LINK AQUACULTURE

FINAL PROJECT REPORT - FINANCIAL YEAR 2001-2002

This form is to be completed by the Project Leader and returned to the LINK Aquaculture Programme Co-ordinator c/o Freshwater Fisheries Laboratory, Faskally, Pitlochry PH16 5LB

Section 1: Project Details

(a) Project Code	SHL09 /EC(12)	CSA Ref	CSA4079
(b) Project Title	*Ostrea* size/age, physiological stress and resistance to *Bonamia ostreae*		
(c) Project Start Date	01.09.97		
(d) Project End Date	31.08.01		
(e) MAFF Project Officer	Dr Mark James		
(f) Name and Address of contractor	School of Ocean & Earth Science Southampton Oceanography Centre University of Southampton European Way Southampton Postcode SO14 3ZH		
(g) Contractor's Project Officer	Dr L. E. Hawkins		

Section 2: Scientific Objectives

Please list the scientific objectives as set out in the original application. If necessary these can be expressed in an abbreviated form. Indicate where amendments have been agreed with the Sponsor's Project Officer, giving the date of amendment.

The project made use of traditional culture methods using hatchery produced oyster spat, reared on proven growing areas, to examine the effects of age and stock density at re-laying, on levels of *Bonamia* infection. The specific objectives of the project were:

1 To quantify the effect of traditional culture methods i.e. stock laid directly on substratum and left undisturbed throughout grow-ng period. To be quantified in terms of disease susceptibility and growth during the course of the project.

2 To quantify the effect of stocking density on the disease susceptibility and growth during the course of the project.

Section 3: Summary of Progress

Please summarise, in layperson's terms:
(a) scientific progress since the last report/start of the project and how this relates to the objectives of the project.
(b) industrial progress - comment on progress from the viewpoint of the industrial partners. What has been the significance of this work to date from an industry angle?

Please provide information on actual results where possible rather than merely a description of activities.

Executive Summary

The project has provided quantitative evidence that supports the proposition that non-intensive traditional methods of rearing the native Flat Oyster *Ostrea edulis* reduce physiological stress and thereby reduce disease-induced mortalities, particularly that caused by *Bonamia ostreae*. During the entire course of the project no unusual mortalities were observed and there was no histological evidence indicating the presence of this pathogen. The suite of indices of oyster health and growth all showed that oysters could be grown to marketable size and quality in three to four years on beds where spat were laid directly on the substratum at densities of up to 30 animals per metre^2. The study also confirmed the anecdotal view that *O. edulis* was not suited to bag and rack culture. The project also indicated that natural local spatfall could not be relied upon to provide the stocks of consistently reliable quality and quantity necessary for a commercially viable enterprise and that such stocks should be obtained from hatcheries. It was concluded that the commercial culture of *O. edulis* was a practical proposition, provided modern hatchery methods were combined with subsequent traditional extensive layings, rather than the intensive rearing that has been used in the recent past.

Introduction

Almost a century ago Hoek (as cited by Orton 1923) made the observation that "...the cultivation of oysters is a culture not a manufacture."; the aim of the present study has been to evaluate efficacy of traditional oyster culture methods as a means of reviving the commercial cultivation of the native Flat Oyster *Ostrea edulis* in British waters. The original intention of the project was to make use of locally spatted *O. edulis* in a study of the effects of stocking density and relaying stress on the growth and disease resistance of oysters laid directly on the substratum at 10, 20 and 30 animals per metre^2. However, the failure of local recruitment in the first year of the project required a modification to the project aims and objectives, as well as pointing to an alternative strategy for potential commercial operations. The project proceeded with hatchery-produced spat laid at the densities given above and monitored on a regular basis until February 2001 using the suite of methods given below. In addition to this work, the Essex Oyster and Seafood Company (EOSFC) requested that a subsidiary study was made of *O. edulis* kept in intertidal bag and rack culture.

Work Programme

After the initial survey and clearance of the trial site at the EOSFC site at Goldhanger Creek on the R. Blackwater near Maldon, Essex, 50,000 *O. edulis* spat (29.67 ± 1.92 mm mean shell length) were obtained from the Seasalter hatchery and laid sub-tidally directly on the bed of the river at three densities (10, 20 and 30 oysters per square metre). These layings were left undisturbed other than by the collection of small numbers for the bimonthly sampling between May 1998 and February 2001. As an additional comparison spat were also placed in bags on racks on the mid and low shore. Both the traditional layings and the bag culture groups of oysters were sampled every two months and their disease susceptibility, health and growth, assessed using the following methods:

1. Measurement of differential haemocyte counts, cell motility and phagocytic ability following both *in vivo* and *in vitro* pathogen challenge.

