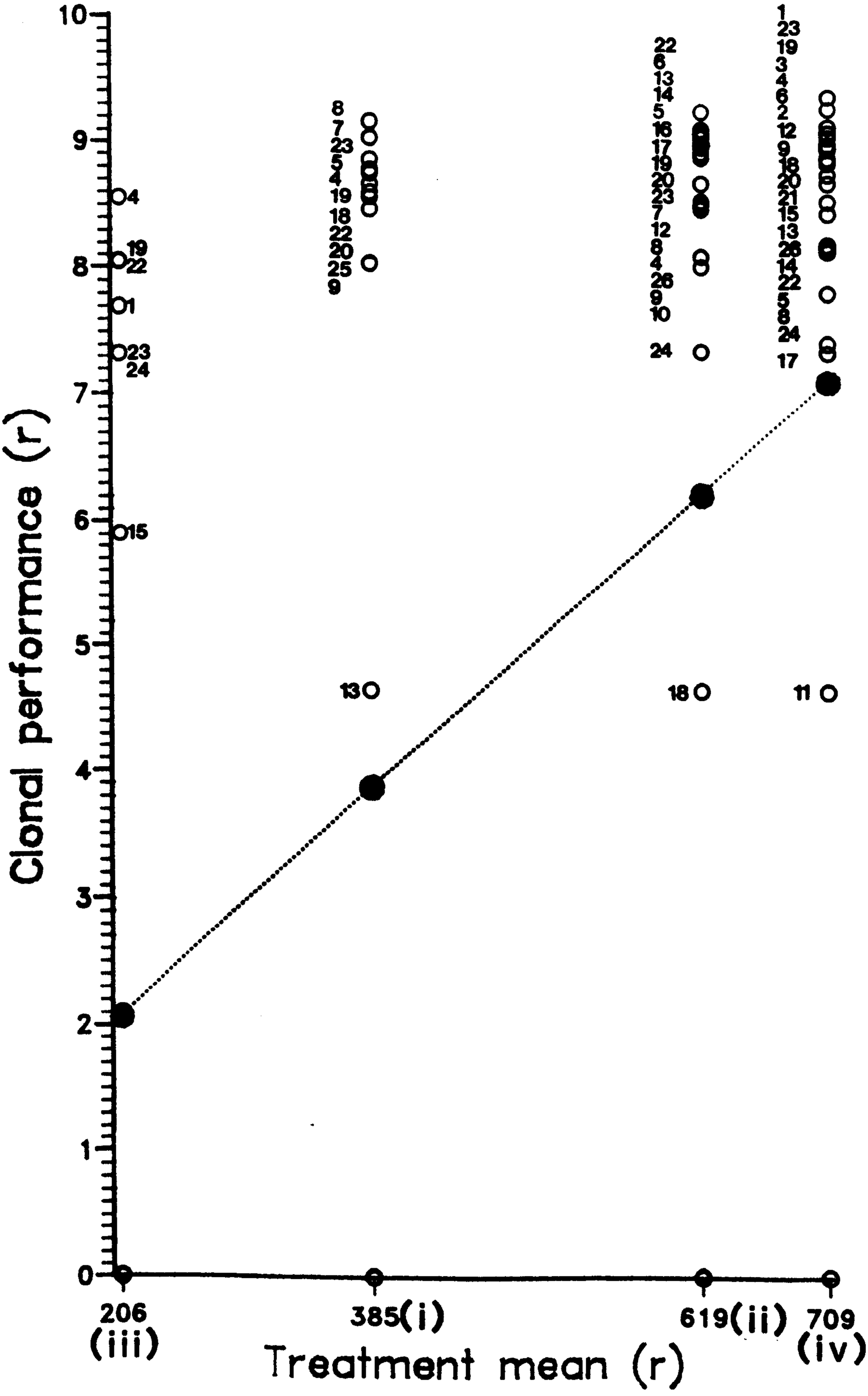
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0

776 (iii)
(ii) 794
798 (i)
827 (iv)

Treatment mean (r)

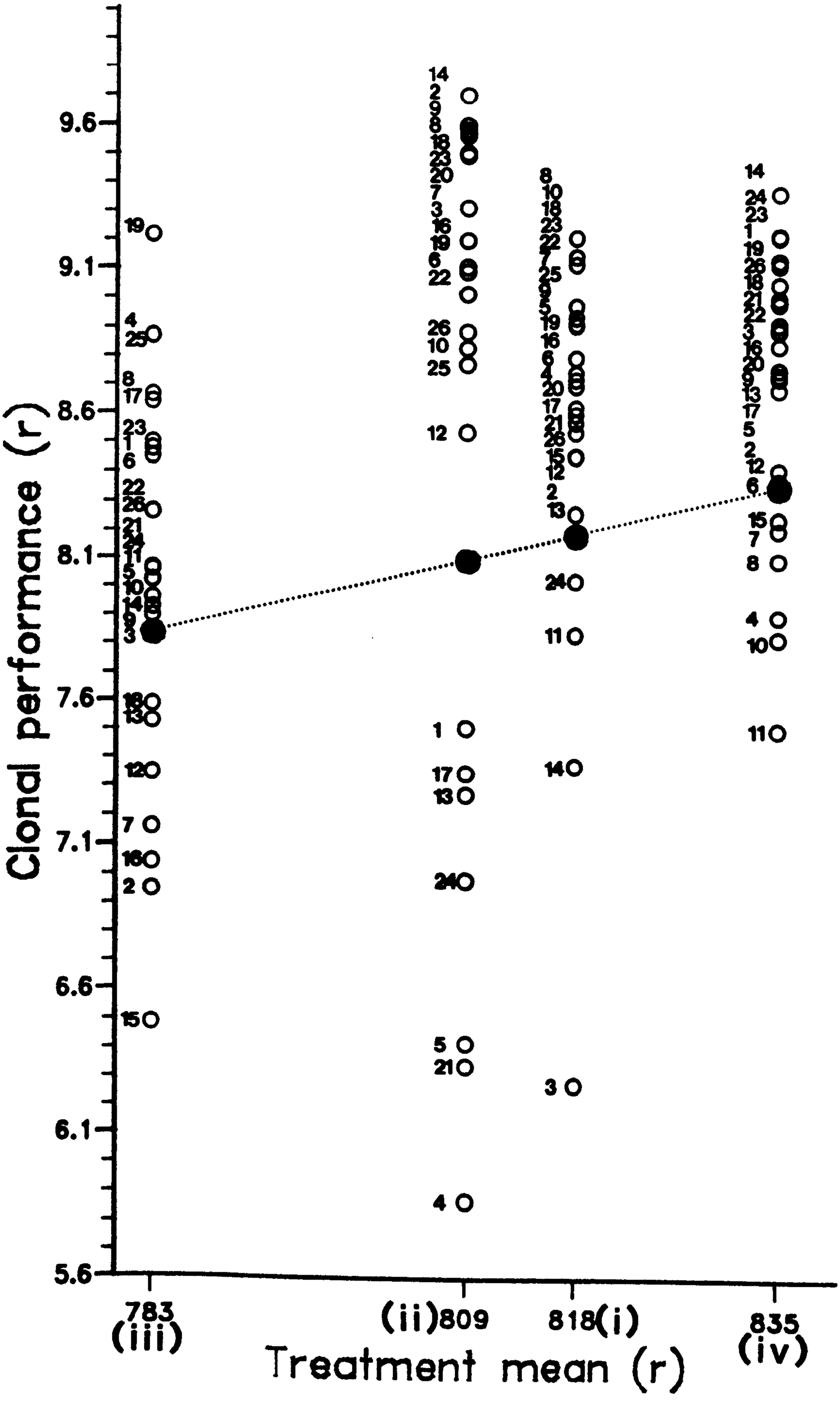
e

Frontal males



f

Total males



g

Females

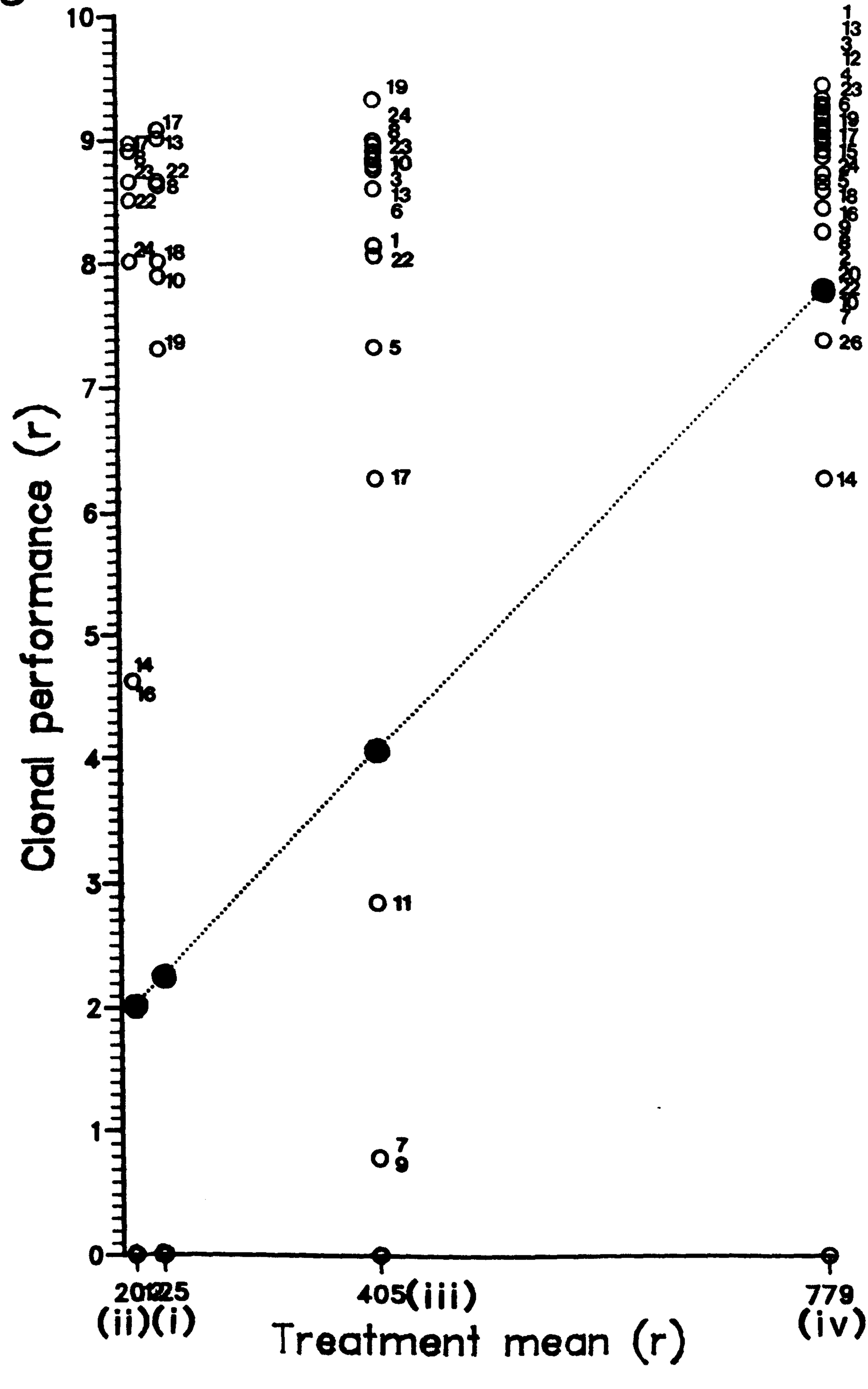

Clonal performance (r)
Treatment mean (r)
10
9
8
7
6
5
4
3
2
1
0
(ii)
(i)
(iii)
(iv)

Figure 3.11

Genetic regression for clonal performance in the four macroenvironments. On each set of axes, the growth rate "r" of each clone is plotted against the mean growth rate of that clone in all macroenvironments. The dashed lines have a slope of +1, and represent the situation where clonal performance is identical to mean clonal performance across all macroenvironments.

a) Total zooids

Linear regression equations:

(i) $y = -0.92 + 1.10x$ $r^2 = 75.3\%$

(ii) $y = -9.01 + 1.95x$ $r^2 = 82.0\%$

(iii) $y = 0.934 + 0.886x$ $r^2 = 76.0\%$

(iv) $y = 8.99 + 0.0696x$ $r^2 = 6.2\%$

b) Total gonozooids

Linear regression equations:

