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DEPARTMENT OF CONSERVATION, FORESTS AND LANDS
FISHERIES DIVISION

CULTURE OF AUSTRALIAN FLAT OYSTERS *OSTREA ANGASI* IN VICTORIA: FINAL REPORT (FISHING INDUSTRY RESEARCH TRUST ACCOUNT 84/77)

N.J. Hickman and C.M. O'Meley

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Internal Report Number 172
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MARINE SCIENCE LABORATORIES

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Marine Science Laboratories
Queenscliff, Victoria 3225
Australia

FIRTA 84/77

CULTURE OF AUSTRALIAN FLAT OYSTERS OSTREA ANGASI IN VICTORIA

PROGRAM AIM

To develop a culture industry for the native flat oyster (*Ostrea angasi*) in Victoria.

PROGRAM OBJECTIVES

1. To determine the best method of obtaining a regular supply of oyster spat.
2. To determine the best methods and areas for on-growing native oysters for human consumption.

SUMMARY OF PROGRAM

In initial investigations the natural reproductive cycle and larval settlement of *O. angasi* in Port Phillip Bay were studied. Gonad development occurred from May to July and overlapped with maturation which extended from May to February. *O. angasi* brooded larvae from October to December and were spent during summer (December to February) when the main larval settlement occurred. The larvae settled on hard substrates not already colonised by other organisms. Attempts to catch commercial quantities of oysters in the wild were unsuccessful because the oysters became too overgrown with marine fouling organisms. A trial to settle oysters in a marine "spatting pond" was also unsuccessful because important variables such as temperature, salinity and phytoplankton species could not be controlled adequately. However, we did develop a pilot-scale hatchery and procedures which will ensure that sufficient *O. angasi* seed will be available during the early development of a mariculture industry. Innovative procedures which improve hatchery production are: feeding larvae and young spat only with actively growing (log phase) algae; fluorometric determination of feeding rates; routine growth measurements and inclusion of the diatom *Chaetoceros gracilis* in the diet of spat.

The growth patterns of *O. angasi* were typical of the *Ostrea* species, namely, growth rates high in summer and low in winter. The explanation for this seasonal pattern is that growth is controlled by temperature rather than by food availability. In Port Phillip Bay, the oysters' condition was good during April - October, but was best during the four months May - August. These months make up exactly the same seasons that *O. edulis* is in best condition in the Northern Hemisphere. However, our analysis of several years data has shown considerable annual variability in the levels of particulate food for oysters; the months when oysters are fat; and the fatness of oysters at different sites. This variability is likely to have a marked effect on the number of months during which top quality oysters can be produced each year.

In Port Phillip Bay, *O. angasi* grew equally well in mid-water and bottom racks when sub-surface floatation was used, but were stunted when grown in bags suspended on surface long-lines in exposed waters. A preliminary study showed that location had a marked effect on growth rate. The husbandry technique which most improved the oysters' growth at sea was density reduction. Density

was found to be more important than grading of oysters by size in promoting faster growth.

Holding oyster seed in land-based nursery upwellers for several months did not affect the oysters' subsequent growth at sea, whereas planting out oyster seed in early summer shortened their time to maturity to less than two years at the latitude of Port Phillip Bay (38°S 145°E). When we selected the fastest growing siblings in the nursery, the growth rate of the resulting adult oysters was no better than that of the slowest growing siblings in the nursery.

This program has demonstrated that hatchery production and simple animal husbandry are capable of dramatically improving the growth performance of *O. angasi*. In our opinion these procedures will be much more useful to oyster farmers than any advances likely to result from selective breeding programs designed to enhance growth rates.

INTRODUCTION AND JUSTIFICATION

The Victorian Department of Conservation Forests and Lands in its Corporate Strategy Statement gives priority to the development of mariculture to provide new fishery opportunities. Evaluation of the biological performance of a species in the location where the proposed development is to occur is essential. This strategy has already led to the establishment of a mussel culture industry in Victoria. The same strategy is being applied to the culture of the Australian flat oyster (*Ostrea angasi*). This species was chosen because it is a marine oyster and Victoria has many large marine embayments. Furthermore Victoria is in the middle of the species' normal geographical range, so that environmental variables such as temperature are unlikely to be a problem. The widespread distribution of *O. angasi* throughout southern Australia could also make the findings of this study applicable in other States. Mariculture of the flat oyster in several States is likely to lengthen the period when oysters are in peak condition, and so benefit both domestic and overseas marketing.

The creation of new fisheries opportunities will become more important as some wild fisheries decline. In Victoria, the knowledge and the experience of fishermen have made them the most successful of the new mussel farmers. Therefore fishermen should regard mariculture as a possible diversification of their activities and not as a threat to their livelihood.

RESULTS AND DISCUSSION

TASKS DESIGNED TO DETERMINE THE BEST METHOD OF OBTAINING A REGULAR SUPPLY OF OYSTER SPAT

Reproduction and Natural Settlement

Because *O. angasi* is genetically very similar to *O. edulis* (P. Dixon pers comm 1985) we reviewed the literature on reproduction in *O. edulis*. We found many anomalies because the sexuality of *O. edulis* has been categorised in many ways (Orton, 1928; Cole, 1942; Loosanoff 1962; Millar, 1964; Perusko, 1967; Wilson and Simons 1985). The difficulty in understanding the different reproductive categories can be illustrated by summarising the early work. Orton (1928) assigned eight categories: hermaphrodite, hermaphrodite female, female with trace of male, male with trace of female, female like male, pure female and

"other" categories. Cole (1942) assigned male, female, transitional and hermaphrodite. Loosanoff (1962) assigned strongly ambisexual categories, predominantly male and predominantly female whereas Wilson and Simons (1985) assigned only male and female categories. Millar (1964) stated the problem succinctly: "As male and female germ cells are often present in the same follicles at the same time, the application of the terms male-phase and female-phase depends on the definition of these terms. Therefore an oyster is said to be in the male phase if at the next release of gametes, these will be spermatozoa, and in the female phase if they will be eggs".

Our histological study of the reproductive cycle of *O. angasi* in Port Phillip Bay (Figure 1) has determined the timing of the main stages of gametogenesis (Figure 2). Oysters were spent during summer and early autumn (December-April), and their gonads developed throughout autumn (May-July) and overlapped with maturation which extended from May to February. The reproductive stages in *O. angasi* are indistinguishable from those of the European oyster *O. edulis* reported by Cole (1942) and Loosanoff (1962).

Differences in reproductive timing could have a large impact on hatchery operation in different States. These differences affect the time needed to condition oysters and the time when hatchery operations could begin. It is likely that hatchery operations in Victoria and South Australia could begin before those in Tasmania, but not as early as those in NSW and Western Australia. The advantages of seed being produced before summer is discussed in the following sections on hatchery production and on-growing.

O. angasi in Port Phillip Bay were brooding larvae from October 1985 to February 1986 (Figure 3). The main brooding period was October and November and the main larval settlement occurred during November and December at Dromana and during December and January in the Geelong Arm. In South Australia, O'Sullivan (1980) showed that *O. angasi* brooded larvae for a longer period (October-March) but did not determine when settlement occurred. In Tasmania Dix (1976) believed the reproductive season was slightly later (December-April) although no seasonal sampling was carried out. It is therefore difficult to compare results in S.E. Australia as reproduction, brooding and settlement patterns were not carried out simultaneously in other studies.

When commercial collectors of the French hat type and PVC plates were deployed in the Geelong Arm during the settlement period, a settlement of 600 oysters per collector was achieved. These oysters became extremely fouled and had very thin shells on the side of the settlement surface. Removal of oysters was time consuming and most were damaged in the process. It is difficult to envisage this method being suitable for mariculture of flat oysters. Prospective farmers have been advised on collecting methods so that they can attempt to refine the techniques if they wish. (N.B. Many successful oyster industries rely on natural settlements).

"Natural" settlement in "spatting ponds" was investigated in a large (100 m x 5 m x 2 m) marine pond in which an algal bloom was created by fertilisation with 25 kg ammonium nitrate, 25 kg of single superphosphate and 5 g of sodium molybdate per 0.1 ha. In December 1984 two hundred *O. angasi* were placed in the pond on four trays spaced at 25 m intervals. Two weeks later two hundred 50 cm square PVC spat collectors were also placed in the pond, and were monitored every two weeks for larval settlement. The procedure was repeated in January 1985 with fewer oysters and spat collectors.