2. Measurement of haemolymph microcidal activity in the form of hydrogen peroxide and lysozyme titres following pathogen challenge.

3. Monitoring of mortality, any major histopathological changes and quantification of levels and tissue distribution of *Bonamia* using the standard staining procedure used at the Fish Diseases Laboratory, Weymouth

4. Measurement of body condition indices of individuals.

The environmental conditions at the site for each sample were also collected to provide the necessary background information on water temperature, salinity, suspended organic and inorganic particulate size fractions and loads, and microalgal cell numbers.

Results

Results of particular relevance to the aims and objectives of the project are described below.
Throughout the project there was no indication of infection by *Bonamia* using histological examination. The success of the traditional approach to the cultivation of *O. edulis* are illustrated by Figures 1, 2 & 3. Using an inter-annual comparison Figure 1 shows that during the first two years of growth on the ground body size was clearly inversely proportional to laying densities in the subtidal groups and in bag & rack culture groups on the mid and low shore, where the degree of tidal exposure markedly affected growth. It is apparent from the February 2001 sampling that laying density no longer had any statistically significant effect on body size, though it should be borne in mind that bimonthly sampling will have reduced initial densities on all three beds and so reduce the influence of density related stress on growth. The data in Figure 1 are of immediate commercial interest as it shows that all groups had reached minimum legal marketable size (Figure 1a) in the third year of growth on the ground, i.e. in relatively short growing period for this species. However, Figure 1b is of equal importance in determining the marketability of the oysters in that there were no obvious differences in the 'quality' of the density trial groups, as measured by mean wet weights of soft tissues. Figures 2 & 3 however indicate that there were still differences between groups when measures of immunocompetence were used, and whilst immunocompetence is not an obvious factor when considering the commercial viability of a culture method the ability to minimise losses to disease is a prime determinant of commercial viability. Figure 2a shows that at low density the cellular defence mechanisms of *O. edulis* were able to cope with an acute pathogen challenge without difficulty but this capability was diminished by increasing stock densities, and by bag culture combined with tidal exposure. The data presented in Figure 3a support the above interpretation, and it should be noted that the high mean values in the tidally exposed groups are likely to have been caused by the increased turnover of large granulocytes in these groups - a phenomenon investigated in previous laboratory studies. These studies found that the generation of destructive oxyradicals by hyperventilation on re-immersion decreases the total large granulocyte population and so increases the relative proportion of active granulocytes.
The information provided by the February 2001 sampling leads to two important conclusions. Firstly, immunological indices continue to reflect the long-term physiological histories of the respective density groupings. It would seem that conditions in the early life stages are crucial in determining the development of effective immunological responses in later stages and therefore are important determinants of long-term survival. This information is not only of interest as a piece of fundamental science but also has major implications for determining the appropriate strategies for commercial cultivation of *Ostrea edulis*. Also, because of the importance of maintaining optimal growth and immunocompetence it was decided that the poor performance of the bag cultures in these respects did not justify a continuation of this non-contracted additional component of the study. Secondly, the February 2001 sample cannot be compared directly with its predecessors because of an extended period of low salinity (20 psu), coupled with unusually high water temperatures in December 2000 and high sediment loads, observed in the last two sampling periods. The effect of such conditions was to eliminate large granulocytes, leaving only a small number of mature forms to react to the stimulus of bacterial challenge (Figure 3b). Thus it would appear that the proportion of phagocytically active cells was greater in the February 2001 challenged group. The results in Figure 3b appear to be anomalous when compared to the previous February sample. However, when these results are compared to data from laboratory studies (Hawkins, et al. 1999) of the combined effects of the relevant temperature and salinity conditions matching those observed in the February 2001 sample there is a perfect

correspondence of effects on challenged and unchallenged groups, in terms of phagocytically active large granulocytes and neutral red retention times (Figure 2b). These observations are an unambiguous demonstration of the need to conduct laboratory studies so that field data to be accurately interpreted, i.e. the essence of the LINK programme in bringing academia and industry together.
Overall, these data provide, for the first time, quantitative information that is needed to inform any management strategy seeking to balance the opposing requirements of maximising yield against greater product quality and minimising losses to disease. It should also be noted that this study has also verified the anecdotal view that *O. edulis* does not grow well in bag culture.