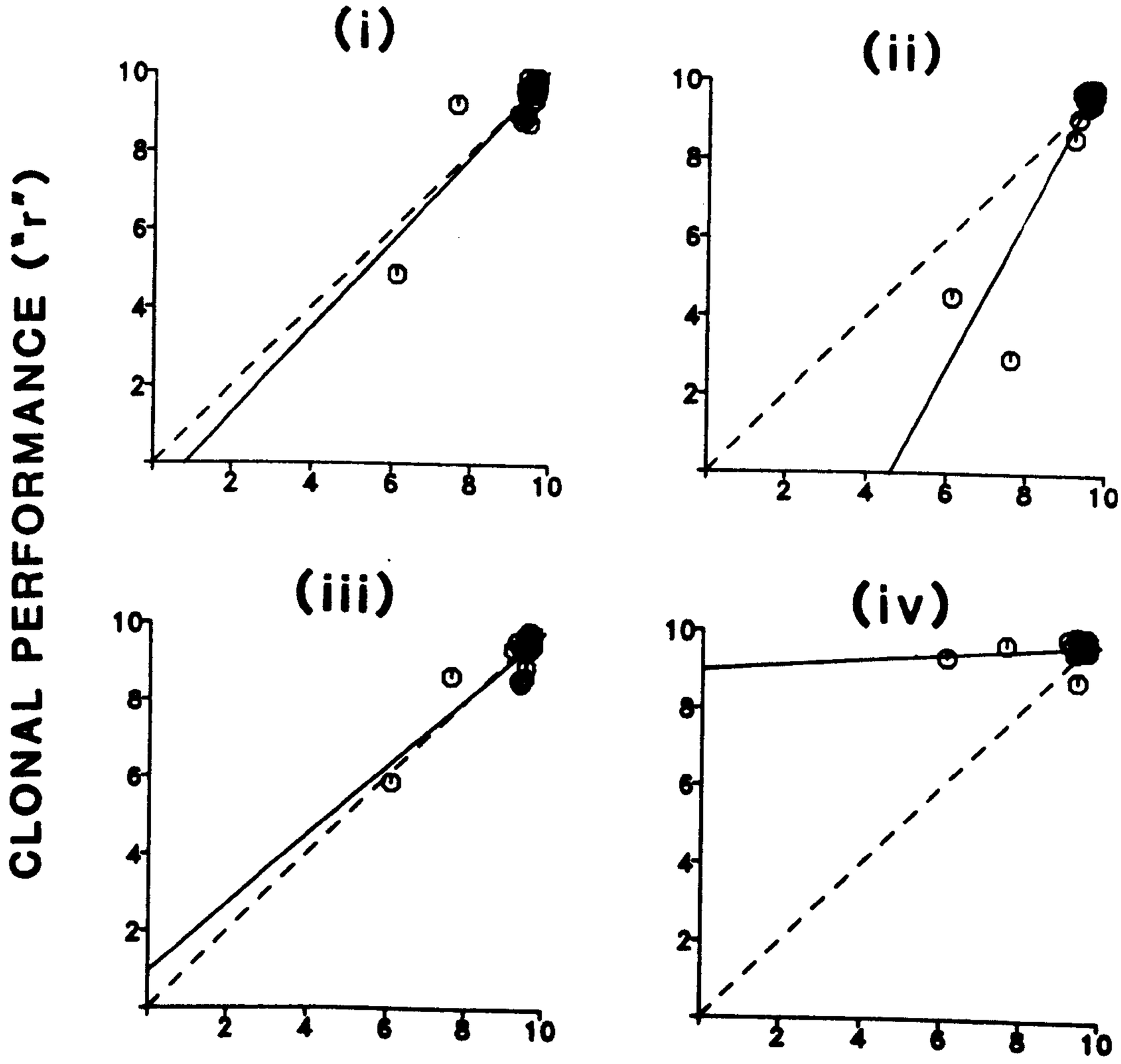
(i) $y = -0.21 + 1.03x$ $r^2 = 23.6\%$

(ii) $y = -5.20 + 1.63x$ $r^2 = 49.0\%$

(iii) $y = 4.31 + 0.437x$ $r^2 = 10.1\%$

(iv) $y = 1.09 + 0.905x$ $r^2 = 18.1\%$

a


(i)
(ii)
(iii)
(iv)
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")
2
4
6
8
10

c) Autozooids

Linear regression equations:

(i) $y = -6.29 + 1.66x$ $r^2 = 80.9\%$

(ii) $y = -7.15 + 1.76x$ $r^2 = 78.0\%$

(iii) $y = 4.18 + 0.545x$ $r^2 = 64.0\%$

(iv) $y = 9.25 + 0.0425x$ $r^2 = 2.6\%$

d) Basal males

Linear regression equations:

(i) $y = -2.81 + 1.35x$ $r^2 = 31.2\%$

(ii) $y = -3.68 + 1.45x$ $r^2 = 35.2\%$

(iii) $y = 4.86 + 0.363x$ $r^2 = 4.8\%$

(iv) $y = 1.63 + 0.832x$ $r^2 = 15.0\%$

e) Frontal males

Linear regression equations:

(i) $y = -2.60 + 1.35x$ $r^2 = 50.2\%$

(ii) $y = 1.12 + 1.06x$ $r^2 = 37.3\%$

(iii) $y = -2.36 + 0.921x$ $r^2 = 36.0\%$

(iv) $y = 3.84 + 0.677x$ $r^2 = 22.9\%$

f) Total males

Linear regression equations:

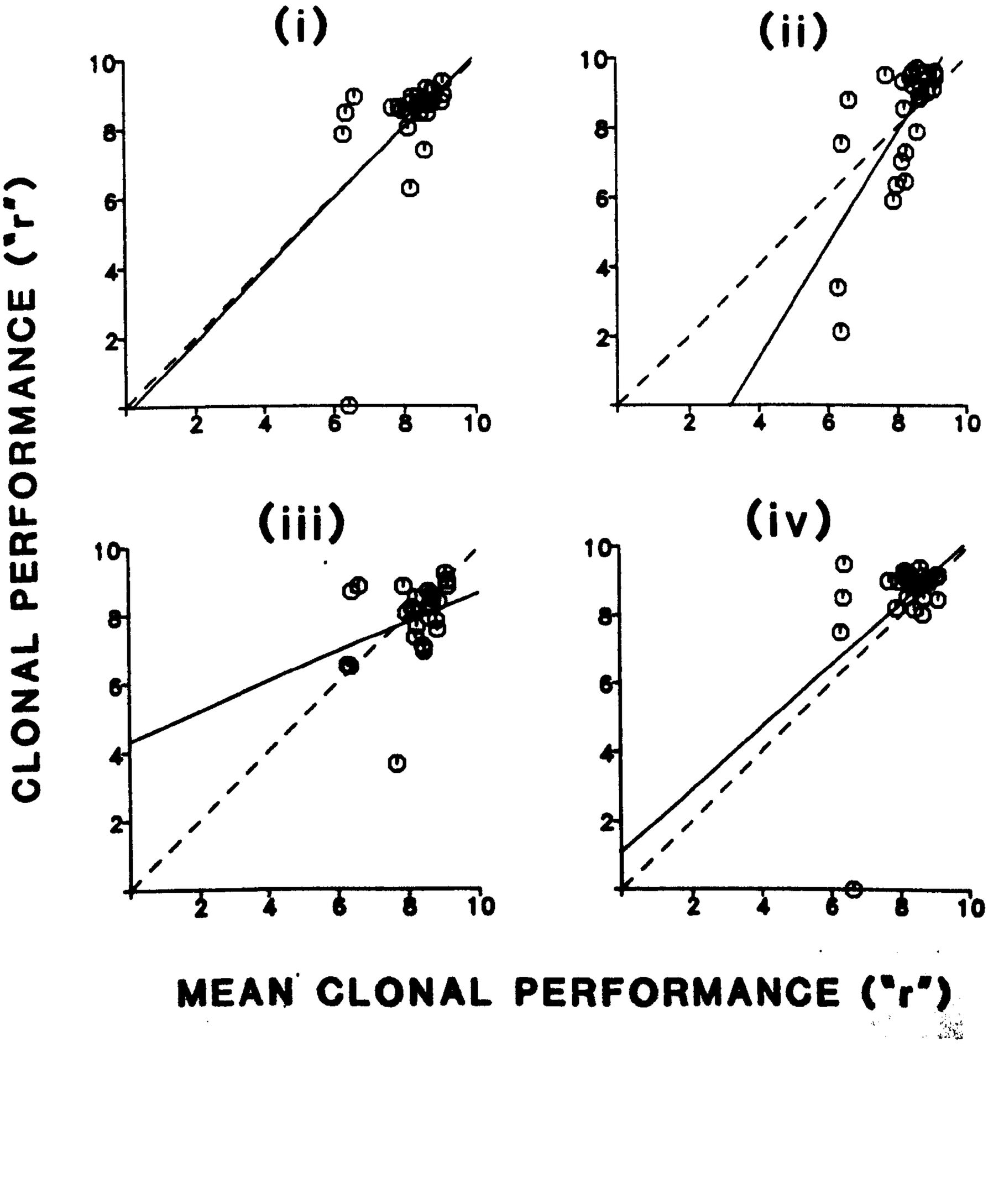
(i) $y = -0.88 + 1.12x$ $r^2 = 25.6\%$

(ii) $y = -5.65 + 1.69x$ $r^2 = 48.1\%$

(iii) $y = 5.96 + 0.231x$ $r^2 = 3.0\%$

(iv) $y = 0.58 + 0.958x$ $r^2 = 19.0\%$

b


(i)
(ii)
(iii)
(iv)
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")
2
4
6
8
10

g) Females

Linear regression equations:

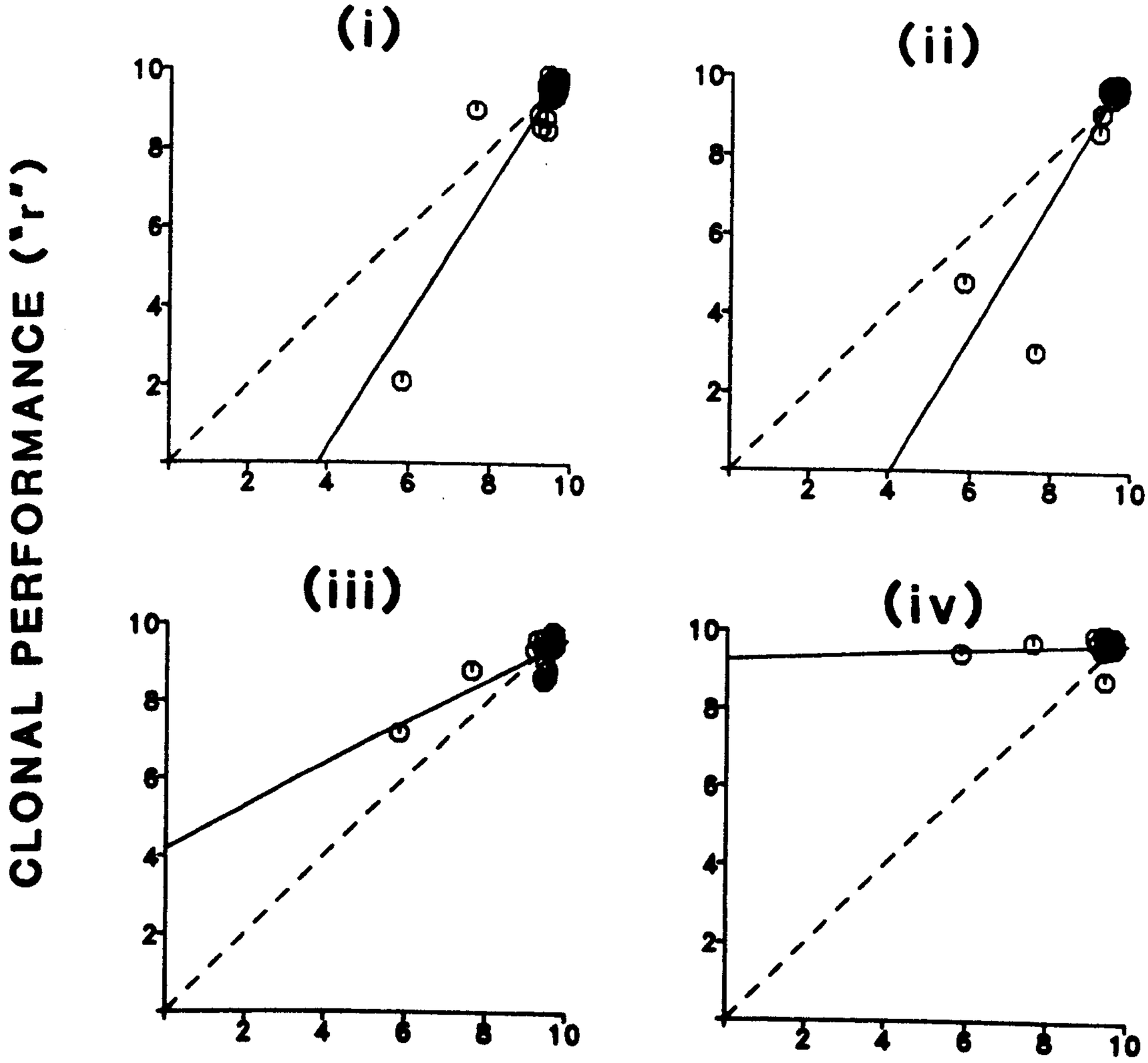
(i) $y = -2.15 + 1.09x$ $r^2 = 55.0\%$

(ii) $y = -1.71 + 0.926x$ $r^2 = 46.0\%$

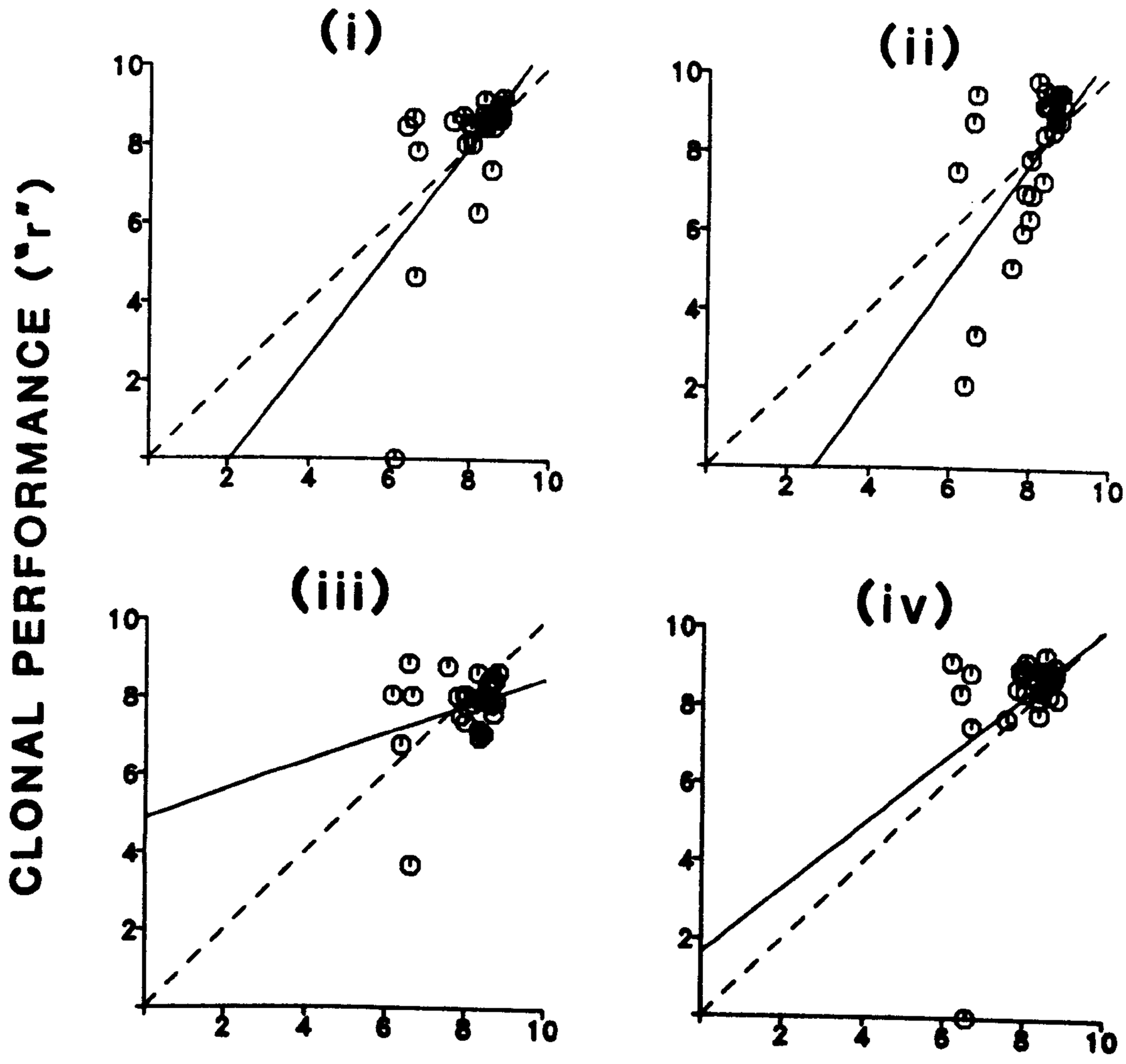
(iii) $y = -1.24 + 1.31x$ $r^2 = 65.2\%$