No larvae settled and work was abandoned. Our failure was probably due to inadequate control of important variables such as temperature, salinity and

phytoplankton species. Nevertheless, the method has been used to produce *O. angasi* in Tasmania and the "Spatting Pond" system has been very successful in Ireland (D.A. Ashe, Oyster Project Manager, Taighde Mara, Galway - personal communication).

Hatchery Production

The Marine Science Laboratories (MSL) has ready access to clean oceanic sea-water by virtue of its location at Queenscliff (38°16'S, 144°39'E) at the entrance to Port Phillip Bay in southeastern Australia (Figure 1). The size of the Bay (almost 2000 km^2) ensures that tidal exchanges make large volumes of high salinity sea-water continuously available. A good choice of site is known to be the most important factor in securing the best quality water for hatchery operations (Holliday 1985).

Sea-water is pumped, by means of two "Mono" pumps (9.3 L/second) from the end of a 200 m long jetty to two 100,000 L enclosed concrete settling tanks. From these tanks, water is pumped through one of two "Permutit" automatic back-flushing sand filters (nominally 30 um) into a 470 m^2 aquarium building. The building is air-conditioned and is supplied with oil-free compressed air. An overall plan of the building and nursery is shown in Figure 4.

The hatchery and nursery are designed around six basic systems: algal culture; sea-water temperature control; broodstock conditioning; larval rearing; larval settlement; and spat rearing. Hatchery structure and operating methods are described in a separate technical report (Hickman and O'Meley 1988). Innovative procedures which improve production will be summarised here.

Only actively growing (log phase) algal cells grown in 200 L bags are used to feed larvae and newly settled spat. At low cell densities (e.g.< 1 million T.ISO cells per mL) cell numbers can be estimated quickly by measuring light transmission through the culture (nephelometry). At high cell densities, or if accuracy is required, a haemocytometer is used for microscopic cell counts.

OUR USE OF ONLY RAPIDLY GROWING ALGAE IS PROBABLY ONE OF THE MAIN REASONS WHY BACTERIAL PROBLEMS HAVE NOT OCCURRED IN OUR HATCHERY. Such problems have plagued hatcheries in Australia (Garland et al. 1983) and overseas (Elston et al. 1981, Lodeiros et al. 1987), causing losses in spat production. Recently (Bratbak 1987) showed that soluble exudates excreted from algae when they stop growing (i.e. stationary phase) are the main substrates for bacterial growth. Similarly, we have found large quantities of exudates in our 200 L bags which contained cells that had stopped growing. Therefore the daily growth of algae in each bag is recorded on a data sheet attached to the bag. Recording the growth rate is important for shellfish hatcheries to prevent bacterial problems from occurring when algal growth slows down. Traditionally, enormous efforts are made to remove or exclude bacteria from the algae, but such a task is impossible.

To maintain the growth of algal cultures in bags, we regularly remove half the algae and refill the bags with 0.2 um filtered sea-water and sterile F/2 media.

Two other important factors minimise bacterial contamination: our use of "oceanic" type water of high salinity and low turbidity; and operating the hatchery when sea-water temperatures (and probably bacterial numbers) are low.

The food consumption of larvae and spat is measured by fluorometry, which is a common method of quantifying phytoplankton in sea-water by measuring the fluorescence of the green chlorophyll pigments (Parsons et al. 1984). The use of

a fluorometer ("Turner Designs" flow-through model) to monitor food consumption has become an indispensable aid in our hatchery. Not only can the larvae be given the correct ration but constant monitoring of food consumption is an extremely effective method of determining the "health" of larvae or spat. IF WE DETECT A DECREASE IN FOOD CONSUMPTION, WE IMMEDIATELY CHANGE ALL THE WATER AND INTRODUCE FOOD FROM A NEW SOURCE. In most hatcheries the larvae have stopped growing by the time trouble is suspected and remedial action is taken too late. We believe that the monitoring of food consumption has been a prime factor in our successful rearing of oyster larvae and spat.

An automated system is used to measure larvae and spat growth. Microscopic images of the larvae are projected through a camera-lucida eye-piece onto an electronic "bit-pad" which is linked to a micro-computer. A "mouse" fitted with a LED light sends measurements of the image directly into the computer. This system allows many measurements to be made quickly and the data can be processed immediately to give the mean and range of sizes of the animals. CAREFUL MONITORING OF GROWTH (OF BOTH LARVAE AND SPAT), ACCOMPANIED BY OPTIMUM FEEDING, GREATLY IMPROVES HUSBANDRY METHODS AND HATCHERY PRODUCTION.

Spat growth rates are highest if the diet consists of at least 30% of the diatom *Chaetoceros gracilis* (Hickman et al. 1987). This species of alga is known to contain essential long-chain polyunsaturated fatty acids (PUFAs) which are thought to stimulate spat growth in the European Oyster *O. edulis* (Enright et al. 1986, Laing and Millicen 1986). We believe that *C. gracilis* also improves the digestibility of algal food by providing siliceous spines which are the right size to assist grinding up food prior to digestion. We base this hypothesis on the frequent observations of less faecal material in spat upwellers when the diet contains a high proportion of *C. gracilis*.

If optimum feeding rates are maintained, spat grow at 1 mm per week in the nursery. Such feeding rates are readily maintained for spat up to 4 mm size but are progressivly more difficult (and expensive) to maintain for spat of 4 - 10 mm. Hatchery operations allow spat to be produced three to four months earlier than "wild" spat can be caught (Figure 3). The resulting oyster seed produced in the nursery are individuals ("cultchless"); consequently problems associated with natural settlement, namely, removing spat from substrates and over-growth, are solved. This is of considerable advantage to the oyster-grower in minimising farm labour costs.

TASKS DESIGNED TO DETERMINE THE BEST METHODS AND AREAS FOR ON-GROWING FLAT OYSTERS FOR HUMAN CONSUMPTION

Seasonality of Growth and Growing Methods

Studies of *O. angasi* grown in Victorian latitudes showed that the species grew much faster than the flat oyster *O. edulis* does in the Northern Hemisphere, where the species is cultured extensively. The seasonal pattern of growth for *O. angasi* (Figure 5) was very similar to that reported for *O. edulis* (Askew 1972, Walne and Mann 1975, Aguis et al 1978, Hall 1984, Wilson 1987). The "biological zero" of 11°C for shell growth of *O. edulis* reported by Wilson (1987) is very close to the temperature at which *O. angasi* also stops growing. There is strong evidence to suggest that temperature plays the main role in determining shell growth in different *Ostrea* species. Castro and Bodoy (1987) used a temperature model to explain growth patterns of *O. peulchana* in Argentina.

If temperature plays a dominant role in seasonal growth patterns it is not surprising that many bivalve studies have shown that shell growth and soft tissue growth may be uncoupled (Borrero and Hillbish 1988 and literature cited therein). Whilst food must be above a certain threshold to allow growth, we found that food had a much greater effect on meat yields. In summer, shell growth often occurred when oyster condition was in decline. Because food levels in the water were low and metabolic rate was high, such shell growth must have taken place at the expense of tissue growth. The factors that control shell growth in bivalves are poorly known (Borrero and Hillbish 1988), and Aquis et al (1978) speculated that differences in the rate of shell growth could be attributed to sea-water having different calcium concentrations.

Intertidal oyster culture beds in Victorian estuaries and embayments would be visually intrusive, but underwater floatation and bottom-mounted racks, which result in similar growth rates, would not be. Consequently there are many sites where deep water or sub-tidal culture could be developed. (Such developments will require growing technology which is the subject of a new joint venture project with industry). In many countries there are conflicts of interest during the development of mariculture in the inshore environment. Because of these conflicting interests, new growing methods are a prerequisite for oyster mariculture development.

Oysters grown in bags attached to surface long-line systems in exposed waters were damaged by wave action. However, mussel farmers have reduced the effect of wave action by decreasing buoyancy (R. McCowan - personal communication 1988). If this problem can be rectified, then deep-water areas suitable for mussel culture could also be used for oyster culture.