Essex Oyster & Seafood Company: The trials are considered to be a great success in that *Ostrea edulis* has grown vigorously on the trial beds with minimal mortality. In the previous 10 years this species had not grown well using more intensive culture methods and had not exceeded 1 ¼" shell size before significant mortalities had intervened. The success of the methods under trial is sufficient to encourage EOSFC to consider investment in full scale commercial implementation at a similar site.

Shellfish Association of Great Britain: The SAGB remain supportive of the work and will assist in the communication of the results of this work to the industry through the medium of its conference programme and other activities.

Conclusions

Overall, the results support the initial project proposition that non-intensive traditional methods of rearing the native Flat Oyster *Ostrea edulis* reduce physiological stress and thereby reduce disease-induced mortalities, particularly that caused by *Bonamia ostreae*. The following conclusions can be drawn from the results:

1. Bag and rack culture is unsuitable for *O. edulis*

2. *O. edulis* can be grown to marketable size and quality in 3-4 years without significant mortalities and no overt signs of bonamiasis when laid directly on to beds, provided stocking densities are kept low (up to 30 animals per metre^2) and there no movements of stock until harvesting. However, it should also be noted that the results show that immunocompetence is inversely proportional to stocking density. Therefore, a commercial operation would have to balance the financial benefit of high stocking density against increased risk of disease related mortality.

3 The quantity and quality of natural spatfalls in British waters are too variable for a commercial venture to rely upon them. The future of *O. edulis* culture lies in the marriage of modern hatchery production of spat with traditional extensive culture, rather than the intensive rearing and movement of stocks between sites that has taken place in the recent past.

References

Hawkins, L.E., Hutchinson, S. and Hauton, C. (1999). Quantitative evaluation of bivalve disease susceptibility and resistance. Final report to MAFF Chief Scientists Group, CSA 2965. 129 pp.

Orton, J.H. (1923). An account of investigations into the cause or causes of the unusual mortality among oysters in English oyster beds during 1920 and1921. *Ministry of Agriculture and Fisheries, Fisheries Investigations, Series II, Vol. VI no. 3. 199pp*