(iv) $y = 5.11 + 0.664x$ $r^2 = 33.8\%$

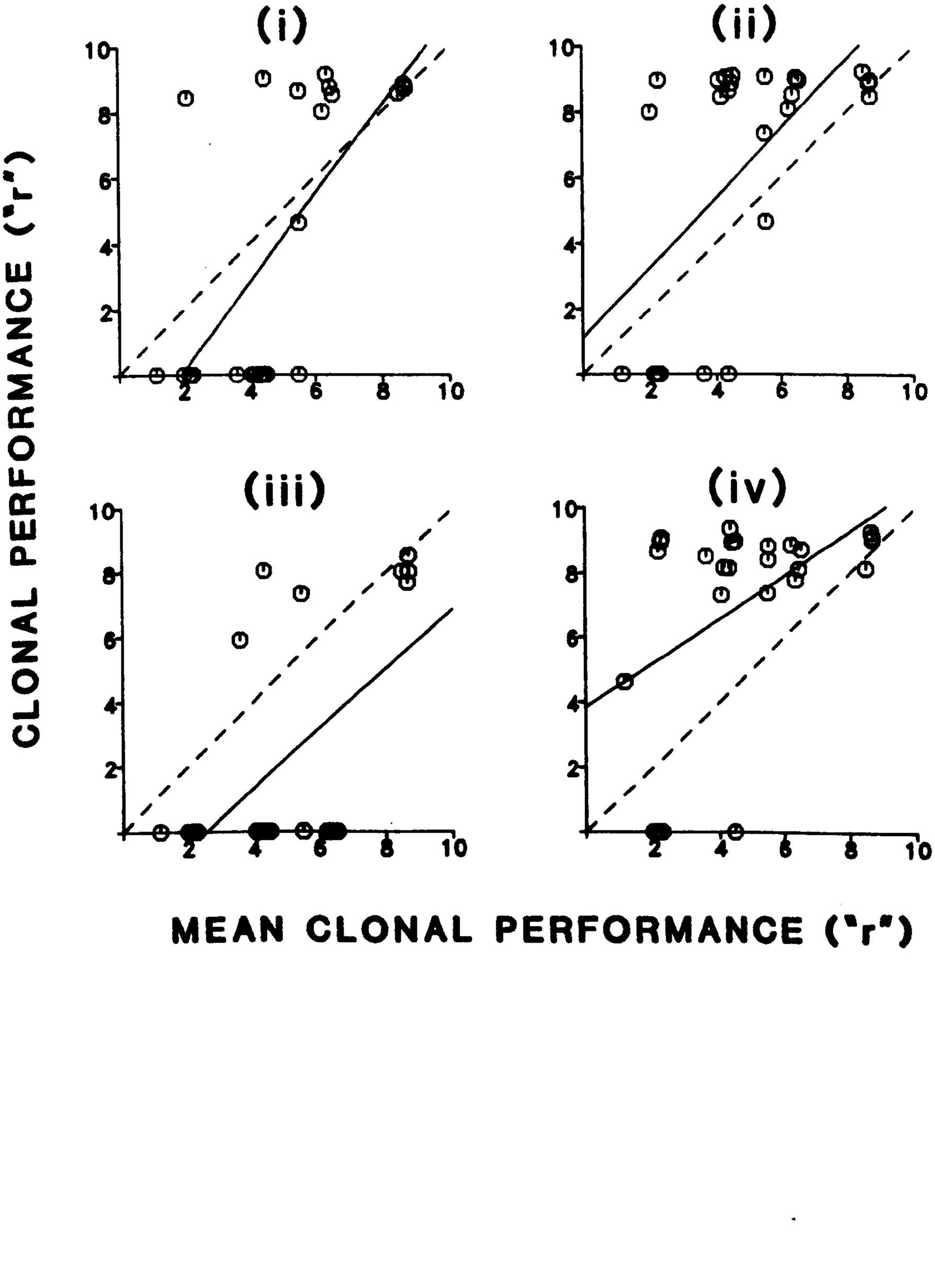
C


(i)
(ii)
(iii)
(iv)
10
8
6
4
2
2
4
6
8
10
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")

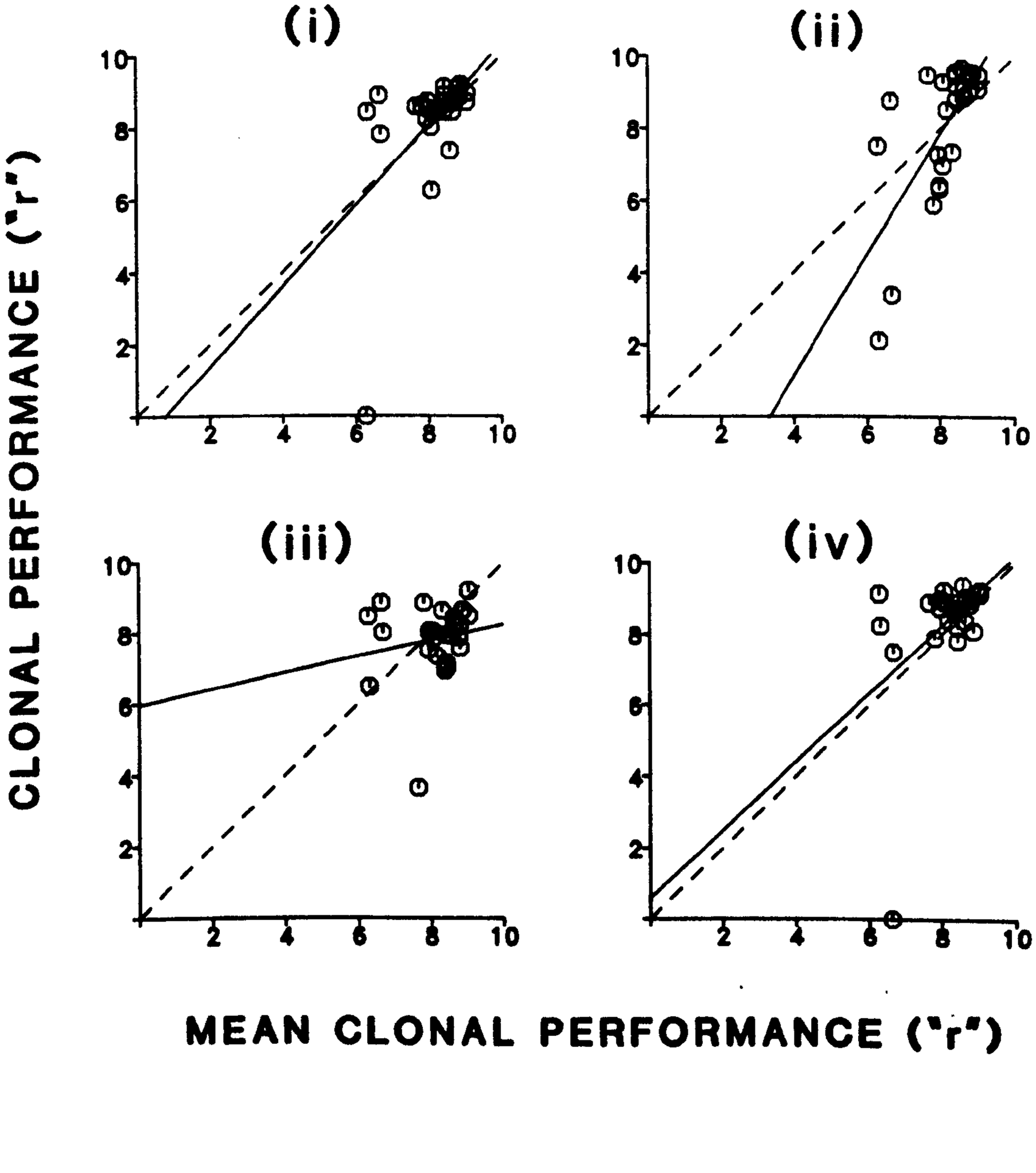
d


(i)
(ii)
(iii)
(iv)
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")
2
4
6
8
10

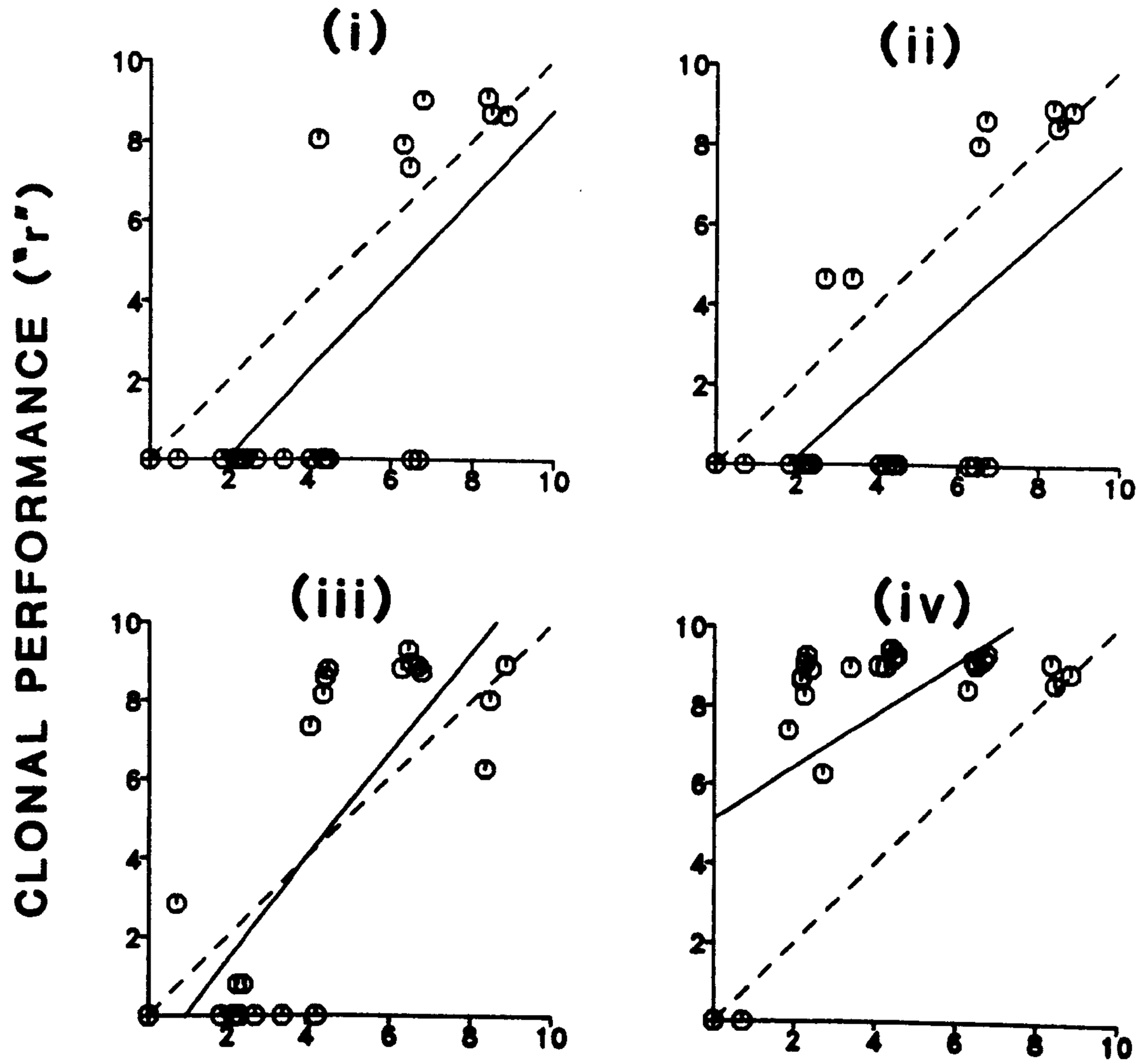
e


(i)
(ii)
(iii)
(iv)
10
8
6
4
2
2
4
6
8
10
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")

f


(i)
(ii)
(iii)
(iv)
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")
2
4
6
8
10

g


(i)
(ii)
(iii)
(iv)
CLONAL PERFORMANCE ("r")
MEAN CLONAL PERFORMANCE ("r")
2
4
6
8
10

Chapter 4:

Intraclonal variation in *Celleporella hyalina* (L.).

4.1 Introduction

In the previous chapter, extensive genotype-environment interactions were found in laboratory reared clones of the marine bryozoan Celleporella hyalina. A detailed examination of relative clonal fitness over four experimental macroenvironments revealed that the performance of individual clones showed extreme variation between macroenvironments. Thus, clones which showed high fitness in one set of environmental variables did not necessarily perform well when placed in a different environmental combination.

If these results are taken as evidence of fine-scale environmental partitioning both within and between clones, then it must be assumed that all clone-mates in the experiment were indeed genetically identical. However, in a previous study (D.J.Hughes 1991, in press), a surprising degree of inconsistency was found when paired clonal replicates were grown within apparently identical macroenvironments, in some cases resulting in clone-mates of widely differing size by the end of the experiment. Since microenvironmental differences seemed to be an unlikely explanation of this variation, it was suggested that somatic mutation may have been involved. The effect of any mutation affecting growth rate would be strongly expressed if regeneration were based on just a few founder zooids, at least one of which carried the mutation.

