New microbial foods for aquaculture

Final Report
to
FRDC

J A Nell, D G MacLennan, G L Allan
S P Nearhos and J Frances

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to

Fisheries Research and Development Corporation

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June 1994

Project title: Isolation growth and evaluation of marine yeasts and bacteria as live foods for aquaculture) (FRDC No 90/61).

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1 SUMMARY

Production of microalgae as live foods for aquaculture species is expensive and time consuming. One possible alternative is the use of marine yeasts and bacteria which are often easier to culture and more productive. Marine yeasts and bacteria were isolated from oyster growing areas in Port Stephens, NSW. Promising cultures were evaluated as new microbial foods for aquaculture at the Brackish Water Fish Culture Research Station. Nine new marine yeasts have shown potential. These were used to replace 86% of the algal diet for oyster spat and produced up to 75% of the growth of algal fed controls. The most promising of these new marine yeasts proved to have a higher food value to oyster spat than live cultures of either food yeast *Candida utilis* or baker's yeast *Saccharomyces cerevisciae*. Six bacterial cultures have also shown potential. These were used to replace 86% of the algal diet for oyster larvae and produced up to 75% of the growth of algal fed controls.

The amino acid composition of the yeasts and bacteria species closely matched that of *Saccostrea commercialis* oyster larvae and spat, indicating a high protein quality. Yeast and bacteria both lacked the polyunsaturated fatty acids recognised as being important for bivalve molluscs, i. e. 20:5(n-3) and 22:6(n-3), and this feature restricts their use in mariculture to a partial replacement for live algae. However, their high levels and good quality of protein indicates their potential to provide important nutrients in a mixed diet. The growth rates obtained by Sydney rock oyster larvae and spat when the new microbial cultures were used as partial algal substitutes were somewhat disappointing. It is thought that poor digestibility of the yeast cell walls is the major factor preventing inclusion of yeasts in oyster diets. To address this issue, $10 000 has been allocated by the Aquaculture CRC to pursue further research and evaluate new marine yeasts as aquaculture feeds.

2 BACKGROUND

Commercially important oysters are farmed under natural conditions and grow on diverse natural diets, which vary depending on season, location and environmental conditions. However, in hatcheries algal diets have been used since the 1940's. The dependence upon the various algal species for feed during the hatchery and nursery phases has caused problems due to high cost and unpredictable supply. This has prompted a search for non-algal food materials, however until the start of this study (July 1990) no satisfactory non-algal diet is available for commercial bivalve culture. Similarly, food cost has been a major limitation to the commercial development of intensive controlled oyster culture.

Various studies have shown that the algal content of adult diets for scallops, mussels, clams and oysters can be reduced by up to half by using

compressed and dry yeasts. The yeast *Candida utilis* has been used successfully to replace up to 50% of the algae in the diet of the American oyster *Crassostrea virginica* spat. In addition research by NSW Fisheries has shown that adult *S. commercialis* can be variably fattened solely on diets containing protein from two types of microbe, the yeast *Candida utilis* and the bacterium *Methylophilus methylotrophus* as its commercial spray dried preparation Pruteen®.

On the basis that such heterotrophic organisms can be used as oyster feeds at different stages of their life history, the major attraction is that yeasts and bacteria can be produced much more rapidly, efficiently and economically ie with a much higher `productivity' than photosynthetic algae because of shorter generation times, higher cell densities and inexpensive growth substrates. Yeasts and bacteria have much faster growth rates than algae (doubling times typically 0.5-4 h compared with 1-4 d for algae).

Successful supplementation of algal diets with the inclusion of pure or mixed cultures of microbes has shown the potential for significantly reducing the cost of live food diets for oysters. This prompted the application to FRDC for the funding of this study.

3 OBJECTIVES

a. To isolate, grow and evaluate marine yeasts, bacteria and other micro-organisms (preferably heterotrophs) for suitability as live feeds for aquaculture and for rapid, low-cost, large-scale production.

b. To screen selected microorganisms for absence of toxicity at a suitable laboratory.

c. To analyse promising micro-organisms for nutritional quality in particular content of protein, total lipids, fibre, carbohydrates, ash, fatty and amino acids, minerals and vitamins at suitable laboratories.

d. To carry out nutritional trials to assess the quality and suitability of selected micro-organisms as food for bivalve larvae, rotifers and for feeding/fattening of adult bivalves, in particular oysters.