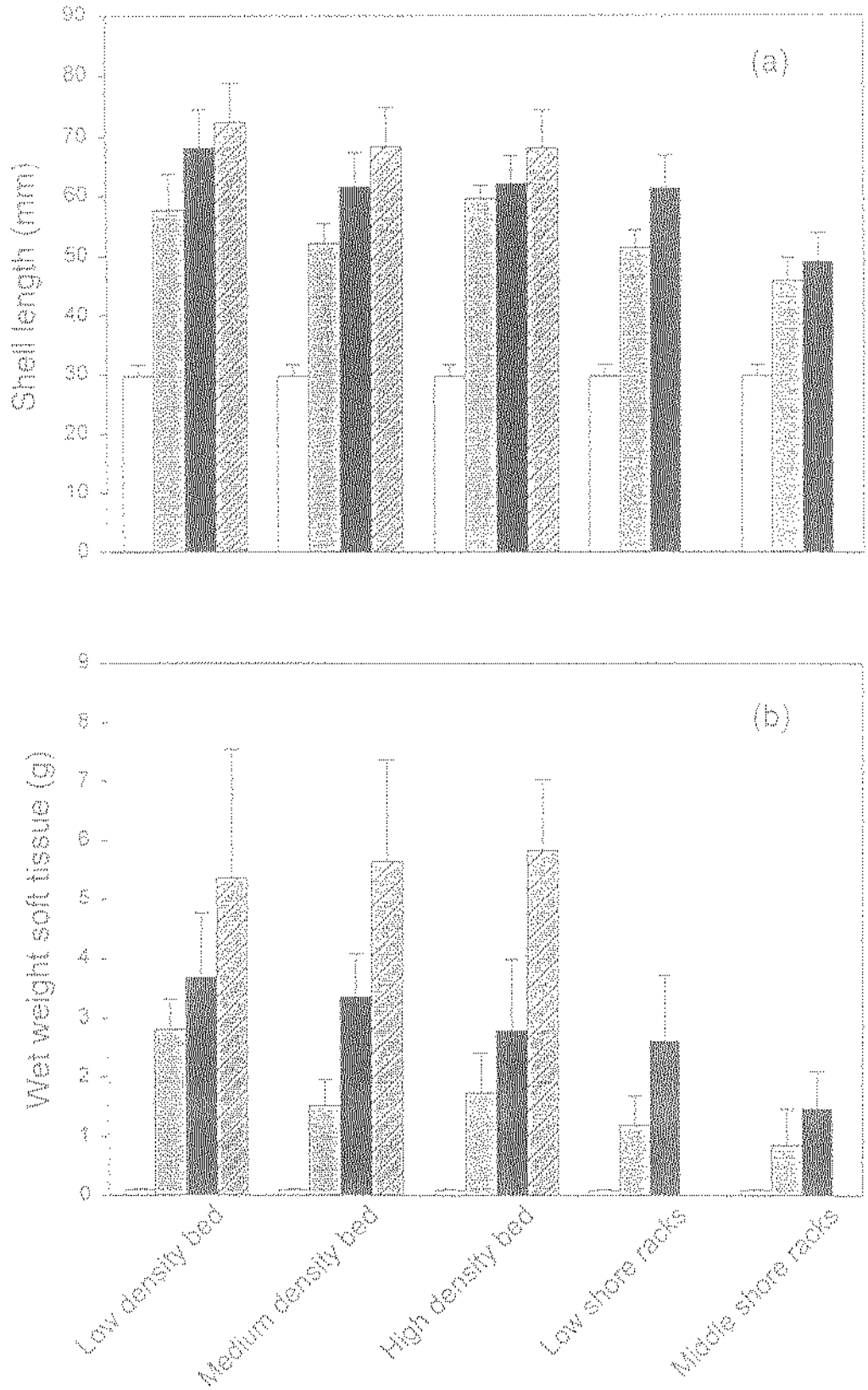

Figure 1
(a) Shell length (mm) and (b) wet weight soft tissue (g) of *Ostrea edulis* laid subtidally at three densities and in bag and rack culture at mid and low shore. Mean values + S.D., n > 16. Clear bar = 1998 initial laying, grey bar = February 1999 subsample, black bar = February 2000 subsample, hatched grey bar = February 2001 subsample.