Intraclonal variation has been well documented in a wide variety of plant species (eg Shephard et al 1980, Scowcroft 1985, Shaw 1990), but evidence of it in animals is generally lacking. Somatic mutation has been proposed as a mechanism by

which trees can maintain heterogeneity within the genet, and thereby provide sufficient variability to control herbivores, and prevent these from evolving successful genotypes for the full-scale exploitation of the host (Edmunds and Alstad 1978, 1981, Whitham 1981). Host plants therefore are able to persist, despite pest populations apparently having a higher rate of evolutionary change (Antolin and Strobeck 1985).

The importance of somatic mutation as a factor in the evolution of clonal animal taxa has not been widely investigated (Slatkin 1984). It appears, however, to play an important role in enhancing diversity under certain ecological settings within plants, both at the individual and at the population level (Whitham and Slobodchikoff 1981, Slatkin 1984, Antolin and Strobeck 1985). Since it could play a similar role in any extended lineage of clonal propagation, somatic mutation is a feature of evolutionary biology which deserves further attention (Hughes 1989).

D.J.Hughes (1991, in press), has stressed the need in further studies of clonal variation, to maximise the number of replicate colonies per clone, in order to allow for the occurrence of intraclonal variation. In the current study, it was decided to examine the degree of variation possible within a single clone of the marine bryozoan Celleporella hyalina, by growing multiple replicates within identical macroenvironments. The evolutionary implications of the results of this study are discussed.

4.2 Materials and methods

4.2.1 Experimental method

While clones were being grown for the experiments on variation in clonal performance (chapter three), one particularly fast-growing genotype emerged, which failed to regenerate in one of the four replicates. Due to the vigorous growth of the survivors, it was decided to propagate multiple replicates of this particular clone. Twenty four replicates were eventually taken from the original colony over a period of 8 months. This allowed six replicates of the same genotype to be grown simultaneously in the four controlled "macroenvironments" described in chapter three. In this way, a detailed study could be made of intraclonal variation in *C. hyalina*.

These clonal replicates were grown alongside the clones from the main experiment, and thus experienced exactly the same conditions. The experimental method employed has already, therefore, been fully described in chapter three.

4.2.2 Analysis of the results

As in the previous chapter, analysis of covariance (ANCOVA) was used in order to examine the principal sources of variation, incorporating all of the data collected over the twelve weeks of the experiment. However, since only one genotype was involved, the analysis in this case was a three-factor ANCOVA with a nested design. The three experimental factors were MACRO, representing the four "macroenvironments", REP, nested within MACRO, representing the six clonal replicates in each macroenvironment, and TIME,

the total number of observations. MACRO and REP were the dependent variables, with TIME as the covariate. These three main effects were fully orthogonal, and were treated as fixed factors. All data were normalised by logarithmic transformation. The sample size was as follows:

MACRO=4, REP=6, TIME=13.

Two interaction terms, MACRO*TIME and REP(MACRO)*TIME result from a general linear model ANCOVA of this type. As before, interaction occurred when the effects of factors were not additive, and therefore, not independent.

4.3 Results

4.3.1 General

Since all of the colonies in the current experiment were derived from a single genotype, it was expected that replicates within a treatment would behave in a similar manner, therefore resulting in very low significance of the REP factor. High significance of the TIME factor was to be expected, due to divergence of colony size over time, and MACRO, rather than REP, should have been the other main factor with respect to differences in colony growth.

However, when the final numbers of zooids of all replicates in each of the four macroenvironments were plotted, obvious differences in intra-clonal performance were apparent, both for total zooid and total gonozooid production (figure 4.1 a,b). In macroenvironments 1-3, there was a gradual, but significant, spread in clonal performance, whereas, in macroenvironment four, replicate three was an obvious statistical outlyer.

4.3.2 Somatic parameters

Surprisingly, the REP term for total autozooid production, was found to have a higher level of significance ($P<0.001$, table 4.1 a), than the MACRO term ($P<0.01$). Similarly, the REP term was moderately significant for colony area ($P<0.01$, table 4.1 b), with no significance of MACRO. This result was much more pronounced in the case of colony perimeter (table 4.1 c). TIME, in the case of all three of these parameters, was responsible for by far the greatest proportion of the total variation (table 4.2), at around 87%,

with the MACRO*TIME interaction term being second greatest in its contribution to the total variation. The contribution from REP, although small, was unexpected.

Frontal autozooids, on the other hand, showed a highly significant effect of MACRO (table 4.1 d), but in this case, the amount of variation accounted for by REP was minimal (table 4.2). Unexpectedly, there were considerable intra-clonal differences in zooid size (table 4.1 e, table 4.2), and REP was responsible for a sizable 13.2% of the total variation.

In general, TIME accounted for the majority of the variation, with highly significant MACRO*TIME interactions. This was to be expected, since the differences in growth between replicates in different macroenvironments became more pronounced with time. Total autozooids, colony area and perimeter showed non-significant REP*TIME interaction terms, whereas frontal autozooids had no significant REP main effect, but showed a highly significant REP*TIME interaction. Again, this was to be expected, since frontal autozooid production usually did not commence until colonies had reached a size of approximately thirty autozooids.

4.3.3 Sexual parameters

Sexual parameters appeared to conform more to expectation than somatic parameters. There was no significant intra-clonal variation in basal male (table 4.3 a) and female (table 4.3 c) production, although REP was weakly significant in the case of frontal males ($P<0.05$, table 4.3 b). Basal male production was greatly affected by MACRO

(p<0.001), which was also responsible for 18% of the total variance (table 4.4). Although MACRO was also highly significant for female production (P<0.001), in this case, the MACRO main effect accounted for only 1.35% of the total variation. For all three types of sexual zooid, both interaction terms were highly significant (P<0.001), although the REP*TIME interaction was reduced in importance in the case of female zooid production (P<0.05).

4.3.4 Sex ratio and reproductive allocation

Sex ratio (females/total males) was found to show slight variation between replicates (P<0.05, table 4.5 a), but MACRO was of primary significance (P<0.001). In this case, there was no significant TIME main effect, but both of the interaction terms were highly significant (P<0.001).

For male reproductive allocation (total males/autozooids) all factors were highly significant (P<0.001, table 4.5 b). For female reproductive allocation (females/autozooids), however, there was no significant REP effect (table 4.5 c), although all other effects were again highly significant, with the exception of the REP*TIME interaction (n.s.). Total reproductive allocation (total gonozooids/autozooids), was most strongly affected by MACRO. (P<0.001, table 4.5). A weak effect was, however, detected for all other terms (P<0.05), although the REP*TIME interaction was again, non-significant. The strong influence of MACRO on male reproductive allocation (table 4.6), suggests that the consistently high significance of MACRO in the case of sex ratio and of total reproductive allocation

was due to environmental variation in basal male production, basal males being the most numerous type of sexual zooids (this study).

4.4 Discussion

Results from the current study demonstrate clearly that, as in a wide variety of plant species (Slatkin 1984), a considerable degree of variation is possible within a single clone, even under identical conditions. However, in the absence of any formal genetic investigation into Celleporella hyalina, caution should be exercised in the interpretation of these results.

Most of the variation demonstrated in the current study may have arisen as a result of developmental differences between clone-mates, involving the differential activation of individual genes. The presence of a distinct statistical outlyer (replicate 3, macroenvironment 4), however, suggests that somatic mutations do indeed occur. Developmental differences could have resulted from division of the parent colony into propagules, each containing meristematic tissues at different states of maturity. If all zooids within the colony possess an identical genome, then the presence of cytological, or other factors which regulate genes controlling the production of polymorphic zooids (Suzuki and Griffiths 1976), may also influence subsequent budding pattern.

Variation resulting from different developmental patterns is suggested by the fact that somatic factors, most notably colony perimeter, showed by far the greatest intraclonal effect, whereas frontal autozooids and most sexual parameters generally did not show a significant amount of intraclonal variation. Frontal autozooids and basal males in particular, zooid morphs which have already been shown to

exhibit a certain amount of environmental determination (see chapter 3), showed variation primarily between macroenvironments but minimum intraclonal variation.

Replicate 3 in macroenvironment 4 reached a much smaller size than its clone-mates in the same treatment, but this probably could not have arisen merely as a result of differential activation of the genome. Somatic mutation seems a more likely explanation, and it is therefore desirable that future studies of intraclonal variation should involve formal genetic analysis. This could be achieved by genetic fingerprinting within multiple replicates of clones, with the serial propagation of variants allowing further comparison of fitness.

Somatic mutations appear frequently in trees on account of the large numbers of cell divisions between the development of new primordia (apical meristems), and have been reported to occur at rates of up to 10^{-3} to 10^{-5} per locus (Antolin and Strobeck 1985). Somatic mutation is thought to be particularly potent in long-lived individuals, such as trees, because these will accumulate the greatest number of somatic mutations, and will therefore contribute the greatest numbers of mutant seed to future generations (Whitham et al 1984). Variability resulting in resistance to predators, derived from somaclonal mutation, may result in selection for increased somatic mutation rates where predation is intense (Antolin and Strobeck 1985). From a theoretical model, these authors predicted that significant variability for somatic mutation rates (per branch), was only selectively advantageous where the rate was 10^{-4}, or greater,

per mitotic division. Since actual rates are probably less than 10^{-5} per cell division in most plant species (Antolin and Strobeck 1985), it was concluded that the conditions under which somatic mutations will contribute significantly to genetic variability are limited. Although Slatkin (1984) had found that substantially increased genetic diversity may arise in populations as a result of somatic mutations, most of this diversity will be among different individuals. Since this variation is lost in each generation as a result of sexual recombination, somatic mutation has severely limited opportunity to generate variation within individuals. It is therefore only in long-lived species, with extended, mitotic lineages, that levels of somaclonal variation might be comparable with variation among individuals (Slatkin 1984)

An apparently necessary factor in the maintenance and perpetuation of somaclonal mutants (Antolin and Strobeck 1985), is the differential exploitation of ramets by pathogens or grazers. But although the asexual proliferation of zooids in bryozoans in many ways resembles vegetative propagation in plants (Hughes 1989), it is most unlikely that even the more persistent bryozoan colonies accumulate significant levels of somaclonal variation as a result of differential grazing by predators. In a short-lived species such as C. hyalina, mutations will be lost rapidly, either as a result of colony mortality, or due to genetic recombination during meiosis. It is therefore unlikely that somatic mutation plays an important role in the evolutionary genetics of marine bryozoans. However, further molecular genetic studies would be required to settle this matter.

Table 4.1

Comparison of growth parameters for clonal replicates, when grown in one of 4 different macroenvironments.

Data are presented as a 3-factor ANCOVA, with the 3 factors MACRO, REP (nested within MACRO), and TIME. Data were transformed to logs throughout.

Sources of variation:- MACRO : Macroenvironment (n = 4)

REP : Replicate (n = 6)

TIME : Time (n = 13)

a) Total autozooids.

Source	DF	Adj SS	Adj MS	F	P
TREAT	3	1.273	0.424	4.72	0.003
REP(TREAT)	20	4.265	0.213	2.37	0.001
TIME	1	437.025	437.025	4859.71	0.000
TREAT*TIME	3	30.689	10.230	113.76	0.000
REP*TIME(TREAT)	20	2.513	0.126	1.40	0.123
Error	264	23.741	0.090		
Total	311				

b) Colony area.

Source	DF	Adj SS	Adj MS	F	P
TREAT	3	0.420	0.140	2.20	0.088
REP(TREAT)	20	2.840	0.142	2.24	0.002
TIME	1	340.947	340.947	5367.54	0.000
TREAT*TIME	3	27.783	9.261	145.80	0.000
REP*TIME(TREAT)	20	1.971	0.099	1.55	0.065
Error	264	16.769	0.064		
Total	311				

Table 4.1 (Cont.)

c) Colony perimeter.

Source	DF	Adj SS	Adj MS	F	P
TREAT	3	0.0399	0.0133	0.71	0.547
REP(TREAT)	20	1.2471	0.0624	3.32	0.000
TIME	1	89.0359	89.0359	4741.05	0.000
TREAT*TIME	3	6.1505	2.0502	109.17	0.000
REP*TIME(TREAT)	20	0.5598	0.0280	1.49	0.084
Error	264	4.9579	0.0188		
Total	311				

d) Frontal autozooids.

Source	DF	Adj SS	Adj MS	F	P
TREAT	3	58.123	19.374	11.16	0.000
REP(TREAT)	20	19.484	0.974	0.56	0.936
TIME	1	987.105	987.105	568.63	0.000
TREAT*TIME	3	418.669	139.556	80.39	0.000
REP*TIME(TREAT)	20	145.962	7.298	4.20	0.000
Error	264	458.284	1.736		
Total	311				

e) Zooids per unit area.

Source	DF	Adj SS	Adj MS	F	P
TREAT	3	1.43122	0.47707	31.16	0.000
REP(TREAT)	20	1.92307	0.09615	6.28	0.000
TIME	1	5.95544	5.95544	388.94	0.000
TREAT*TIME	3	0.26822	0.08941	5.84	0.001
REP*TIME(TREAT)	20	0.94613	0.04731	3.09	0.000
Error	264	4.04232	0.01531		
Total	311				

Table 4.2

Proportion of sum-of-squares (see table 4.1) accounted for by each source of variation for clonal replicates grown in 4 different macroenvironments. For each variable, the values given are percentages of the adjusted S.S.

Abbreviations: Auto - autozooids, Area - colony area, Peri - colony perimeter, Af - frontal autozooids, ZPU - zooids per unit area.

VARIABLE

SOURCE	Auto	Area	Peri	Af	ZPU
MACRO	0.25	0.11	0.04	2.78	9.83
REP	0.85	0.73	1.22	0.93	13.20
TIME	87.49	87.26	87.30	47.28	40.88
MACRO*TIME	6.14	7.11	6.03	20.05	1.84
REP*TIME	0.50	0.50	0.55	6.99	6.50
ERROR	4.75	4.29	4.86	21.95	27.75

Table 4.3

Comparison of sexual parameters for clonal replicates, when grow in one of 4 different macroenvironments.