4 DESCRIPTION OF INTELLECTUAL PROPERTY and TECHNICAL SUMMARY OF ALL INFORMATION DEVELOPED

All information obtained during the life of the project is contained in this report and will be published in the scientific literature. The most promising and interesting culture have been lodged with the "Australian Collection of Microorganism" at the University of Queensland, St Lucia, Qld and can be obtained by any microbiologist.

5 COVERING LETTER TO FRDC

Brackish Water Fish Culture Research Station
NSW Fisheries
Taylors Beach Road
Salamander Bay NSW 2301

Fisheries Research and Development Corporation
PO Box 9025
DEAKIN ACT 2600

June 1994

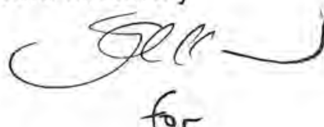
Dear Sir

I hereby submit the Final Report for Project "Isolation growth and evaluation of marine yeasts and bacteria as live foods for aquaculture; FRDC No 90/61". All the objectives as set out in the original research grant application have been achieved. The growth rates obtained by Sydney rock oyster larvae and spat when the new microbial cultures were used as partial algal substitutes were somewhat disappointing. It is thought that poor digestibility of the yeast cell walls is the major factor standing preventing inclusion of yeasts in oyster diets. To address this issue, $10 000 has been allocated by the CRC for Aquaculture to pursue further research and evaluate mew marine yeasts as aquaculture feeds.

Summary of total funds and other contributions to this project:

	$
FRDC	377 952
NSW Fisheries	221 000
Agritechnology Pty Ltd	128 000
Total	726 952

Yours sincerely



Dr John A Nell

6 ISOLATION OF NEW MICROBES

a **Isolation of various microbes from Port Stephens, NSW**

S P Nearhos

NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, NSW, 2301, Australia

SAMPLING

Only northern Port Stephens sites were sampled in the pilot study. These sites were selected because of their relative remoteness from dense human populations and so too from associated nutrient rich effluent and enteric bacteria. Five sites were chosen (Map 1): Site 1. Carrington; Site 2. Fame Point; Site 3. Pig Station #3; Site 4. Inner Corrie Island, and Site 5. Outer Corrie Island.

The sites represent a progression from inner through mid- and outer- Port Stephens' waters and, although water mixing and circulation studies were not to hand, the gross geography and so sedimentation patterns are consistent with these delineations. Oyster industry activities are largely confined to mid- and inner- Port Stephens, respectively these are generally used for spat catching and oyster grow-out.

The Carrington site (1) was on a rocky shore adjacent to a mud flat and oyster fattening leases. Here samples were taken from abiotic (water and sediment), and biotic (oysters, marine algae and various mangrove parts: leaves; roots and inter-tidal bark) materials. Fame Point (2) is an intermediate site between inner- and mid- Port Stephens' waters. Samples collected here included water, sediment and algae. Pig Station #3 is a site (3) where oyster fattening is conducted. At sites (4) and (5) corresponding to inner- and outer-sites on Corrie Island, materials sampled included water, sediment and seagrass. A further sample of mid-channel plankton material was collected using a 100 μm plankton net. At each site additional measurements were made for temperature, pH and salinity. All samples were labelled and stored at 4° C in bottles or plastic bags.

SAMPLE TREATMENTS

Sample materials were processed through shaken and stationary enrichments and by direct inoculation to selective media.