Husbandry Procedures

Summer shell growth could not be retarded by trimming the shells when they were growing most actively. The trimmed shell was immediately replaced. Therefore, even if the practice of "rumbling" oysters (which some growers practice) does slow shell growth, it probably does so by damaging the oyster in the process. There was also no evidence that reducing shell growth improved meat yields. Therefore it is unlikely that these husbandry methods can be used to slow shell growth in the hope of improving meat yields.

The growth of hatchery seed from two settlements (which grew at different rates in the nursery) was affected more by the time of planting out (Figure 6) than by the holding of the seed in upwellers for one or two months during summer (Figure 7), although the "oceanic" sea water did not sustain any shell growth during the holding period. This will have many advantages for oyster nurseries concerned with the quality of seed (which due to economics cannot be fed) which has to be held on flow- through sea water. It appears that once the seed has grown to 10 mm length then the seeds' quality can be maintained by holding the seed on a "maintenance" ration for several months at least. Even after six months holding in upwellers, seed which had grown at different rates in the nursery grew at the same rate when they were put to sea. But after seed had been held for longer than nine months, their mortality rates increased in relation to the time for which they had been held in the upwellers. We were unable to select in the nursery for faster growing *O. angasi* as Newkirk (1981) was unable to select at the larval stage for *O. edulis*.

The husbandry technique which improved growth rate the most on the oyster farm was density reduction (Figure 8). Although grading by size appeared to accelerate the growth of larger oysters (Figure 9), the gains in growth performance were not great when compared to the method of simply halving the

bag density. Therefore the variability in growth rate which is common in oysters is not greatly reduced by grading the oysters. Oyster farmers wishing to enhance their oysters' growth should concentrate on optimising bag density. Farm management may dictate that oysters be graded by size but growth rates are more likely to be improved by density reduction.

Production of hatchery seed for "planting out" in early summer can greatly improve returns to capital on the oyster farm by including two summers in a grow-out period of only eighteen months. If this is accompanied by the simple husbandry technique of density reduction then the oysters will reach commercial size when meat yields should be at their best (see following section).

Meat Condition

In Port Phillip Bay, flat oysters are in good condition from April to October, but are in best condition during May - August, the months corresponding to the season for best condition in *O. edulis* in the Northern Hemisphere. However, our analysis of several years data has shown that each year there were considerable differences in the levels of particulate oyster food; in the months when oysters are fat; and in the fatness at different sites. The variation in such factors is likely to have a marked effect on the number of months when top quality oysters can be produced each year.

Different growing treatments had the greatest effect on the oysters' condition during the summer months (Figure 10). If oysters are unable to completely stop summer shell growth even when they are in poor condition (often when food levels are low and metabolic costs high) then this could explain why summer mortality is often common in *Ostrea* species. In these circumstances post-spawning stress would also exacerbate the situation particularly in large oysters.

Heavy Metals in *O. angasi* from Port Phillip Bay

Analysis of six heavy metals in cultured oysters collected from two sites over 1.5 years showed that cadmium was a potential public health problem (Figure 11). Concentrations of other metals of possible concern (lead, copper, and zinc) were below the health limits on all occasions. *Ostrea* species are known to have high bio-accumulation factors for heavy metals so particular care must be taken in selecting suitable growing sites for this species. (N.B. Oysters in remote Shark Bay in W.A. have cadmium levels above the health limit, so this potential problem is not always associated with pollution inputs.)

EVALUATION OF THE PROGRAM

Hatchery production is the best method for ensuring a regular supply of *O. angasi* spat. By successfully designing and operating a pilot commercial hatchery, we have comprehensively met the first objective of the program. Our success has stimulated commercial interest in Victoria and Western Australia where there are presently no oyster culture industries.

The second objective "to determine the best methods and areas for on-growing oysters for human consumption" was not met completely during the present program. However, the promising results from our work has prompted the development of several new studies, namely: "The Victorian Site Assessment Program"; "The Sanitary Survey Program"; and a program to develop new systems for the growing of *O. angasi* commercially. Our assessment of the biological performance of *O. angasi* in Victoria has shown that the species has

excellent culture potential. We have demonstrated the following: *O. angasi* can be grown equally well in mid-water and on bottom racks; early "planting" of hatchery seed can dramatically improve growth rates; simple husbandry techniques can further improve growth and reduce mortality; and several large marine embayments are suitable for on-growing.

We conclude that there is a sound basis for developing a flat oyster culture industry. The "Commercial Aquaculture Development Unit" of the Victorian Fisheries Division has been established to assist commercialisation, and will attempt to resolve many of the conflicting interests which will accompany developments in the sensitive coastal zone.

DISSEMINATION OF INFORMATION

We have prepared annual reviews of the work carried out; these reviews were made available from the M.S.L. librarian. We have also conducted annual workshops for growers, entrepreneurs, and other interested parties. Five papers have been presented at inter-State conferences: A.N.Z.U.S. (Townsville); Australian Mariculture Association (Lismore); and First National Shellfish Conference (Perth) (See Appendix). In addition to the present report, scientific papers are being prepared for publication. These papers will appear in the M.S.L. "Internal Report Series" prior to external publication.