Data are presented as a 3-factor ANCOVA, with the 3 factor MACRO, REP (nested within MACRO), and TIME. Data were transforme to logs throughout.

Sources of variation:- MACRO : Macroenvironment (n = 4)

REP : Replicate (n = 6)

TIME : Time (n = 13)

a) Basal males .

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	253.766	84.589	67.53	0.000
REP(MACRO)	20	35.976	1.799	1.44	0.105
TIME	1	502.727	502.727	401.35	0.000
MACRO*TIME	3	100.039	33.346	26.62	0.000
REP*TIME(MACRO)	20	191.502	9.575	7.64	0.000
Error	264	330.686	1.253		
Total	311				

b) Frontal males.

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	3.816	1.272	0.65	0.586
REP(MACRO)	20	65.194	3.260	1.66	0.041
TIME	1	1571.817	1571.817	798.93	0.000
MACRO*TIME	3	53.540	17.847	9.07	0.000
REP*TIME(MACRO)	20	147.382	7.369	3.75	0.000
Error	264	519.396	1.967		
Total	311				

c) Females.

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	34.875	11.625	6.00	0.001
REP(MACRO)	20	32.252	1.613	0.83	0.672
TIME	1	1319.509	1319.509	681.58	0.000
MACRO*TIME	3	607.831	202.610	104.66	0.000
REP*TIME(MACRO)	20	72.629	3.631	1.88	0.014
Error	264	511.090	1.936		
Total	311				

Table 4.4

Proportion of sum-of squares (see table 4.2), accounted for by each source of variation for clonal replicates grown in 4 different macroenvironments. For each variable, the values given are percentages of the adjusted S.S.

Abbreviations: Mb - Basal males, Mf - Frontal males, Fem females.

	VARIABLE		
SOURCE	Mb	Mf	Fem
MACRO	17.94	0.16	1.35
REP	2.54	2.76	1.25
TIME	35.54	66.57	51.18
MACRO*TIME	7.07	2.26	23.58
REP*TIME	13.54	6.24	2.82
ERROR	23.38	22.00	19.82

Table 4.5

Comparison of sexual investment for clonal replicates, when grown in one of 4 different macroenvironments.

Data are presented as a 3-factor ANCOVA, with the 3 factor MACRO, REP (nested within MACRO), and TIME. Data were transformed to logs throughout.

Sources of variation:- MACRO : Macroenvironment (n = 4)

REP : Replicate (n = 6)

TIME : Time (n = 13)

a) Total sex ratio (females/total males).

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	116.574	38.858	12.78	0.000
REP(MACRO)	20	110.511	5.526	1.82	0.019
TIME	1	4.387	4.387	1.44	0.231
MACRO*TIME	3	246.172	82.057	26.99	0.000
REP*TIME(MACRO)	20	153.116	7.656	2.52	0.000
Error	264	802.509	3.040		
Total	311				

b) Male reproductive allocation (total males/autozooids).

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	281.029	93.676	59.22	0.000
REP(MACRO)	20	63.841	3.192	2.02	0.007
TIME	1	306.757	306.757	193.91	0.000
MACRO*TIME	3	26.820	8.940	5.65	0.001
REP*TIME(MACRO)	20	92.637	4.632	2.93	0.000
Error	264	417.637	1.582		
Total	311				

c) Female reproductive allocation (females/autozooids).

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	42.325	14.108	7.28	0.000
REP(MACRO)	20	32.703	1.635	0.84	0.659
TIME	1	237.773	237.773	122.74	0.000
MACRO*TIME	3	383.115	127.705	65.92	0.000
REP*TIME(MACRO)	20	63.020	3.151	1.63	0.047
Error	264	511.433	1.937		
Total	311				

d) Total reproductive allocation (total gonozooids/autozooids).

Source	DF	Adj SS	Adj MS	F	P
MACRO	3	4.2115	1.4038	7.06	0.000
REP(MACRO)	20	7.1597	0.3580	1.80	0.021
TIME	1	0.9397	0.9397	4.73	0.031
MACRO*TIME	3	2.1072	0.7024	3.53	0.015
REP*TIME(MACRO)	20	4.0005	0.2000	1.01	0.455
Error	264	52.4952	0.1988		
Total	311				

Table 4.6

Proportion of sum-of squares (see table 4.5), accounted for by each source of variation for clonal replicates grown in different macroenvironments. For each variable, the values given are percentages of the adjusted S.S.

Abbreviations: TSR - total sex ratio, MRA - male reproductive allocation, FRA - female reproductive allocation TRA - total reproductive allocation.

SOURCE	TSR	MRA	FRA	TRA
MACRO	8.13	23.64	3.33	5.94
REP	7.71	5.37	2.57	10.10
TIME	0.31	25.81	18.72	1.33
MACRO*TIME	17.18	2.26	30.16	2.97
REP*TIME	10.68	7.79	4.96	5.64
ERROR	55.99	35.13	40.26	74.03

Figure 4.1

Performance of clonal replicates grown in macroenvironments (i) to (iv).

a) Total zooids.

b) Total gonozooids.

a

NO.ZOOIDS

(i)

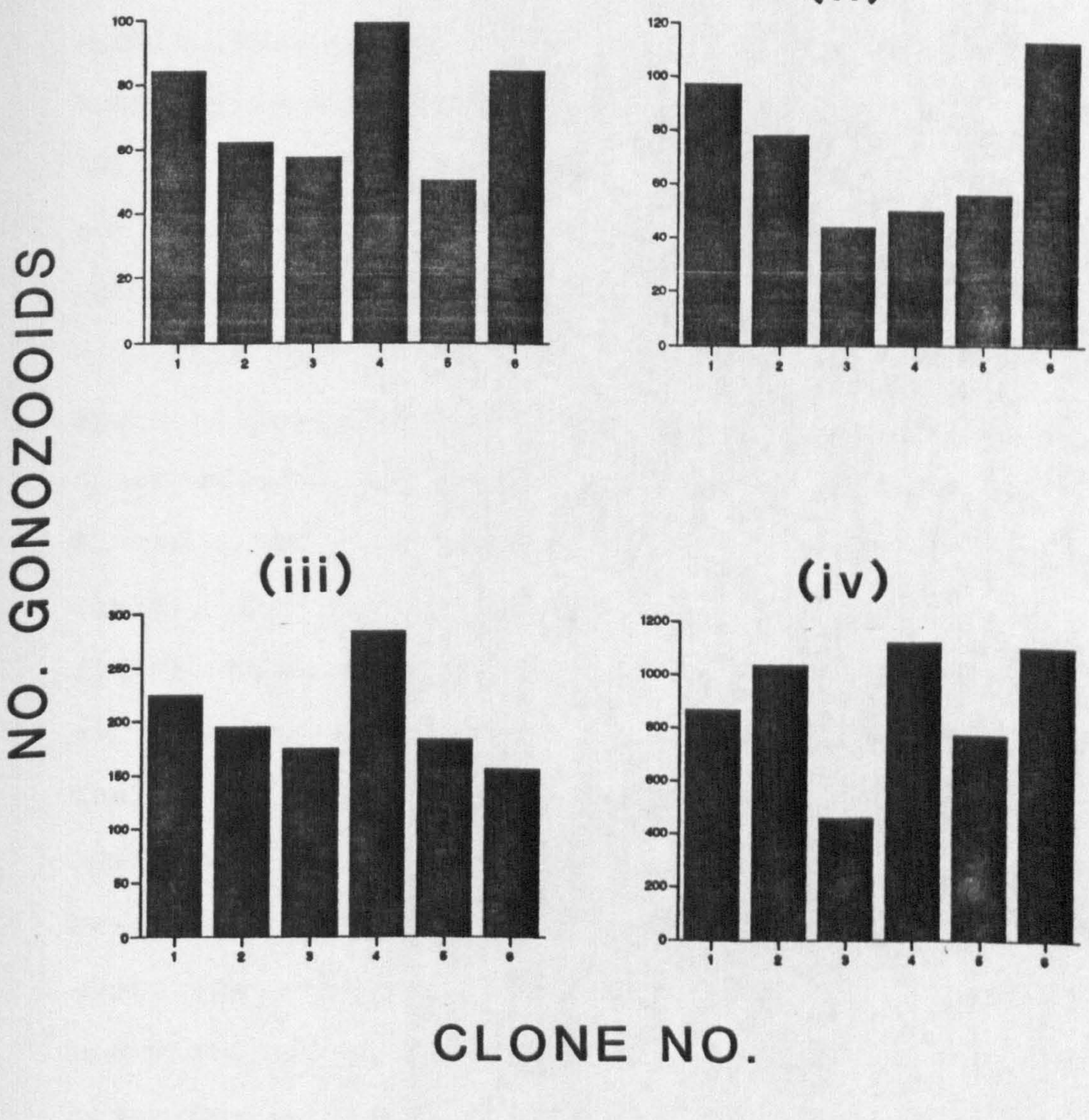
(ii)

(iii)

(iv)

CLONE NO.

b



Chapter 5:

The possibility of self-fertilisation in *Celleporella hyalina*.

5.1 Introduction

Substantial temporal overlap between male and female reproductive functions is a common feature of many hermaphroditic sessile invertebrates (see Ghiselin 1974). It is possible, therefore that some degree of self fertilisation may occur in these forms. Huxley (1856) first noted the existence of simultaneous hermaphroditism in the zooids of Bugula avicularis. Other early reports pointed towards autogamy in both freshwater (Allman 1856, Braem 1897, Marcus 1934), and marine bryozoans (reviewed by Silén 1966). It is now considered, however, that most bryozoans are at least potentially outbreeding (Silén 1972, Ryland 1976).

This current opinion results from direct observations of sperm release and from genetic studies. The first account of sperm release in Bryozoa was given over a century ago by Hincks (1860), and subsequently by Joliet (1877) and Marcus (1938), but clear evidence was first published by Silén (1966). More recently, Silén (1972) reviewed sperm release in all species where it had been observed, including eleven Cheilostomata, three Ctenostomata and two Stenolaemata. An additional two species of Cheilostomata have subsequently been reported by Chimonides and Cook (1981). In all cases, sperm are released through the tentacles. Fertilisation in non-brooding species occurs either in the intertentacular organ during ova discharge, or externally once the egg has been released (Silén 1972, Ryland 1976). Fertilisation in brooding species however, is thought to be internal (Silén 1972, Ryland 1976, Chimonides and Cook 1981, Dyrynda and King 1982, D.J.Hughes 1987). Sperm can probably locate the ova by

chemotaxis (Clarke 1981), similar to the method employed by hydroids (Miller 1982).

The gene frequencies for isozyme loci in the two cheilostomes Bugula stolonifera and Schizoporella errata were found to conform to the Hardy-Weinberg equilibrium, supporting the idea of routine outbreeding (Schopf 1977). Although sperm release provides the potential for outbreeding, it is possible that inbreeding may occur in natural populations, since sterility mechanisms have yet to be described for any bryozoan (Ryland 1976, Bell 1982).

Self fertilisation has been reported in many simultaneously hermaphroditic animals, both under experimental conditions, and in natural populations (eg Ghiselin 1969, Clark 1978, Bell 1982). Amongst colonial invertebrates, autogamy is known to take place exceptionally in tunicates (Sabbadin 1971, Berrill 1975), and has also been found in a hermatypic coral (Kojis and Quinn 1981).

Selfing represents the most extreme form of inbreeding, and may result in levels of heterozygosity falling to almost zero within a few generations (eg Falconer 1981). Increased levels of homozygosity usually results in inbreeding depression (Charlesworth and Charlesworth 1987). Complete selfing, when maintained in the long-term, will eventually result in the same evolutionary inability to adapt to changing ecological conditions, as is the case in parthenogenetic populations, but with no possibility of perpetuating heterozygous genotypes (Maynard-Smith 1978).

Certain advantages may, however, result from selfing under specific ecological settings, ameliorating some of the

costs already mentioned. Recessive deleterious genes can progressively be purged from a population if inbreeding is maintained over a number of generations, and as a result, inbreeding depression quickly declines. Self fertilisation can also present sessile organisms with the opportunity to avoid the problems of sperm dilution in free spawning species, and copulation or sperm transfer in species with internal fertilisation. Also, when selfing is routine, or at least is available as an emergency option, isolated individuals can rapidly found populations sexually on entering new habitat space (Ryland and Bishop 1990). It may also be the case that locally favourable genotypes are perpetuated by selfing, or some form of inbreeding in a sessile organism. Such favourable genotypes would otherwise be diluted or disrupted by outcrossing, therefore resulting in outbreeding depression (Shields 1982, Grosberg 1987). Habitual inbreeding may be particularly relevant in spatially heterogeneous, but temporally stable habitats. The extent to which selfing actually occurs may, therefore, reflect the balance of these costs and benefits in a particular ecological setting.

Celleporella hyalina (L.) is a simultaneous hermaphrodite with separate male, female and feeding zooids. Sperm have, however, been found in all three zooid types (Marcus 1938), and intra-colonial sperm migration has been suggested as a means of self-fertilisation (Marcus 1938, 1941, D.J.Hughes 1987). Sperm release in Celleporella has never been observed, but it is assumed that this process takes place through the male lophophore (D.J.Hughes 1987),

with sperm subsequently entering the females of neighbouring, and probably also the same colony (Ryland and Gordon 1977). Both intra-colonial sperm migration, or the re-entry of liberated sperm could result in selfing in this species. C. hyalina can be found in dense monospecific stands growing on the kelp Laminaria saccharina (Cancino 1986). This type of aggregation in bryozoans has previously been interpreted as a means of minimising the chance of selfing (Ryland 1973, Cancino et al 1991). If such be the case, then it would be expected that C. hyalina is routinely outbreeding in natural populations.

The present study was designed to investigate the possibility of self-fertilisation in isolated colonies of C. hyalina. A previous study of isolated colonies suggested that C. hyalina was indeed routinely outbreeding (Cancino 1983), but the colonies were not placed in isolation until sexual zooids had started to appear, and a very small sample size was used. In addition, it is possible that the colonies experienced malnutrition, on account of the experimental method employed.

5.2 Materials and methods

The colonies of Celleporella hyalina used in the present experiment were derived from two sources, both of which are assumed to have been produced sexually. The first set of nine colonies was founded from a laboratory population which was originally settled from naturally occurring colonies in the Menai Straits in October 1989. The second set of twelve experimental colonies were founded by larvae settled from colonies taken directly from the Menai Straits in July 1990.