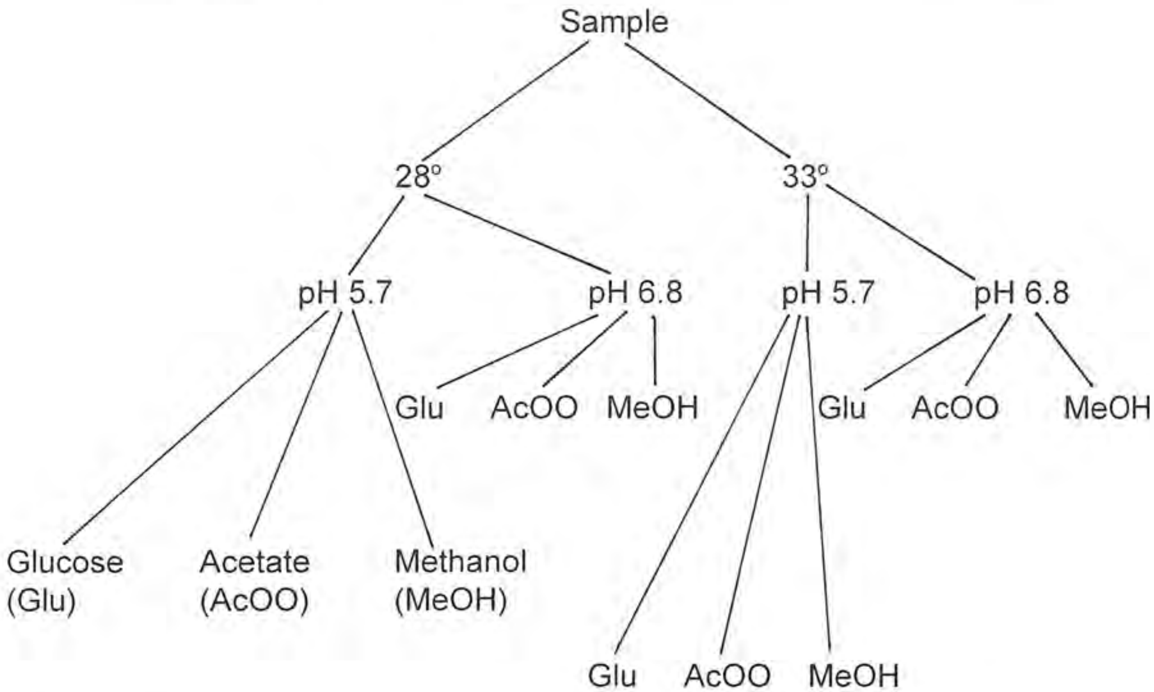
Shaken enrichment

Experimental Design

It was proposed that environmental samples be exposed to chemically defined media altered according to temperature, pH and carbon source so as to in shaken incubation, selectively enrich for microbes; specifically yeast (low pH), or bacteria (high pH), favoured under such conditions.

Five sediment, three water and one plankton samples were processed through two water-cooled shaker incubators, (Paton Scientific), these operated at 28° and 33° C. Additional selective pressures were imposed in these by variation in a chemically defined medium (MacLennan) of pH and carbon source. These procedures are summarised in Figure 1.

FIGURE 1 Sample Treatments Through Enrichment Cultures in Shaker Incubators.



A total of ninety two, 250 ml erlenmeyer flasks were used. Each flask contained 80 ml defined medium prepared as in Figure 1. Sediment samples were inoculated into all 12 enrichment treatment permutations in portions of *ca.* 10 g wet weight. Three water samples, from Carrington, Pig Station #3 and Inner Corrie Island were membrane filtered (0.45 μm) with pads in 300 ml portions. These filters and pads were aseptically cut into eighths and equal parts were distributed to 8 flasks of culture media varied according to pH and temperature but containing only glucose or acetate as carbon sources. Eight similar flasks were each inoculated with a 4 ml portions from the plankton suspension. Flasks in these incubators were set to shake at 100 RPM and then incubated 5d at respective temperatures.