FIGURES

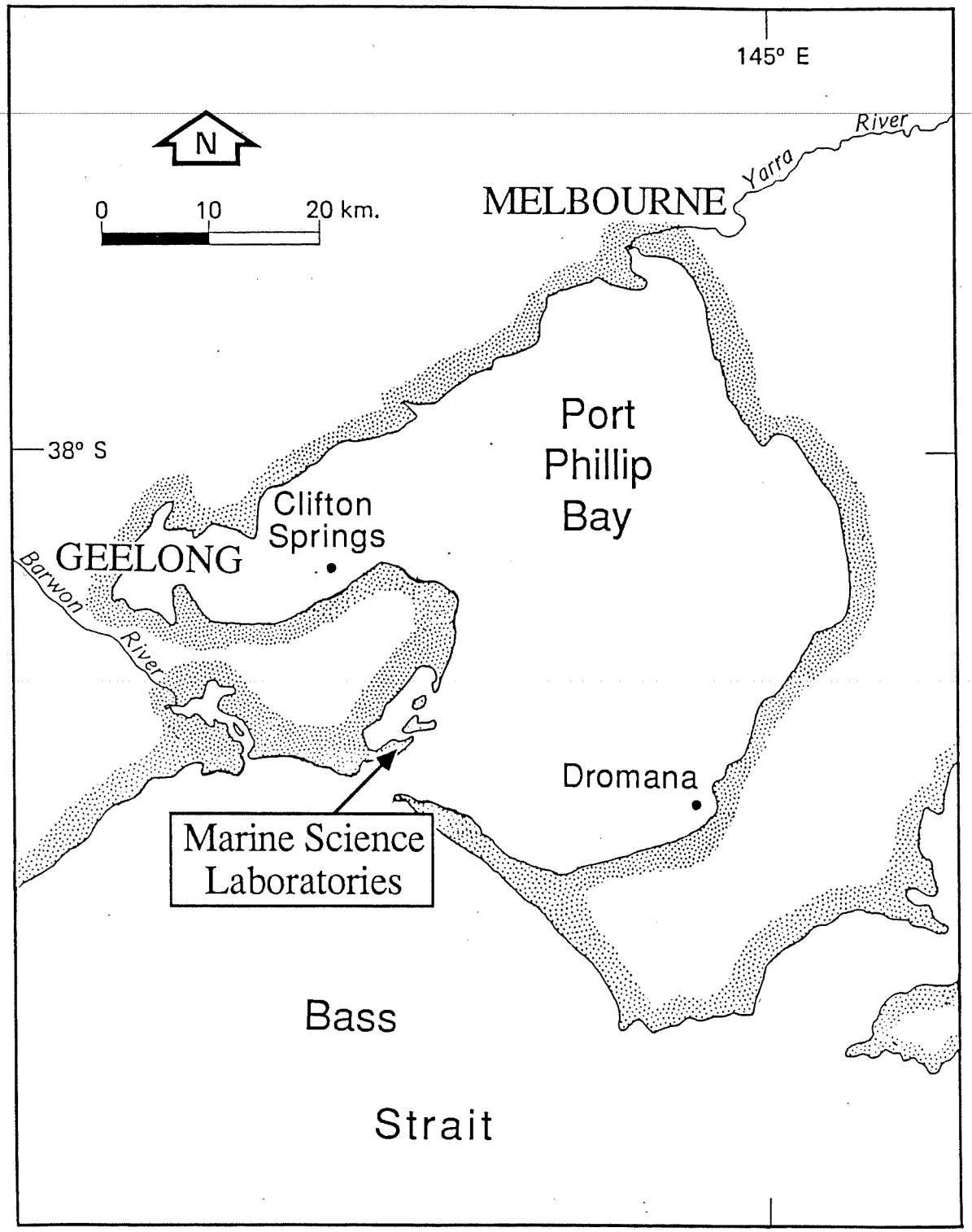


Figure 1. Location of study areas

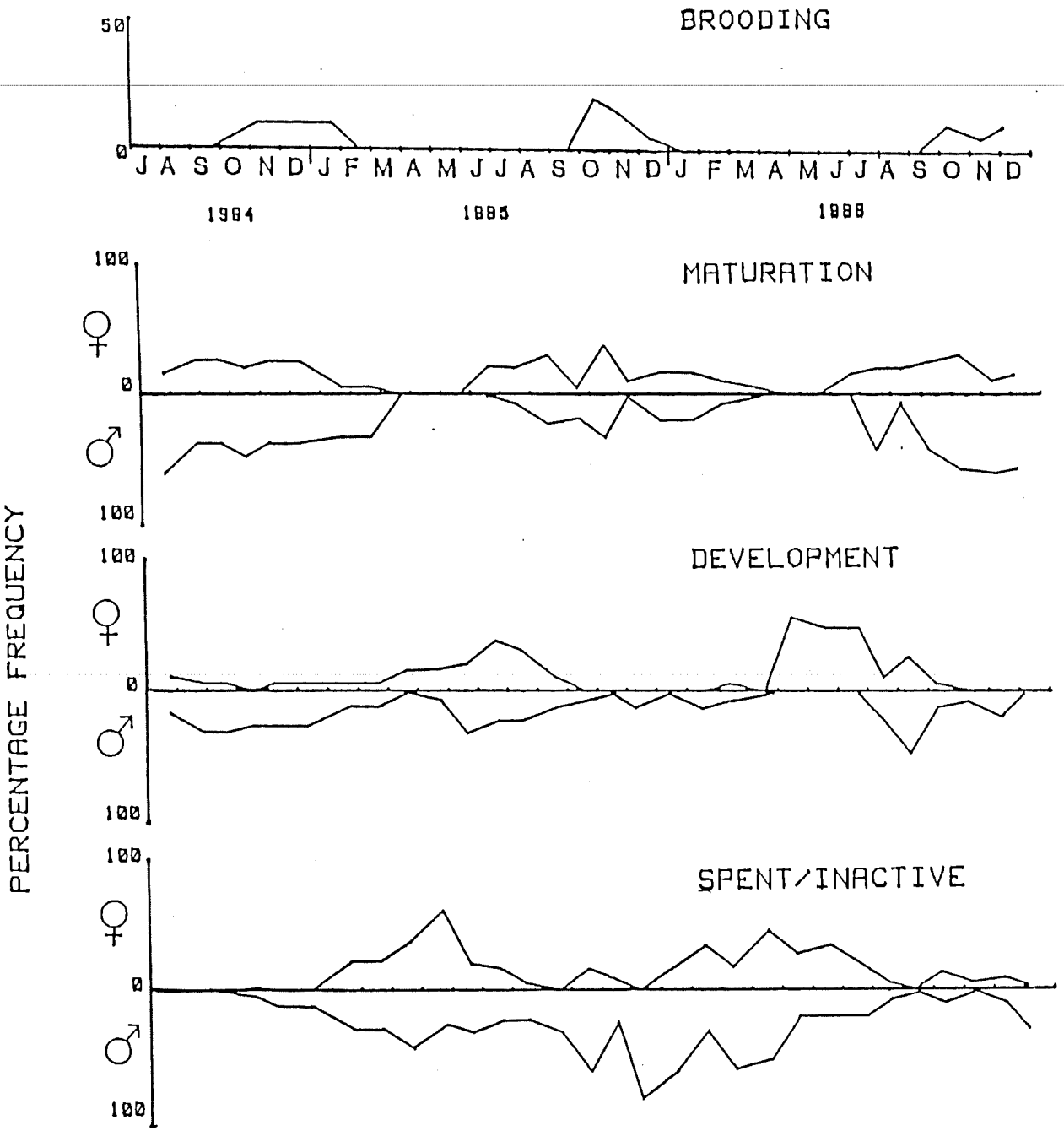


Figure 2. Breeding cycle of *O. angasi* in Port Phillip Bay, Victoria.

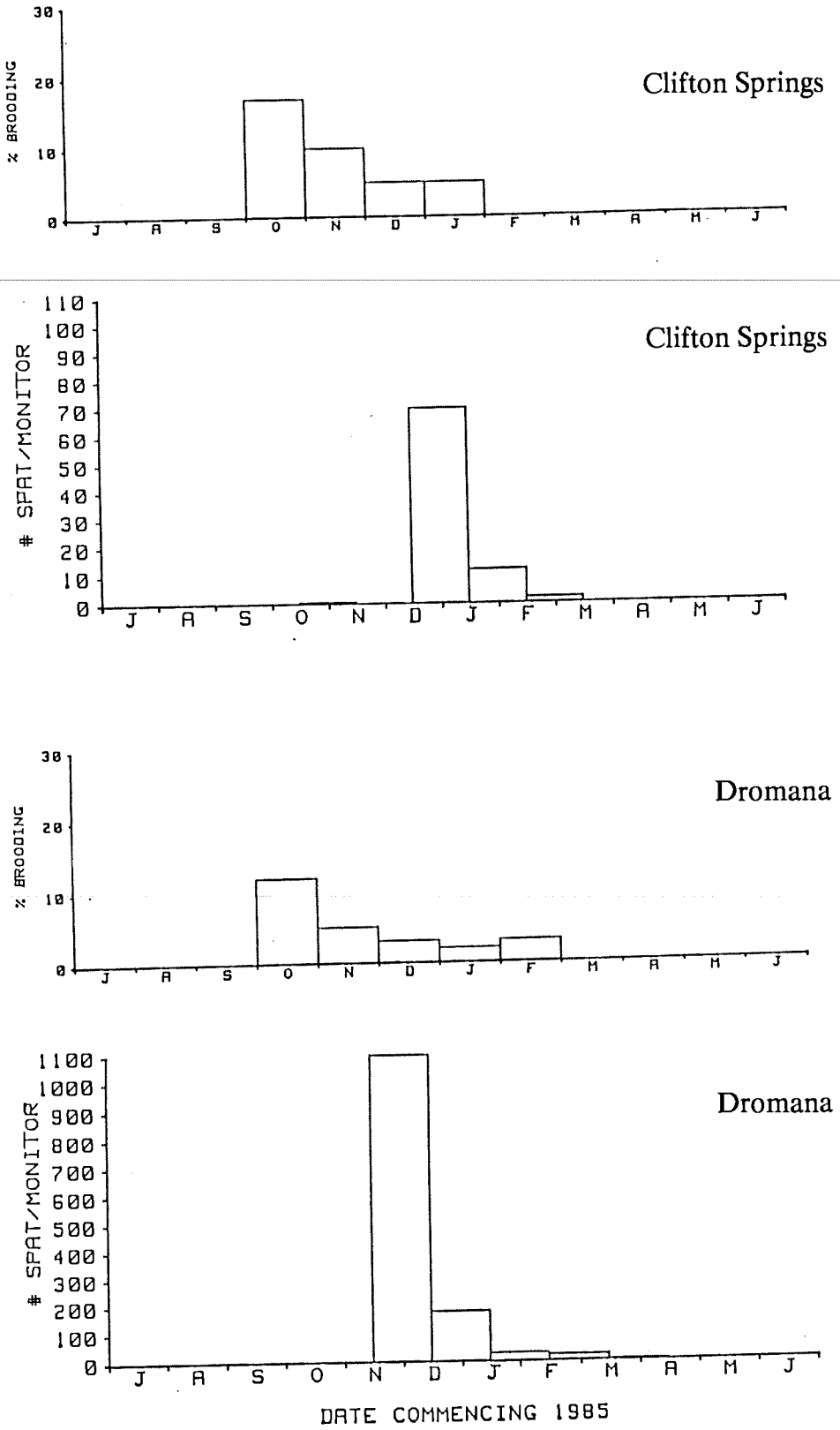


Figure 3. *Ostrea angasi* larval brooding and spat settlement at Dromana and Clifton Springs. (Numbers on spat monitor refer to spat per collector).