Larvae were settled by standard means (see chapter 1), and were allowed to metamorphose on glass microscope slides in a plastic culture vessel, containing 0.2 μm filtered, U.V.-irradiated seawater, to which the algal food Rhodomonas baltica had been added at a concentration of 100 cells.μl^{-1}. This allowed the primary zooids to commence feeding immediately on completion of metamorphosis (see chapter 1).

Transition to a fully operational state becomes conspicuous when the gut of the primary zooid fills with Rhodomonas. At this stage, well-spaced primary zooids were selected to generate experimental colonies. The glass was cut with a hand-held, diamond-pointed cutter into approximately 1.5cm * 1.5cm pieces, with each primary zooid being located centrally. These pieces were then placed into glass vials containing Rhodomonas baltica at 100 cells.μl^{-1}. The vials were sealed and mounted vertically on a rotating cylinder. The cylinder was placed in a constant-temperature room at 15°C under continual illumination. Rotation of the apparatus prevented the algae from settling and maintained circulation

around the colonies. In this way, the colonies stayed very clean, and rarely required brushing (see chapter 1).

Preliminary experiments showed that colonies would grow normally with only one change of algal medium per week, for a period of up to five weeks. Thereafter, depending on colony size, signs of retarded growth sometimes started to appear, suggesting inadequate food supply. It was decided therefore, that the algal medium should be changed three times weekly from the fifth week onwards, in order to maintain a relatively constant cell concentration.

Precautions had to be taken at all times in order to ensure that no contamination occurred between experimental colonies. When counting zooids at weekly intervals, therefore, culture vessels were handled as little as possible. Each colony was removed from its glass vial using a pair of forceps which had been passed through a flame, and placed in a sterilised petri-dish for counting under the dissection microscope. Only one colony was ever removed from its vial at any one time, and all surfaces were cleaned with alcohol before and after each colony was counted. Particular care was taken not to touch the glass chips or the actual colonies manually, and all switches and dials on the microscope were cleaned carefully after use.

Each week, feeding autozooids, brown bodies, fully formed buds, frontal autozooids, basal males, frontal males, females and embryos were counted, and each colony was drawn with the camera lucida. Area and perimeter were calculated electronically from the drawings, using a digitiser.

Both sets of colonies were grown in isolation for a period of ten weeks, after which they were split into two groups. The first group was removed from isolation and grown in 'common garden' conditions (Grosberg 1988), for a further eighteen days, with counts of sexual zooids and embryos taken every three days. The same schedule was applied to the second group, but in this case, isolation was maintained for a further nine days to serve as a control.

5.3 Results

Both sets of colonies grew rapidly, maintaining a compact, circular form, with a low perimeter:area ratio (figure 5.1 a, b). Autozooids and sexual zooids were produced as normal (figure 5.2, figure 5.3), although the production of frontal male and female zooids often decreased once the colonies were taken out of isolation. The removal from isolation was also generally accompanied by a deceleration (a virtual cessation in some cases) of colony growth. Whether this occurred as a result of a switch of investment into the nutrition of newly outcrossed progeny is unclear, as the deceleration in growth started before colonies were taken out of isolation in several cases (figure 5.1).

Embryos appeared as early as the fifth week in colonies three and four of the first series (table 5.1). The other colonies in this series started to produce embryos on the sixth week of the experiment, with the exception of colony five, which did not produce any embryos until week eight, and colony eight, which produced no larvae during the period of isolation.

The highest percentage of ovicells containing larvae was attained by colony number one on the seventh week of the experiment (table 5.1), but the figure subsequently fell from 38% to 33% by the end of the period of isolation. At the end of the tenth week in isolation, only 9% of ovicells were occupied, compared with 60% at the end of the experiment, after colonies had been grouped together. This was not a general, temporal increase in the number of embryos, since

brooding activity remained low in the control colonies during the nine days of prolonged isolation (figure 5.4).

No embryos were produced until week five by colonies one and two in the second series (table 5.2). The remaining colonies started producing embryos between weeks six and eight, with the exception of colony number seven, which never produced any larvae under conditions of isolation. Relatively fewer females were produced by the second series, and although the percentage of ovicells containing larvae under conditions of isolation sometimes appears high, this was usually due to a high level of occupation in very small numbers of ovicells. For example, the one hundred percent occupation rate in colony three from weeks eight to ten results from two ovicells containing two embryos.

In this second series, the percentage of occupied ovicells rose from 28% after ten weeks in isolation to 56% at the end of the experiment in common garden. This is in accordance with the first series. Again, the lack of increased larval output from the control colonies until placed in common garden, shows that the increase in larval output was not a temporal phenomenon (figure 5.5).

A very high incidence of abortion appeared to occur across both series when in isolation. This was particularly conspicuous in colonies containing large numbers of females and very low numbers of embryos. Although the number of embryos often appeared to be constant from week to week, the ovicells that contained the embryos were often different in identity. Since these embryos could only have been brooded for a maximum of thirteen days, as opposed to the three weeks

normally required for successful brooding, it was assumed that they were aborted.

No settlement was ever detected from any of the isolated colonies, but this may simply have been due to the fact that the glass vials containing the isolated colonies were difficult to inspect, especially when trying to avoid the possibility of contamination. If viable larvae had been produced, however, some degree of settlement would have been expected on the glass chips, on which the parental colonies were growing.

5.4 Discussion

The production of larvae by colonies grown in isolation could be accounted for by three possible explanations namely, sperm storage, parthenogenesis or self-fertilisation. If it is assumed that no contamination occurred between colonies during the experimental procedure, then it is most unlikely that the larvae were produced as a result of outcrossing, unless precocious sperm storage were to occur, since colonies were isolated as primary zooids. For this process to operate, sperm would have to enter the primary zooid, presumably via the feeding lophophore (D.J.Hughes 1987) and remain in storage for periods sometimes in excess of seven weeks, passing through subsequent zooid generations in order to fertilise the ova when eventually produced. Although sperm storage occurs in many hermaphroditic invertebrates (eg Ghiselin 1969, Clark 1981) with highly efficient utilisation of the stored sperm (eg Parker 1970, Ward and Carrel 1979), this is usually restricted to those groups in which sperm are transferred by copulation. Records of sperm storage are rare among aquatic animals, however, and the sperm stored may originate from many sources, since transmission is generally not via copulation. Exogenous sperm storage has been reported, however, in the hermaphroditic, spirorbid polychaete Spirorbis spirorbis (L.) (Daly and Golding 1977, Picard 1980), and by females of the gonochoristic, sabellid polychaete Fabricia sabella (Ehrenberg) (Kahmann 1984). More recently, Bishop and Ryland (1991) reported exogenous sperm storage in the compound ascidian Diplosoma listerianum. Sperm storage, possibly

facilitated by sperm chemotaxis (Miller 1982), would allow sperm from a variety of sources, and possibly in low external concentrations, to accumulate over a period of time. This would remove any need for synchronisation of male and female production to ensure efficient egg fertilisation, and possibly would also increase the genetic diversity of the progeny (Bishop and Ryland 1991). Precocious insemination has certainly been recorded in the Bryozoa on several occasions (Marcus 1938, 1941, Correa 1948, Dyrynda 1981a, Dyrynda and King 1982). Marcus (1938), found sperm in association with ovocytes 200-300 times smaller than the fully mature ovocyte.

Parthenogenesis is widespread among invertebrate taxa (Bell 1982, Hughes and Cancino 1985), but in bryozoans has only been reported as likely to occur in Crisia (Robertson 1903). However, absence of data can not be taken as evidence of the lack of parthenogenesis in the Bryozoa. It should also be noted that even in extensively studied organisms (eg slugs), what was first thought to be self-fertilisation eventually proved to be apomictic parthenogenesis (Nicklas and Hoffman 1981).

Autogamy is normally suppressed in most hermaphroditic animals where outcrossing is possible (Williams 1975, Heath 1977, Maynard-Smith 1978). Selfing, nevertheless, is still a common phenomenon in the invertebrates (eg Clark 1978, Bell 1982 for reviews). Amongst the Bryozoa, self-fertilisation seems probable among the Phylactolaemata (Braem 1897, Marcus 1934 cited in Silén 1972, Clark 1981, Bell 1982), and has been suggested as probable in the Cheilostome Epistomia bursaria (Dyrynda and King 1982). In a recent study, Maturo

(1991), reported a wide variety of bryozoans as capable of producing viable larvae in isolation.

If it can be assumed that sperm storage and parthenogenesis do not take place, and that no contamination occurred during the experimental procedure, then the current study verifies Cancino's (1983) observations that self-fertilisation is possible in Celleporella hyalina. However, the low number of embryos produced, the high incidence of abortion, and the rapid increase in larval output when returned to a 'common-garden' environment all suggest that this species is routinely outbreeding, with self fertilisation possibly available as an emergency option. Retention of the male lophophore, despite its apparent infrequency of emergence, would further indicate the importance of gamete dispersal to C. hyalina.

In a recent study by Cancino et al (1991), it was reported that although colonies of C. hyalina produced male zooids after twelve weeks in isolation, female zooids were never produced, even after a period of sixteen months, whereas non-isolated colonies produced females and viable larvae. This case of non-simultaneous hermaphroditism was also found to be true of Membranipora isabelleana. The different levels of selfing observed between individual colonies in the current study, may to some extent reflect differences between the reproductive behaviour of various genetic individuals (Ryland and Bishop 1990, this thesis chapter 3). The results from Cancino et al's (1991) study may suggest geographic variation in reproductive strategy in C. hyalina. There also remains the possibility, however, that

the few colonies which did produce a relatively large number of embryos under conditions of isolation, may have been the result of outcrossing due to contamination. Genetic differences may also be important with respect to resource allocation, with those genotypes favouring greater investment into somatic growth investing less in the production of sexual zooids and in the nourishment of autogamous offspring. Since no settlement of the larvae produced in isolation was ever observed, it may be the case that the larvae produced by autogamy are of reduced fitness, as a result of inbreeding depression (Charlesworth and Charlesworth 1987).

The relatively extensive growth and compact form of most of the isolated colonies may simply reflect a greater proportional investment into somatic growth than would normally be found under natural conditions, in the absence of any opportunity for outcrossing. The onset of sexual reproduction in Membranipora membranacea has been found to be closely associated with extrinsic limitations for further growth and survival (Harvell and Grosberg 1988). Cancino et al (1991) found arrested oocyte development in some bryozoans, where full oocyte maturation could be triggered by the presence in the same container of other genetic individuals, despite no physical contact between the different clones. A similar phenomenon has been reported in clones of the colonial ascidian Diplosoma listerianum (Ryland and Bishop 1990), when kept in reproductive isolation. Such observations suggest that fertilisation in these organisms may sometimes be mediated by allorecognition mechanisms (eg Grosberg and Quinn 1986).

Table 5.1

Percentage of ovicells containing larvae, series 1. The horizontal lines demarcate the switch from isolation to communal location.

		Colony no.								
		1	2	3	4	5	6	7	8	9
Day	21	*	*	*	*	*	*	*	*	*
	28	*	0	0	*	*	*	0	*	*
	35	0	0	3	18	*	*	0	0	0
	42	9	11	3	25	*	3	7	0	0
	49	38	3	5	13	0	2	10	0	0
	56	32	3	3	14	11	2	6	0	1
	63	35	8	2	18	12	3	10	0	1
	70	33	2	2	16	17	2	12	0	1
	73	36	6	6	18	17	2	9	0	1
	76	41	28	19	43	45	2	7	0	2
	79	75	56	46	58	76	1	11	0	2
	82	80	64	59	72	71	1	11	0	3
	85	82	64	61	44	54	23	13	2	28
	88	74	65	63	75	57	66	44	12	86

Table 5.2

Percentage of ovicells containing larvae, series 2. The horizontal lines demarcate the switch from isolation to communal location.

		Colony no.											
		1	2	3	4	5	6	7	8	9	10	11	12
Day	28	*	*	*	*	*	*	*	*	*	*	*	*
	35	12	3	*	*	0	0	*	0	*	0	*	0
	42	90	1	*	2	0	43	*	0	0	83	*	0
	49	68	0	*	35	0	82	0	0	19	45	0	0
	56	44	3	100	56	2	0	0	2	18	68	8	2
	63	39	0	100	69	5	82	0	1	18	45	17	6
	70	41	0	100	69	3	54	0	0	12	46	6	1
	73	43	0	50	77	5	64	0	0	13	42	0	1
	76	49	2	67	80	20	72	0	1	13	36	6	3
	79	74	25	50	76	58	65	0	1	14	39	10	3
	82	76	35	50	78	54	77	2	2	18	46	23	8
	85	82	58	50	89	58	72	8	7	35	53	33	3
	88	84	72	50	88	58	75	42	32	52	48	50	23