Stationary Enrichment

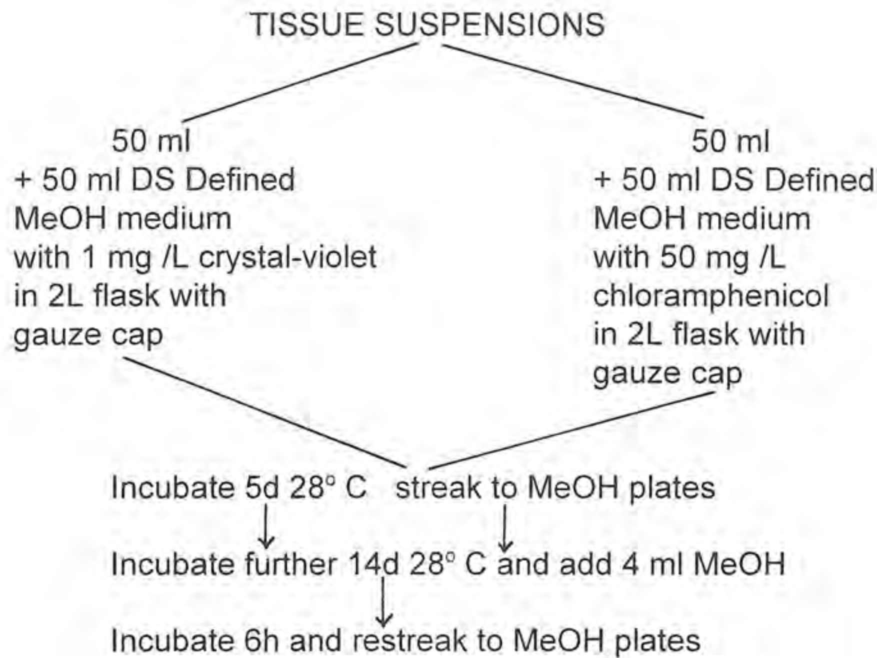
Experimental Design

Enrichments were prepared using similar volumes of methanol-containing defined medium, but instead ensuring aerobic conditions through the high surface-area to volume ratio which resulted from using 2L gauze stoppered flasks. Two further selective criteria were imposed; the first treatment group used crystal violet to favour Gram-negative over Gram-positive bacteria, the second used chloramphenicol as a selective inhibitor to favour yeasts over bacteria. Samples processed through these enrichments were plant or animal tissues not screened (except plankton) through shaking culture enrichments.

Tissue samples were placed in 100 ml sterile phosphate buffered saline (PBS) at pH 7 and macerated or rinsed by treatment with a tissue homogeniser (ultraturrrax). Tissue suspensions (50 ml) were dispensed aseptically to an equivalent volume of double strength (DS) methanol (MeOH) enrichment broth prepared as shown in Figure 2.

Two series of culture inoculations were performed, the first was to recover all isolates possible and the second, after all carbon source was depleted, was timed to recover the fast growing organisms which responded to a fresh dose of methanol.

FIGURE 2 Sample Treatment Through Stationary Enrichments



The plant and animal tissue samples audited by this procedure were from seaweed, mangroves leaves, oysters, mangrove bark and roots, brown algae, seagrass, plankton and a gastropod egg-sac.

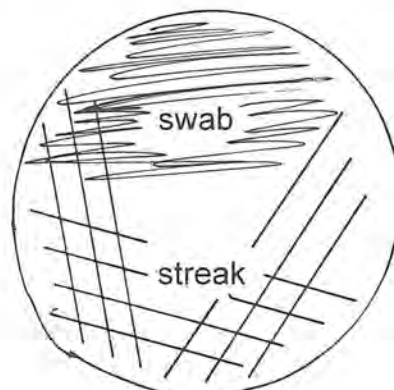
Direct isolation

Experimental Design

Direct isolations were made on to complex media with standard formulations to select for yeasts (chloramphenicol) and Gram-negative bacteria (crystal violet) and also on to Methanol defined medium plates. Rose-bengal Chloramphenicol agar was selected for yeasts and Difco marine agar with 1 mg/l crystal violet selected for Gram-negative bacteria.

Inoculations were performed as shown below by first swabbing then streaking plates.

FIGURE 3 Inoculation Pattern Used for Direct Isolation Media



Direct inoculations were prepared from all water and sediment samples and tissue samples inoculated to stationary enrichments.

Media were incubated at 28° C for the periods: Crystal violet marine agar 48 h , MeOH and Rose Bengal Chloramphenicol agar medium 7d.