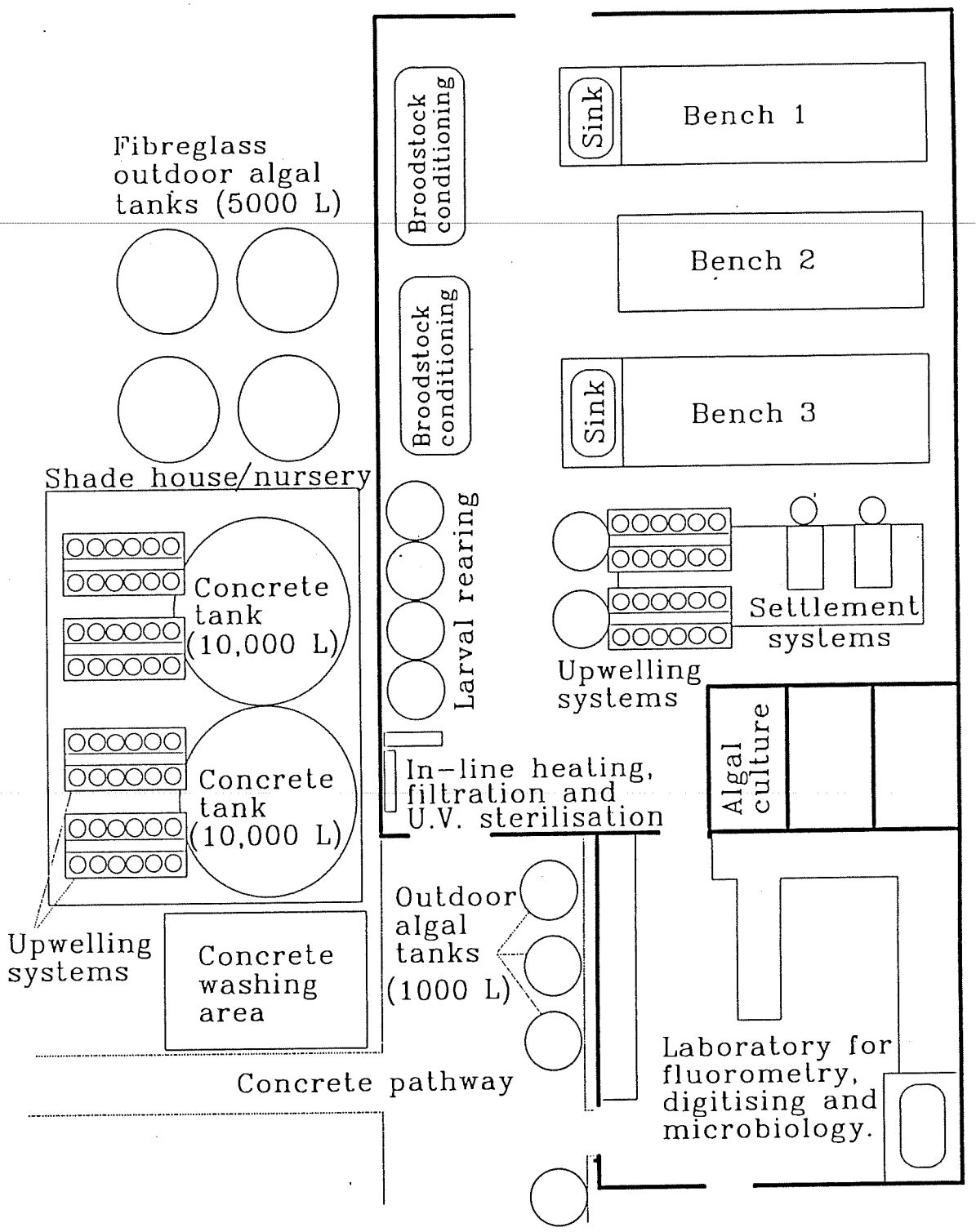
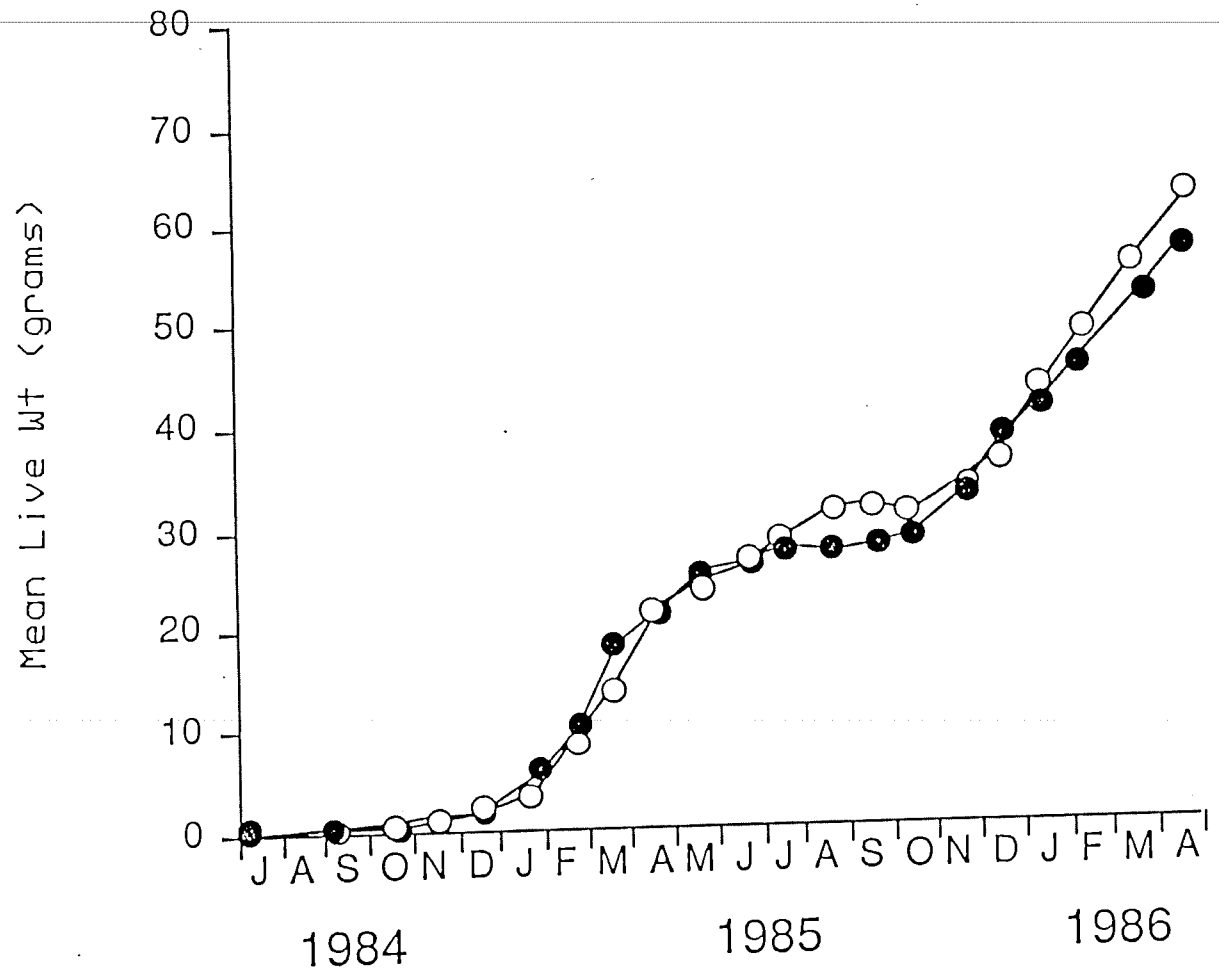


Figure 4. Plan of aquarium showing pilot hatchery and nursery. (Schematic diagram - not to scale)



open circles = Dromana
closed circles = Clifton Springs

Figure 5. Growth of *O. angasi* in mid-water at two sites in Port Phillip Bay.

