FIRC 36

No 19 84 . 48 .....

- ☐ NEW PROPOSAL
- ☐ CONTINUING PROJECT
- ☒ FINAL REPORT
- ☐ PROGRESS REPORT

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Bacteria in shellfish hatcheries

ORGANISATION: Uni of Tasmania

PERSON(S) RESPONSIBLE: C.D. Garland & T.A. McMeekin

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1984/85		29,936
1985/86	31,004	31,004

RELATED APPLICATIONS: 79/70 – Feasability study of a commercial shellfish hatchery ($4,500)

82/70 - Bacterial pathogens of oyster larvae and spat (82/83 $39,155 · 83/84 $41,233)

RECEIVED 5 / 11 / 1986 DISTRIBUTED ...../...../19...

Secretary
Fishing Industry Research Committee

F139--12/84

FINAL REPORT

FIRTA GRANT 84/48

Bacteria in Shellfish Hatcheries

by

C.D. GARLAND and T.A. McMEEKIN
Department of Agricultural Science, The University of Tasmania

Period of Grant: July 1984 to June 1986

GENERAL AIMS

(1) To develop a code of sanitation aimed at reducing the prevalence of bacteria in a commercial shellfish hatchery.

(2) To improve the survival and growth of abalone (Haliotis ruber) during the larval and post-larval phases.

GENERAL COMMENT

Aim 1 Studies on the impact of bacteria on larval shellfish, principally oysters, at the commercial hatchery at Bicheno operated by Shellfish Culture Pty. Ltd., proceeded very successfully and are summarised below.

Aim 2 Unfortunately no significant study of abalone was undertaken because larvae could not be produced. Despite several trips to obtain potentially competent broodstock from the wild, and efforts to condition animals in cages in coastal waters, all attempts to induce abalone to spawn and produce larvae failed. The reasons are given in the Final Report of 84/50 'Abalone Container Culture' (Wilson and Sumner, Department of Sea Fisheries, Hobart).

RESULTS

Bacteriological Studies of Larval Pacific Oysters (Crassostrea gigas) reared in a commercial hatchery at Bicheno

Previous work by us (Garland et al., 1983, Aust. J. Mar. Fresh. Res. **34**: 483-487; Final Report FIRTA 82/48, Garland and McMeekin) showed that marine

bacteria caused severe mortalities in larval oysters reared under intensive (hatchery) conditions. The major source of bacteria was considered to be the cultures of microalgae fed to larvae. Other sources of bacteria, such as the intake seawater and surfaces of pipes and culture tanks, were also regarded as significant.

At the start of FIRTA 84/48 (July 1984) our strategy was to reduce the prevalence of bacteria in the hatchery by the following measures:

(1) The use of axenic (bacteria-free) starter cultures of microalgae, since these were free of bacteria pathogenic to larvae. Thus the introduction of pathogens into the mass algal culture system via the starter culture route should be eliminated.

(2) The use of 0.2 um membrane-filtered seawater as a growth medium for mass culture of microalgae, since it would be likely to reduce the bacterial level in microalgae cultures fed to larvae. We did not attempt to mass culture (500 litre bags) microalgae in the axenic state because (i) of the high cost of large-scale germ-free culture equipment and (ii) the introduction of large amounts of extracellular organic material, present in axenic microalgal cultures, into the larval culture medium would be likely to be very perturbatory to animals.

(3) General sanitary measures, e.g. cleaning the surfaces of pipes and tanks, forced/heated air-drying of equipment, location of air-conditioners, drains, etc.

The measures proved to be very successful. Production of ready-to-set larval oysters increased five-fold, from 30.5 million in 1983-84 before the grant commenced, to 106.5 million in 1984-85 (first year of grant), to 158 million in 1985-86 (final year of grant). Also, the rearing of larvae in batches became much more predictable. In 1985-86, 7 of 8 batches yielded the target (or close to target) number of larvae viz. 5% of fertilized eggs. The batches were culled vigorously during rearing, according to a larval size vs. larval density schedule.

The success was attributed to:

(1) The absence of specific larval pathogens including Vibrio anguillarum, V. tubiashii and a red Pseudomonas sp. from the feed microalgae cultures.

(2) A reduced bacterial level in the feed microalgae cultures. In 1985-86, approximately 90% of feed cultures contained $< 2 \times 10^6$ bacteria ml^{-1}. Epidemiological data gained by us strongly indicated that cultures with $> 2 \times 10^6$ bacteria ml^{-1} caused 15-100% larval mortality within 1-7 days of feeding. Cultures exceeding the threshold bacterial level were fed most commonly in 1983-84 but their incidence was greatly lowered later by means of the use of 0.2 um membrane-filtered seawater as the growth medium.