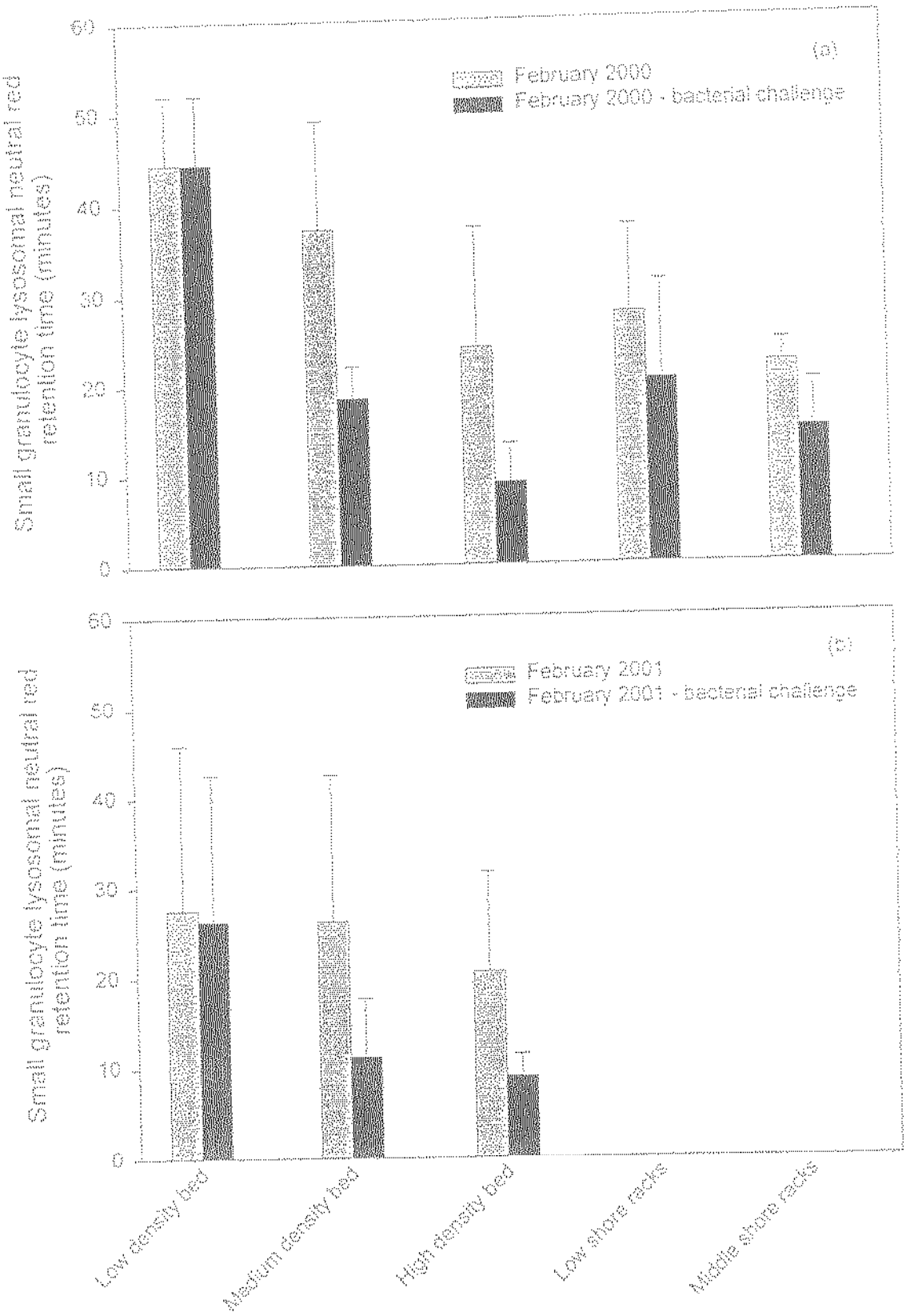

Figure 2
Small granulocyte lysosomal neutral red retention time (minutes) of *Ostrea edulis* laid subtidally at three densities and in bag and rack culture at mid and low shore sampled at (a) February 2000 and (b) February 2001 (bag and rack cultures discontinued in 2000). Mean values + S.D., n > 8.