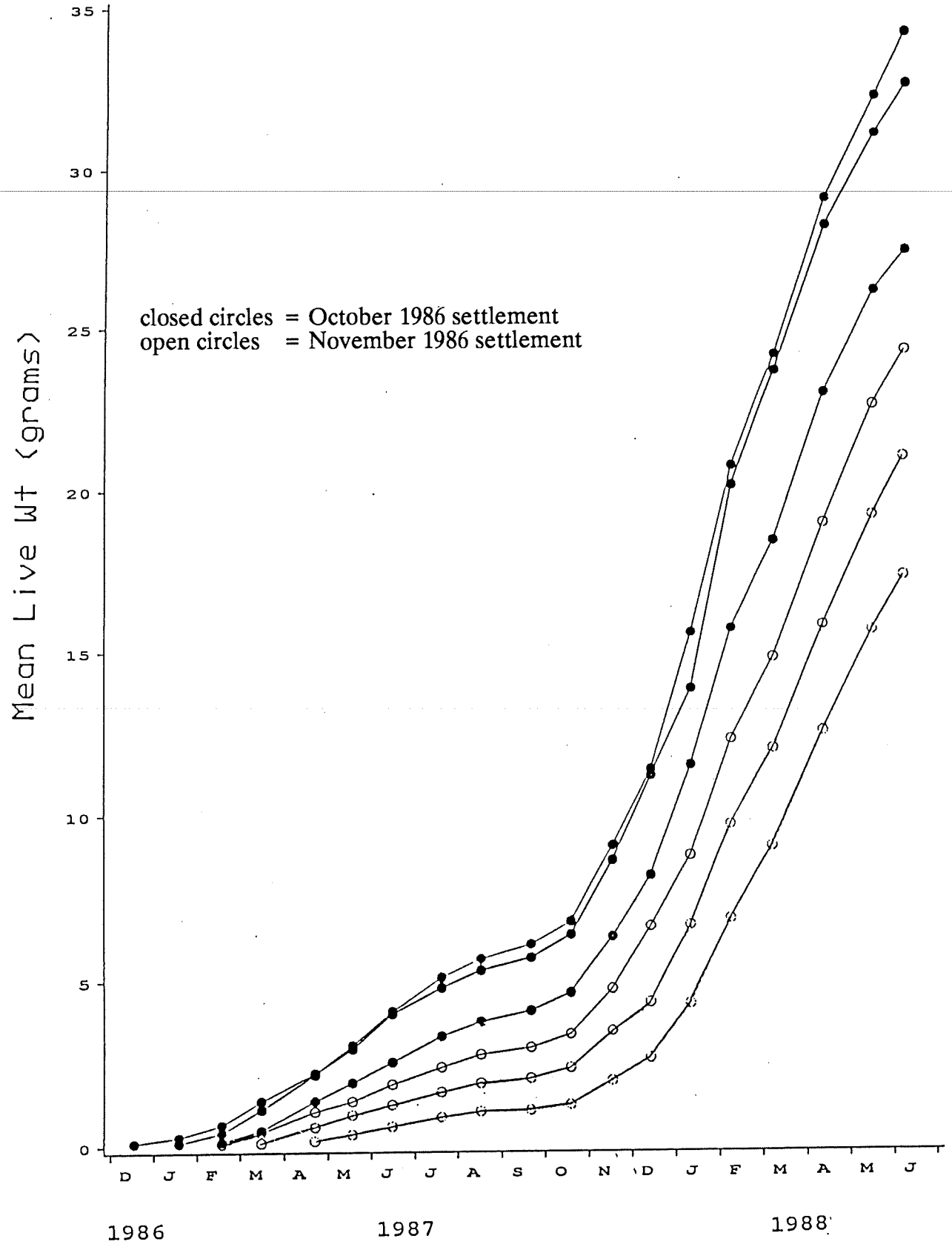


Figure 6. Growth of *O. angasi* which were put to sea at Clifton Springs at monthly intervals at a size of 10 mm. For each settlement the fastest growers in the nursery were put to sea first and the slowest growers were put to sea last.

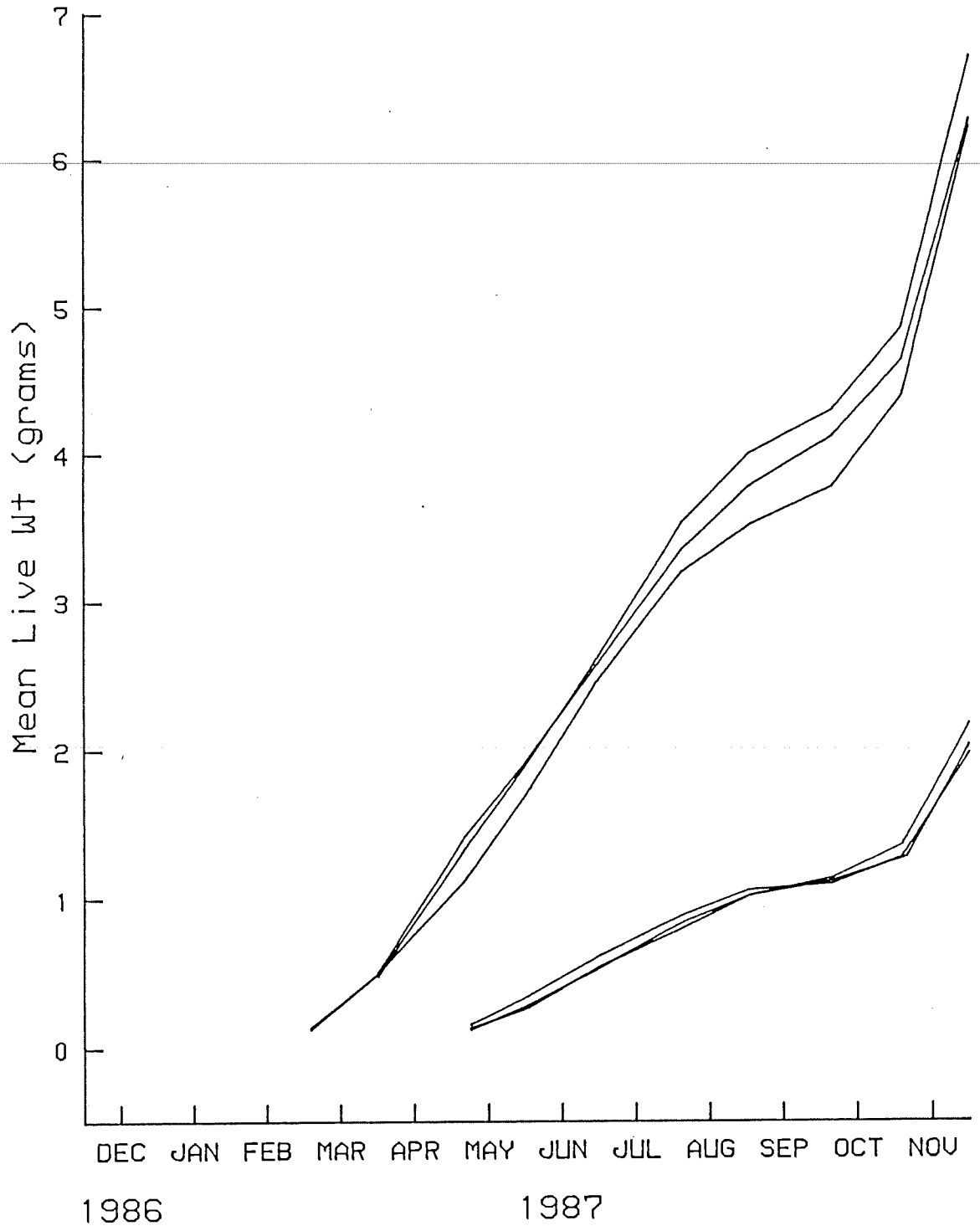


Figure 7. Oysters which were put to sea as shown in Figure 6 were also held in on-shore upwellers and put to sea at the same time.