(3) Improvements in general hygiene. In particular, materials (equipment, surfaces, etc.) were disinfected by means of washing in hot water (if possible), followed by forced-air heat-drying (typically at 70°C). The use of chemical disinfectants, such as hypochlorite, was not encouraged.

DISSEMINATION OF RESULTS

(1) Two papers have been submitted to the journal 'Aquaculture'. The Abstracts are appended; complete copies may be obtained from G. Stablum, Secretary, FIRC.

(2) A 'Manual of Hygiene for Shellfish Hatcheries' has been written and more than 60 copies distributed to laboratories and hatcheries in Australia. A Table of Contents is appended; a complete copy may be obtained from G. Stablum, Secretary, FIRC.

(3) Formal talks on bacterial disease of larval oysters have been presented to NSW Fisheries Salamander Bay (May 1985, May 1986), WA Fisheries Perth (May 1985) and Marine Research Laboratories Queenscliffe (May 1986).

FUTURE ACTIVITIES

Results obtained during FIRTA 84/48 indicate various future lines of research and development, particularly -

(1) The necessity for an Australian culture collection of axenic microalgae which laboratories and hatcheries can readily access. In the short term, this collection will be established by means of FIRTA 86/81. The transfer of the collection to a commercial operator in late 1989 is planned.

(2) A detailed study of the microbiological characteristics of 0.2 um membrane-filtered seawater. This work is being investigated by Dr. Garland on FIRTA 85/19.

(3) Experimental verification that a bacterial level of 2×10^6 ml^{-1} microalga culture (independent of bacterial biotype) induces larval mortalities, and a study of the pathogenic mechanisms responsible. This work is being investigated by Dr. Garland on FIRTA 85/19.

(4) The need to continue the dissemination of results via international scientific journals, 'Australian Fisheries' and CSIRO bulletins. Lectures and workshops will also be conducted in various sites around Australia by Dr. Garland and colleagues on FIRTA 85/19 and 86/81.

T.E. Lewis, C.D. Garland, T.D. O'Brien, M.I. Fraser, P.A. Tong, C. Ward, S.L. Cooke and T.A. McMeekin. 1986. The use of 0.2 um membrane-filtered growth medium for improved control of bacterial levels in feed microalgae cultures. Aquaculture, submitted

Abstract

Feed microalgae, cultured in 500 l bags and harvested semi-continuously (in log phase) for oyster larvae, were studied over three production seasons in a commercial hatchery. Total heterotrophic bacterial counts (plate-viable method) were expressed in $\log_{10}$ units. In the 1983-84 season, the pasteurised-cooled seawater used as growth medium for feed microalgae contained 4.08 ± 0.78 bacteria ml^{-1}. The highest count reached in feed microalgae at harvest occurred in bags of Chaetoceros calcitrans ($7.19\ ml^{-1}$); Isochrysis galbana, Dunaliella tertiolecta and Chroomonas salina supported 6.27 bacteria ml^{-1} or more. The overall mean bacterial count of feed microalgae was 6.38 ± 0.77 ml^{-1}. In 1984-85 0.2 um membrane-filtration replaced pasteurisation. The filtered growth medium had a bacterial load of 1.54 ± 1.72 100 ml^{-1}. Counts in clones at harvest were significantly lower than those of the previous season; Thalassiosira pseudonana supported 6.14 bacteria ml^{-1}. The overall mean bacterial count in feed microalgae was 5.79 ± 0.62 ml^{-1}, significantly lower than in 1983-84. In 1985-86, bacterial levels in feed microalgae, including the new clones Tetraselmis suecica and Pavlova (Monochrysis) lutheri, were similar to those of the previous season; the overall mean bacterial level in the feed microalgae was 5.87 ± 0.35 ml^{-1}. The decreased variability of the mean was attributed to daily integrity testing of the final filter.

In most instances bacteria in feed microalgae reached plateau levels which were maintained for 1 to 12 weeks. The use of the membrane-filtered growth medium did not adversely effect the harvest characteristics of the clones studied. In 36% of specimens of all clones examined by scanning electron microscopy, bacteria colonised the surface of microalgae. Vibrio spp. were detected very rarely in feed microalgae.

INTRODUCTION

The production of marine molluscan larvae of commercial importance in high density (hatchery) conditions can be savaged by bacterial infections (Tubiash, 1975; Brown, 1983; Garland et al., 1983, 1987). Whether as primary pathogens, opportunists or harmless agents, bacteria can enter the larval culture system via four major sources: the intake seawater used for rearing animals; the stocks of microalgae; the fomites (such as the surfaces of pipes and culture tanks), and the broodstock used for spawning (Elston, 1984). It is most important to know the quantitative levels of bacteria in these sources below which it is safe to proceed with animal rearing, but above which measures to disinfect the source must be taken. However, very few data are available to provide guidelines.