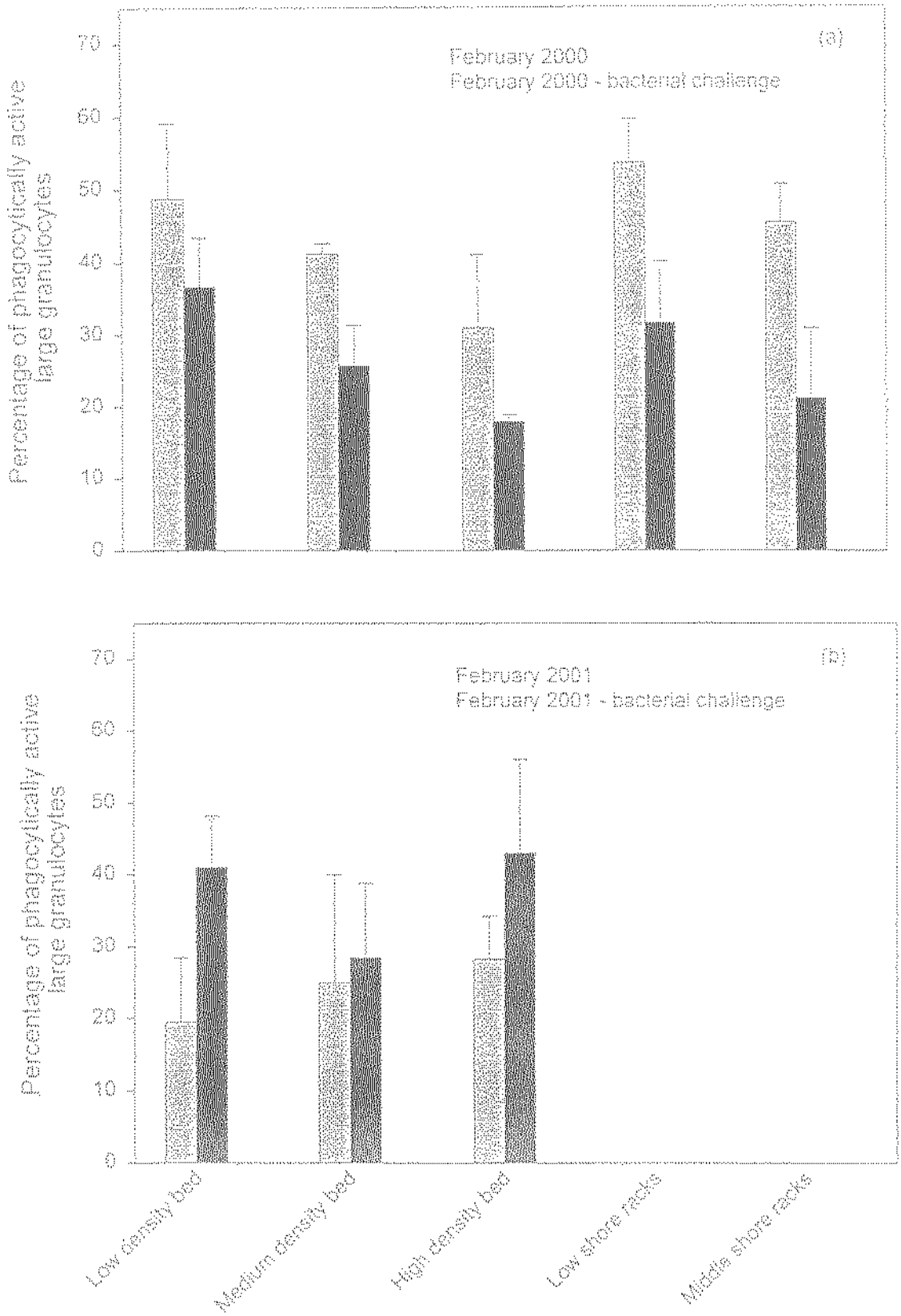

Figure 3
Percentage of phagocytically active large granulocytes of *Ostrea edulis* laid subtidally at three densities and in bag and rack culture at mid and low shore sampled at (a) February 2000 and (b) February 2001 (bag and rack cultures discontinued in 2000). Mean values + S.D., n > 8.

Section 4: Amendments to Project

Are the current scientific objectives appropriate for the remainder of the project? | Yes

If NO, explain the reasons for any changes giving the financial, staff and time implications.

Contractors cannot alter scientific objectives without the agreement of the Sponsor's Project Officer

Section 5: Progress in Relation to Targets

(a) List the primary milestones for the year/period under report as stated on the original application form

It is the responsibility of the contractor to check fully that ALL primary milestones have been met and to provide a detailed explanation if this has not proved possible.

Milestones		Target Date	Milestones Met?	
Number	Title		in full	on time
1	Survey of main channel, layout of trial beds and deposition of experimental stock	09/98	Yes	Yes
2	Bimonthly sampling of trial beds	28/02/2001	Yes	Yes
3	Preparation of reports, dissemination of results	31/08/2001	Yes	Yes

Section 5: Progress in Relation to Targets (continuing)

(b) Do the remaining primary milestones look realistic? | N/A

(c) If you have answered NO at (a) or (b), please provide an explanation.

Section 6: Project Costs and Staffing Input

In this reporting period, what was:

(a) the approved expenditure?	N/A
(b) the actual expenditure?	N/A
(c) * the approved staff input?	University of Southampton 0.49 man years Essex Oyster & Seafood Company 0.19 man years
(d) * the actual staff input?	University of Southampton approximately 1 man year Essex Oyster & Seafood Company 0.19 man years

* staff years of direct science effort

Section 7: Publications and Other Outputs

(a) Please give details of any outputs eg published papers/presentations during this reporting period.

Hawkins, L.E., Hutchinson, S. & Devall, C.A. (2000). Flat oyster culture - An evaluation of traditional methods. *Shellfish News*. 10: 5-7.

Section 7: Publications and Other Outputs (continuing)

(b) Have opportunities for exploiting Intellectual Property arising out of this work been identified? Yes

If you have answered **YES**, please give details.

The results of the project will be presented at the next SAGB Shellfish Cultivation conference in May 2002 and an update of the article published in Shellfish News has be requested by the editor.

The extensive data set will be used to provide information for scientific papers submitted to peer-reviewed journals and the information will be made available for incorporation into cd based information pack and economic model, should the appropriate funding be obtained.

(c) Has any other action been taken to initiate Technology Transfer? No

If you have answered **YES**, please give details.

Section 8: Future Work

Please comment briefly on any new scientific opportunities which may arise from the project.

The results of this and other bivalve shellfish projects in the LINK programme point unequivocally for the need for the production of hatchery reared stock of known quality rather than the reliance on natural spatfalls. The data from this project provides further support for the proposal submitted to DEFRA Chief Scientist's Group for funding to develop new approaches to minimise disease related mortality in shellfish hatcheries without recourse to pharmaceutical products.

Section 9: Declaration

I declare that the information I have given is correct to the best of my knowledge and belief.
I understand that the information contained in this form may be held on a computer system.

Signature		Name	Dr L. E. Hawkins
Date		Position in organisation	Senior Lecturer