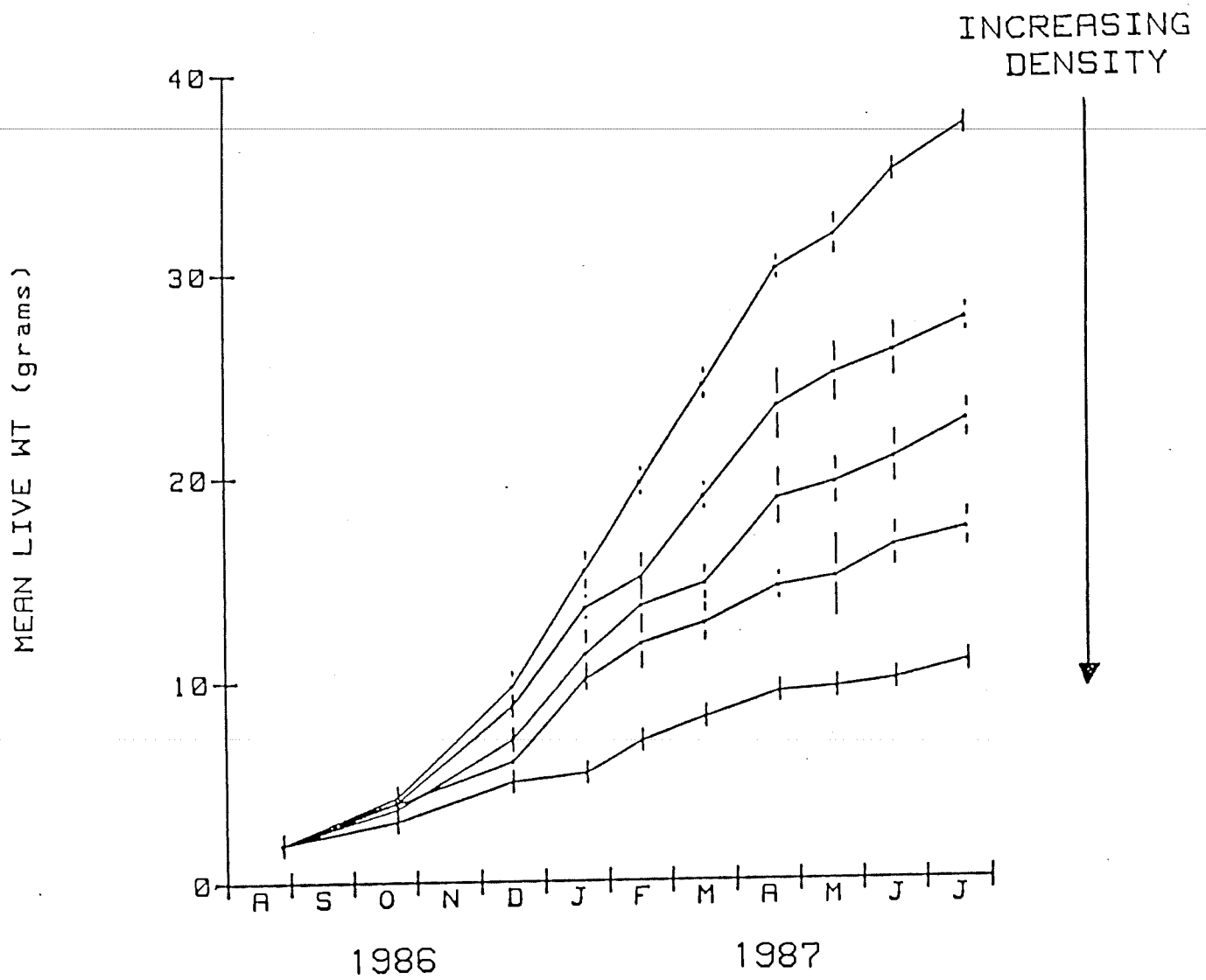


Figure 8. Effect of bag density on *O. angasi* growth.