In the case of microalgae it is well established that their extracellular products promote bacterial growth (Cole, 1982; Jones, 1982). Prieur and Le Roux (1975) and Murchelano and Brown (1969) examined bacteria present in small flasks and carboys of microalgae grown in static conditions in a laboratory for rearing marine bivalve larvae. However, in many hatcheries microalgae for

ABSTRACT

C.D. Garland, T.E. Lewis, M.I. Fraser, T.D. O'Brien, P.A. Tong, C. Ward, T.A. McMeekin and T.G. Dix. 1986. Epidemiological evidence suggesting that levels of mixed bacteria in feed microalgae cultures limit hatchery production of larval oysters (Crassostrea gigas). Aquaculture, submitted

Abstract

Larval production in a commercial hatchery over three seasons was limited by bacterial infection, sometimes severely. Feed microalgae cultures (500 l bags) were identified as the vector for transmission of bacterial disease. When microalgae cultures exceeding a threshold value of 6.3 $\log_{10}$ (or 2×10^6) bacteria ml^{-1} were fed to 1-7 day old animals, the growth rate was greatly reduced. Small (15-25%) losses of larvae occurred within 1-7 days of feeding, or major (30-70%) or total losses within 1-5 days. If no further cultures of microalgae exceeding the bacterial threshold were fed, surviving animals grew satisfactorily to the ready-to-set stage. If cultures exceeding the threshold level were fed, small, major or total losses of surviving larvae occurred within 1-7 days. During the study technology involving 0.2 um membrane-filtered seawater as the growth medium for feed microalgae and daily integrity testing of the 0.2 um filter was introduced in the hatchery. This resulted in the proportion of feed microalgae cultures exceeding the bacterial threshold ($\rangle$6.3 $\log_{10}$ ml^{-1}) decreasing from 55.6% to 20.6% to 10.9% in successive seasons, associated with the larval survival rate increasing from 1.3% to 3.2% to 4.6%. Bag cultures of all clones used (Isochrysis galbana, Chaetoceros calcitrans, Pavlova lutheri, Thalassiosira

pseudonana, Dunaliella tertiolecta, Tetraselmis suecica, Chroomonas salina) were implicated in the transmission of bacterial disease to one or more batches of larvae. Mixed bacterial biotypes, rather than single strains, were responsible for disease but these rarely included Vibrio or Flavobacterium/Cytophaga spp., and never red pigmented Pseudomonas spp. There was no evidence that bacteria in intake seaweater, fertilized eggs or fomites caused larval losses, or that non-bacteriological factors were responsible.

INTRODUCTION

Bacteria of the genera Vibrio, Pseudomonas and Alteromonas can cause high mortality in hatchery-reared bivalve larvae (Brown, 1983; Garland et al., 1983; Elston, 1984). Bacterial pathogenicity can be due to direct tissue invasion, toxins or both. In the case of vibriosis, the most commonly reported disease so far, bacteria attach to the periostracum, pallial infection begins at the shell periphery but progresses systemically and foci of bacteria form in the digestive gland (Elston, 1984). Toxigenic bacteria include a Vibrio sp. which produces a heat-labile exotoxin of molecular weight 68,000 (Brown and Roland, 1984) and a Pseudomonas sp. which releases prodiginine on cell lysis (Brown, 1981b). To date losses of larvae due to bacterial disease in Northern Hemisphere hatcheries have been attributed generally to single causative agents such as V. anguillarum, V. tubiashii, V. alginolyticus and unidentified individual strains of Vibrio and Pseudomonas (Jeffries, 1982; Brown, 1983; Elston, 1984; Hada et al., 1984).

Previously we reported bacterial pathogens of larval Pacific

MANUAL OF HYGIENE FOR SHELLFISH HATCHERIES

BY

T.E.Lewis

C.D.Garland

and

T.A.McMeekin

(1986)

Department of Agricultural Science,
University of Tasmania,
Box 252C, G.P.O.,
HOBART,
Tasmania, 7001,
Australia.

FUNDED BY
THE FISHING INDUSTRY RESEARCH TRUST ACCOUNT,
DEPARTMENT OF PRIMARY INDUSTRY,
CANBERRA, A.C.T., AUSTRALIA.

Printed by
University of Tasmania Printing Department

ISBN 0-85901-339-1

TABLE OF CONTENTS