Figure 5.1

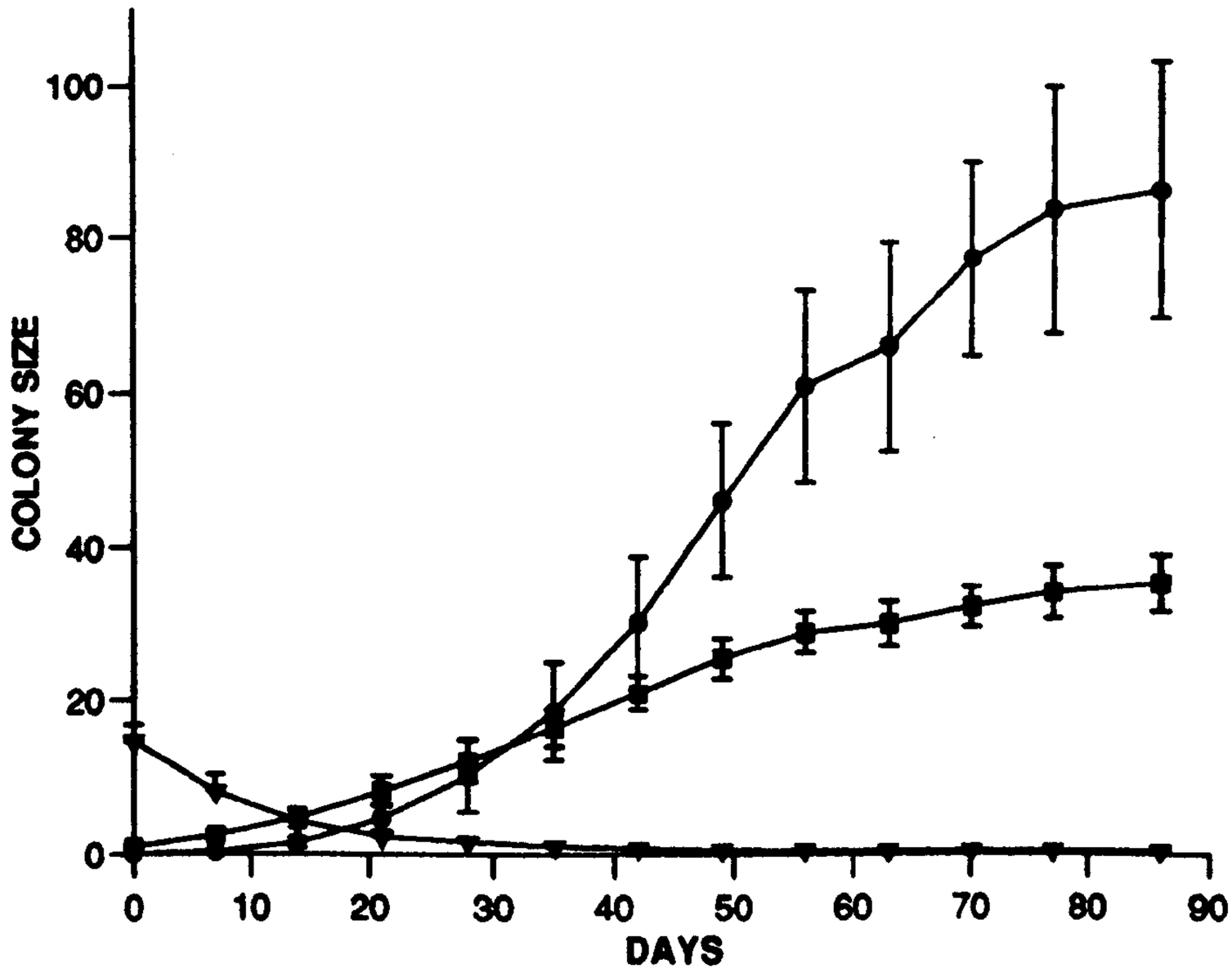
a) Mean area, perimeter and the perimeter:area ratio, series 1, a set of nine colonies derived from a laboratory grown population, originally founded from naturally occurring colonies, October 1989. Data points are mean values ±95% confidence limits.

Area (circles), perimeter (boxes) and perimeter:area ratio (triangles).

b) Mean area, perimeter and the perimeter:area ratio, series 2, a set of twelve colonies founded from larvae taken from colonies growing in the Menai Straits, July 1990. Data points are mean values ±95% confidence limits.

Area (circles), perimeter (boxes) and perimeter:area ratio (boxes).

a


COLONY SIZE
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
DAYS

b

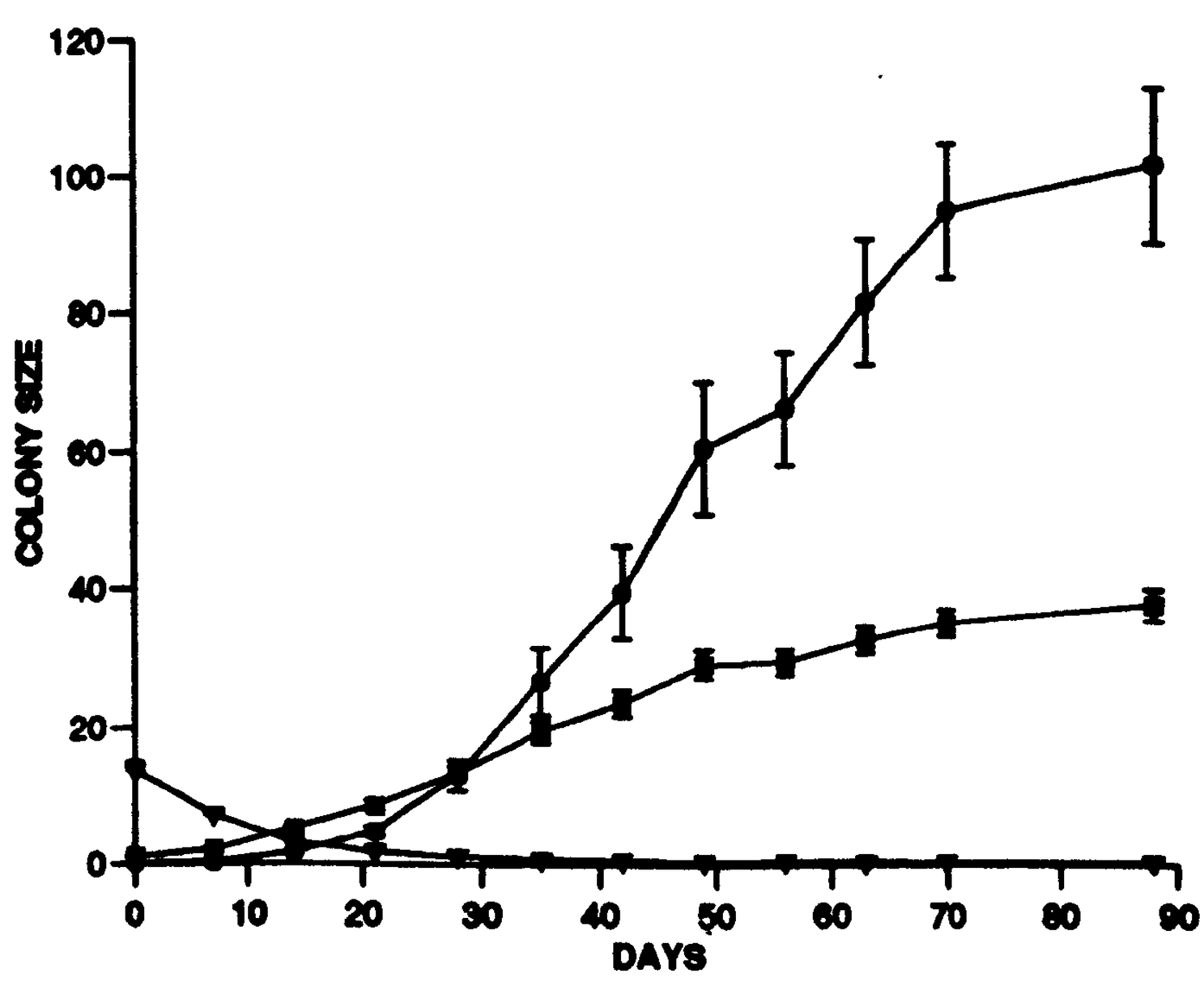

COLONY SIZE
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
DAYS

Figure 5.2

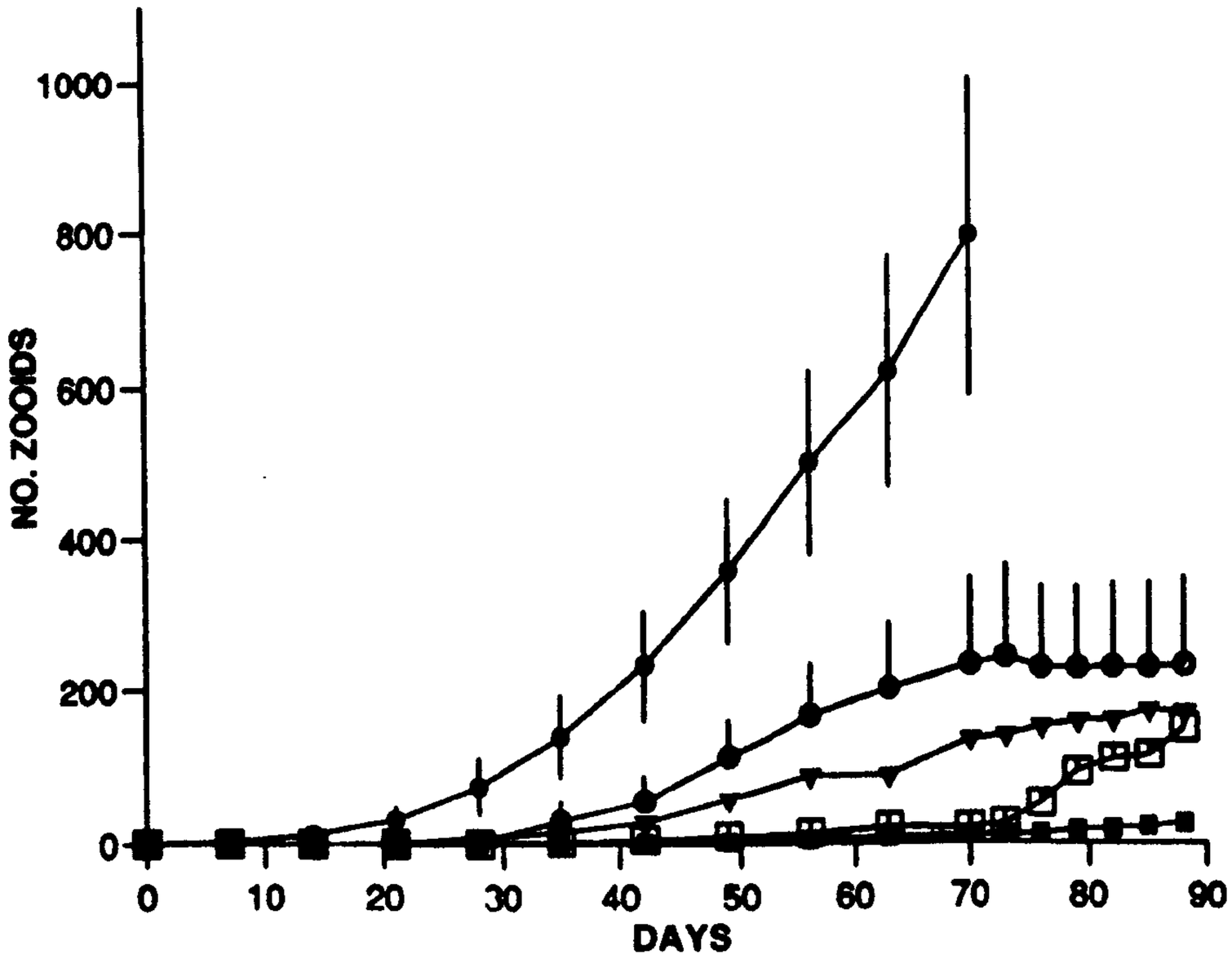
Colony growth series 1, a set of nine colonies derived from a laboratory population originally founded from naturally occurring colonies, October 1989. Data points are mean values ±95% confidence limits.

Autozooids (solid circles), basal males (solid boxes), frontal males (solid triangles), females (open circles), and embryos (open boxes).

Figure 5.3

Colony growth series 2, a set of 12 colonies founded from larvae taken from colonies growing in the Menai Straits, July 1990. Data points are mean values ±95% confidence limits.

Autozooids (solid triangles), basal males (boxes),frontal males (circles), females (diamonds), and embryos (open triangles).


1000
800
600
400
200
0
NO. ZOOIDS
0
10
20
30
40
50
60
70
80
90
DAYS

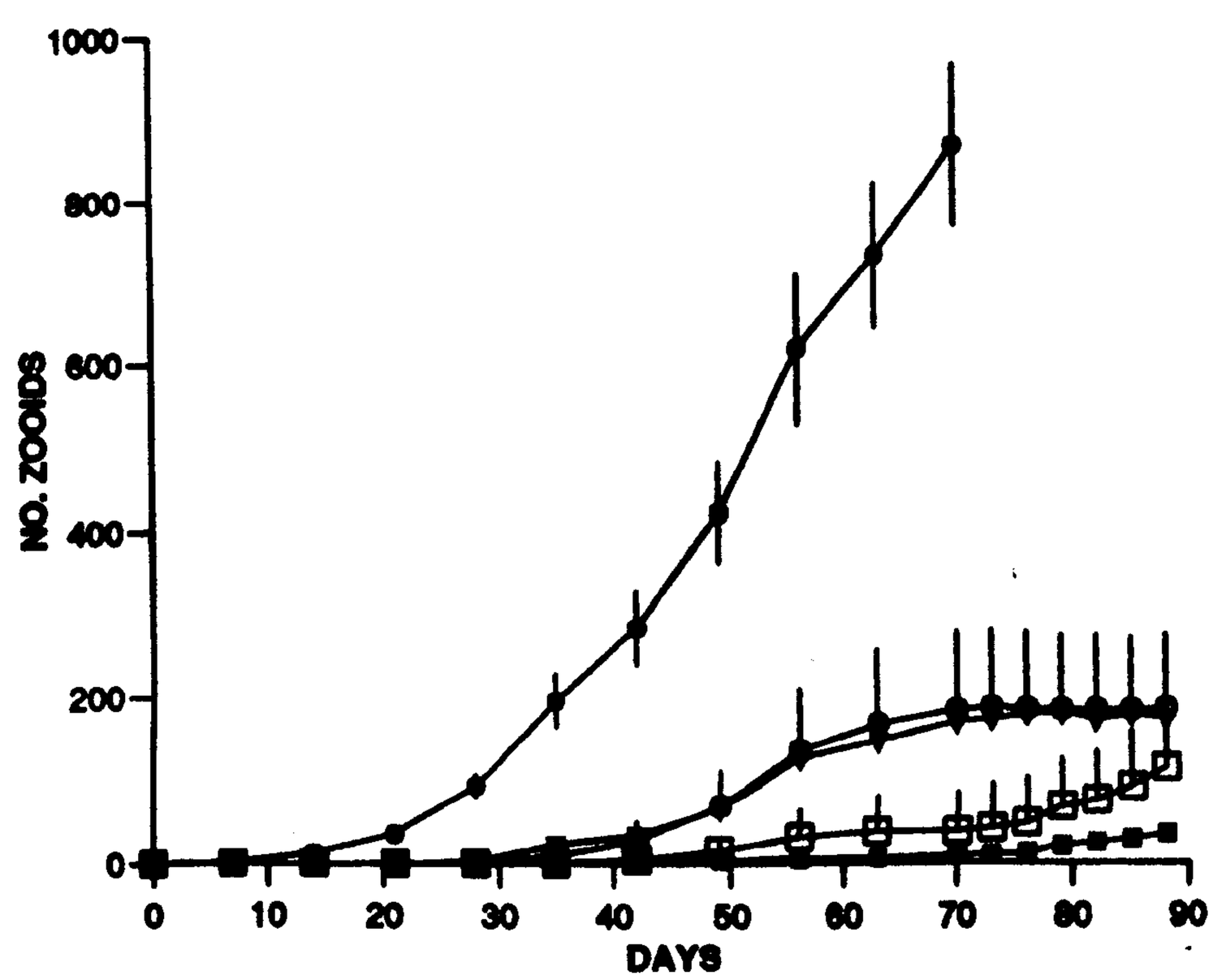

1000
800
600
400
200
0
NO. ZOOIDS
0
10
20
30
40
50
60
70
80
90
DAYS

Figure 5.4

Mean number of ovicells (solid lines), plotted against the mean percentage of ovicells containing larvae (broken lines), series 1.