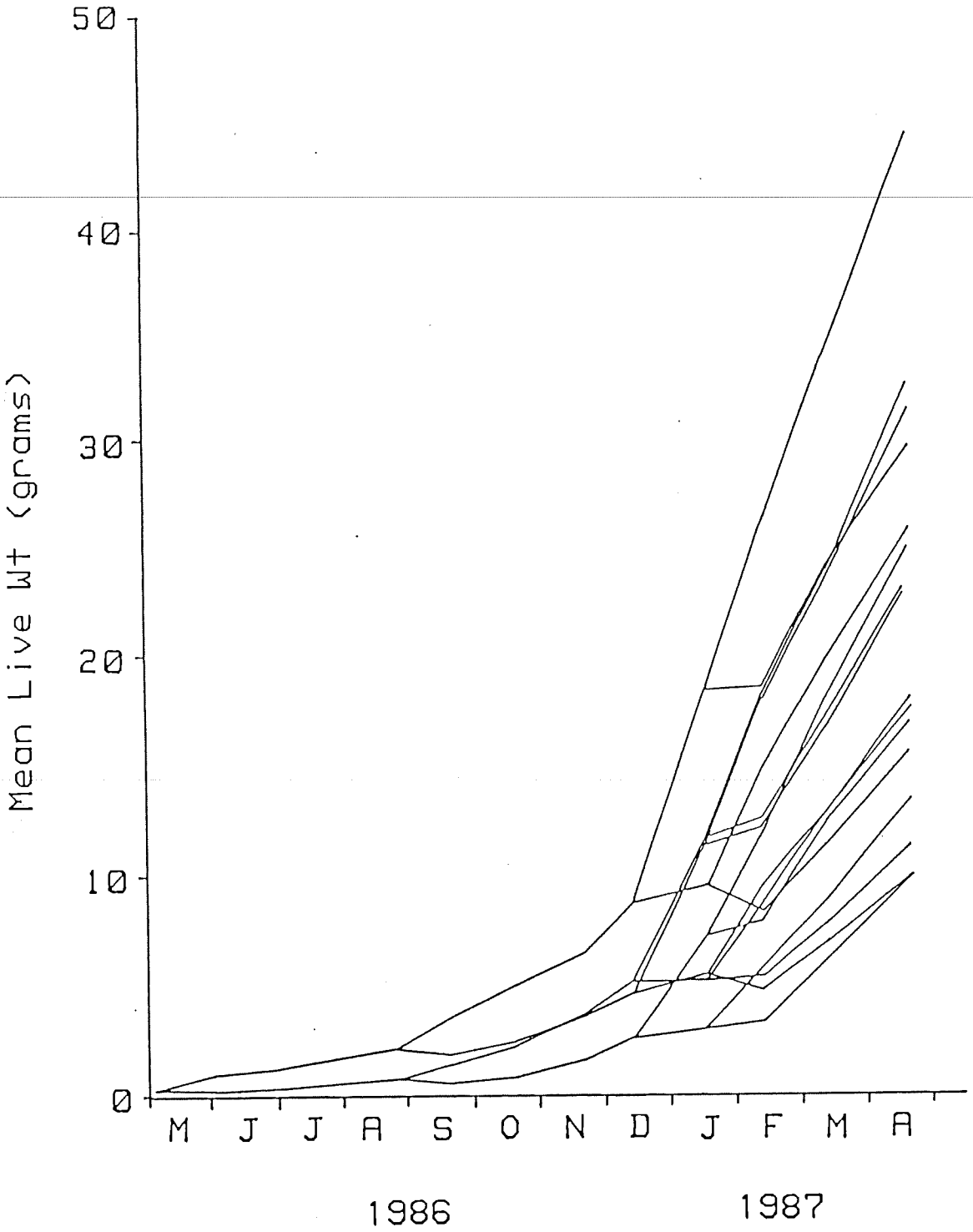
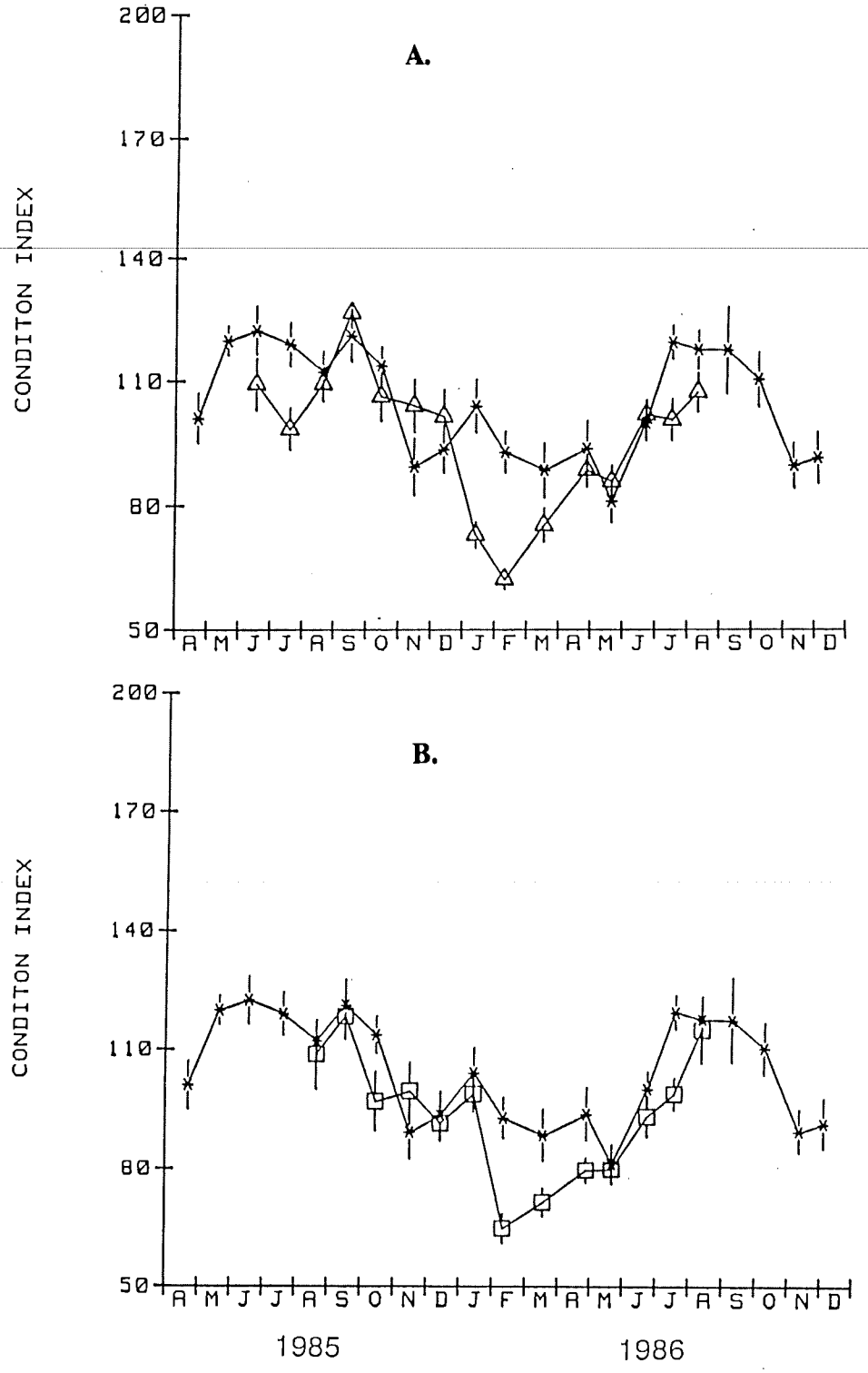
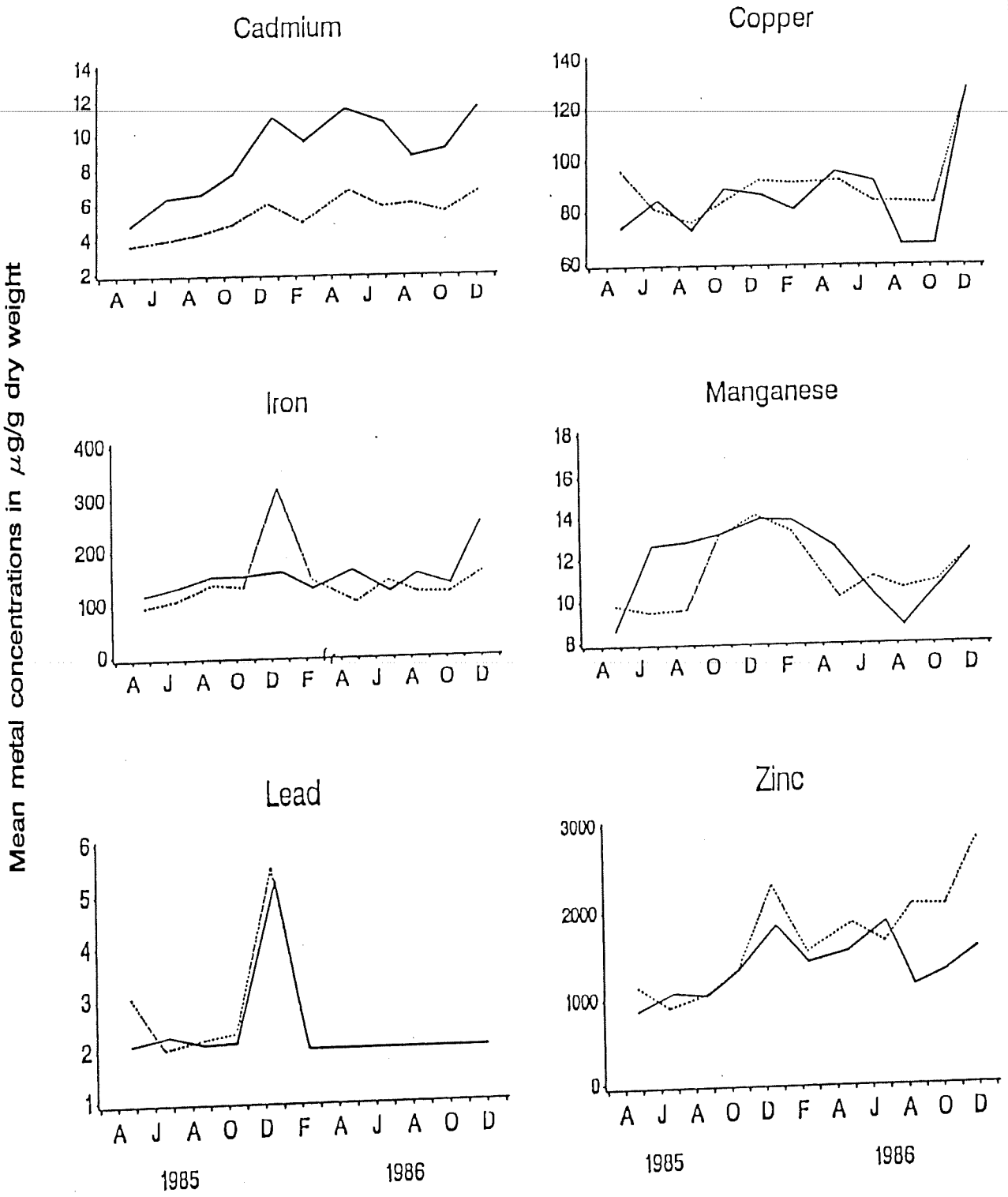


Figure 9. Effect of grading on *O. angasi* growth.



A. Density effect
B. Mid-water compared to bottom oyster tray
Stars = low density mid-water, triangles = high density, mid-water and squares = low density on bottom oyster trays. (x and S.E. plotted, n = 10)

Figure 10. The effect of density and position in the water column on condition of *O. angasi*.



Note: lead values less than 2 are plotted as 2.

Figure 11. Mean heavy metal concentrations (n=10) in cultured *O. angasi* from two sides in Port Phillip Bay.

ACKNOWLEDGEMENTS

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