Group 1 was isolated for 70 days (triangle), while group 2 was isolated for 79 days (box).The overall means are also given (circles).

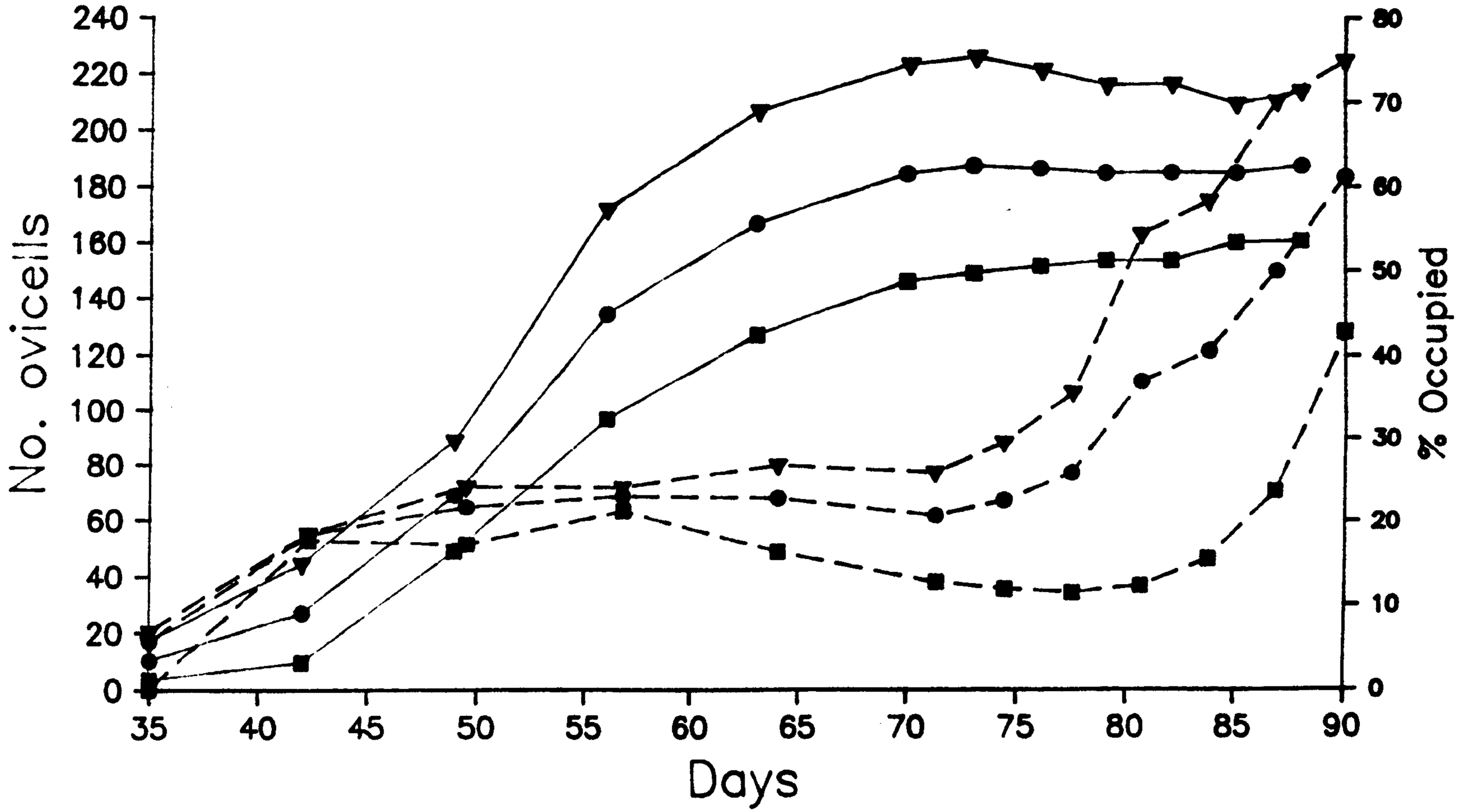
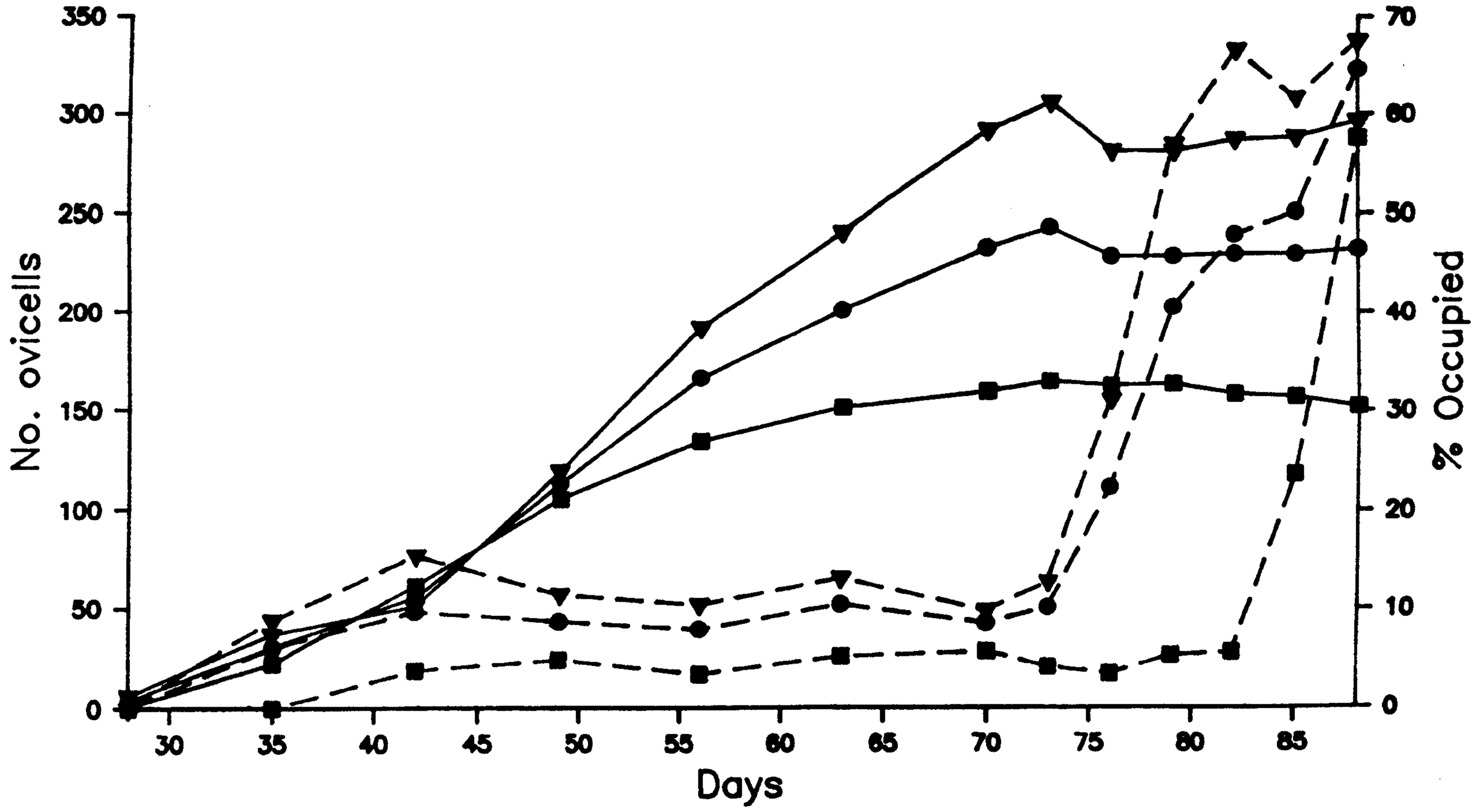

No. ovicells
% Occupied
Days
0
20
40
60
80
100
120
140
160
180
200
220
240
0
10
20
30
40
50
60
70
80
35
40
45
50
55
60
65
70
75
80
85
90

Figure 5.5

Mean number of ovicells (solid lines), plotted against the mean percentage of ovicells containing larvae (broken lines).

The first group (triangles) were taken out of isolation after 70 days, while the second group remained in isolation for 79 days (boxes). The overall means are also plotted (circles).


No. ovicells
% Occupied
Days
350
300
250
200
150
100
50
0
70
60
50
40
30
20
10
0
30
35
40
45
50
55
60
65
70
75
80
85

General discussion

Laboratory studies of growth and reproduction in the cheilostome bryozoan Celleporella hyalina (L.) were able to elucidate and confirm aspects of life-history patterns previously reported for this species from studies carried out in the natural environment.

Rhodomonas baltica proved to be outstanding as a diet for C. hyalina, and colonies were maintained in the laboratory during the entire experimental period without showing any signs of malnutrition. This also appeared to be the first reported case of laboratory culture of a marine bryozoan resulting in the production of viable larvae. The culture method (chapter 1), therefore, should allow great flexibility in future studies on the growth and reproduction of C.hyalina. Moreover, this method also proved successful with a number of other encrusting bryozoans. It may, therefore, prove particularly useful in the study of factors determining the onset of reproduction in such species, some of which are already known to demonstrate environmentally induced gametogenesis (D.J.Hughes 1986, Harvell and Grosberg 1988).

C. hyalina was found capable of growing and reproducing in cell concentrations ranging from 10 to 300 cells.μl^{-1} (chapter 2). As has been demonstrated in other bryozoan species (Best and Thorpe 1983, 1986a), feeding in C. hyalina appears to be an active process, rather than the passive filtration of seawater, with higher cell concentrations stimulating a higher filtration rate. High perimeter to area ratios in colonies grown on diets of low nutritional adequacy (chapter 1), and at exceptionally high or low cell densities

(chapter 2), corresponds with previous findings that colony growth tends to be lobate in colonies settled during the winter months, becoming circular during the spring and summer (Cancino and Hughes 1988). These findings from laboratory-grown colonies suggest that food supply may indeed be a limiting factor in marine bryozoan communities, during certain times of year. Similarly, increased allocation to male function during the winter months (Cancino and Hughes 1987, 1988), in sea-grown colonies, corresponds to the current finding that basal male zooids were the favoured sexual zooid type under conditions of low resource (chapters 1-3).

Inter-colonial variation in growth parameters was a consistent feature revealed by experiments investigating the effects of diet on colony growth (chapters 1 and 2). The replication of individual genotypes and comparison of their growth in controlled 'macroenvironments' (chapter 3), confirmed previous reports of genetically based variation in life-history parameters in C. hyalina (D.J.Hughes 1989). An incidental, but important, finding from this study was that zooid size in C. hyalina cannot be assumed to be invariant, but is strongly temperature-dependent and, moreover, shows a considerable degree of inter- (chapter 3) and intra-clonal (chapter 4) variation.

The high level of genotype-environment interaction and the surprising degree of inconsistency in the ranking of clonal performance between experimental macroenvironments (chapter 3), suggests that fine scale niche-partitioning may be possible between colonies at a highly localised scale.

Furthermore, the finding that ranking of clonal performance also varied according to the measure of performance used, suggests that reproductive success as a male or a female may be environmentally determined. This would provide a genetic basis for selection acting on variation in life-history traits, resulting in sexual selection on very fine spatial and temporal scales. The serial propagation of a single genotype (chapter 4) further revealed that a considerable degree of variation was possible within a single clone in apparently identical macroenvironments. Although the presence of one statistical outlyer hinted that somaclonal mutations do sometimes occur, it was concluded that most intraclonal variation of this type arises as a result of developmental differences, possibly depending on the maturity of meristematic tissues in the initial propagules.

The demonstration of fine-scale niche-partitioning in C. hyalina provides further evidence that this may be one of the fundamental aspects underlying the maintenance of sexual reproduction in this and all other sexual species (Bell 1982, 1987). Although it was found that C. hyalina may be capable of a limited amount of self-fertilisation (chapter 5), the low numbers of selfed embryos produced suggest that selfing is used only as an emergency option (Bishop and Ryland 1990). Moreover, the high rates of abortion and the lack of evidence of the settlement of larvae produced by selfing, suggest that such larvae are of reduced fitness, possibly as a result of inbreeding depression (Charlesworth and Charlesworth 1987).

It is suggested that future studies of C. hyalina should involve formal, biochemical-genetic analysis. This may

involve the serial propagation and genetic fingerprinting of laboratory grown colonies, in order to ascertain whether embryos produced in isolation are in fact the result of outcrossing, and also, the determination of the degree of relatedness in natural populations. Such studies may be able to discern fine-scale population differences in exploitation of the natural environment, possibly associated with the quality and durability of substratum occupied, and therefore, the probable colonial longevity (Cancino 1986). Differences in reproductive strategy have recently been demonstrated on a wide geographic scale, with distinct differences in the responses of isolated colonies of C. hyalina in the U.K. (chapter 5), and Chile (Cancino et al 1991).

Future work on the relative performance of clones in any species should aim to maximise the number of genotypic replicates used per treatment, so allowing for the extensive range of intraclonal variation possible even within a controlled environment (D.J.Hughes 1991, in press, chapter 4, this thesis). The serial propagation of 'mutants' may also shed more information on whether somatic mutation ever has a considerable role to play in the evolutionary biology of colonial animals, comparable with that which has been demonstrated in long-lived plant species (Antolin and Strobeck 1985).

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