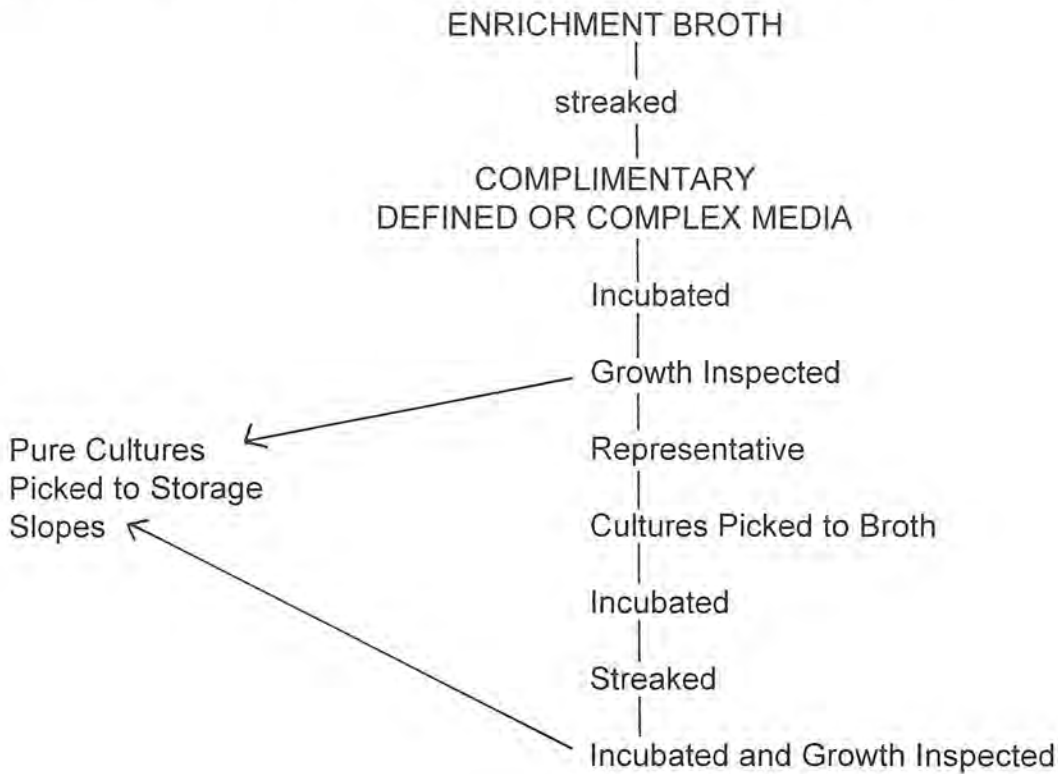
MICROBIAL ISOLATION AND PURIFICATION

Defined and complex culture media were prepared as broths in 7 ml culture bottles and as solid media in petri dishes. Defined media were colour-coded for easy carbon source recognition .

Shaken and stationary culture enrichments were purity streaked onto complimentary solid agar media. Plates were incubated up to 5d and representative colony types were picked into storage slopes if pure, or if purity was doubtful, picked into broths, reincubated and then purity streaked.

Representative cultures of each colony type from direct isolation media were picked into broth and purity streaked as above. These procedures are summarised in Figure 4.

FIGURE 4 Flow Chart For Microbial Isolation and Purification



SCREENING PROCEDURES

Isolates in pure culture were processed through a screening procedure which posed the following questions:

1. What is the broad taxonomic grouping of the isolate?
2. Does the isolate have pathogenicity markers?
3. Does the isolate produce toxins which might poison or otherwise affect the feeding organism?
4. What is the feed value of organisms screened through steps 1-3?

Screening characters

A Description of Colonies on Solid Media.

- colour
- shape
- size
- texture
- odour
- pigment production
- luminescence
- agar hydrolysis
- swarming growth

B Description of Cells by Microscopic Examination.

- size
- shape
- motility
- flagellar insertion mode
- Gram state

for filamentous forms:

- hyphae septate
- filamentous / discrete cells
- conidial form
- birth scar
- splitting cells

C Biochemical Features.

Catalase
Oxidase
Oxidation / Fermentation

The colony, cellular and biochemical features listed above will allow some sort of identification handle to be placed upon isolates. In some cases this information can be used to exclude groups of organisms deemed to be unsuitable. Further groups of organisms found in subsequent procedure to be acceptable may then be specifically sought according to these, or more refined identification procedures.

Pathogenicity markers

Four pathogenicity screening assays were selected. These are casein hydrolysis, colony colour on congo red medium, growth in the presence of EDDA and neuraminidase production. The assay for casein hydrolysis is a test for protease. Generally pathogenic micro-organisms are able to produce such proteolytic enzymes. Most non-pathogens also can produce proteases, however non-protease producers almost without exception are non-pathogenic or not pathogenic in the conventional sense.

The selection of colony colour on congo red medium was because this substrate has been suggested previously as both an indicator of nitrogen fixation (white) and of pathogenic strains (red).

A common symptom of microbial infections is anaemia, one of the causes of this symptom is competition for limiting quantities of iron between the host and invading microbes. Both produce specific proteins, siderophores, which chelate or bind to these scarce iron molecules. Ethylene diamine diphenylacetic acid (EDDA) has an iron binding affinity similar to the hosts natural proteins ie. transferrins and lactoferrins. If an organism then is able to grow under conditions of limited iron, in the presence of this compound, the inference can be that it may also have the potential to become a pathogen if infection occurs. The selection in our case is for organisms which cannot grow in the presence of EDDA.

At the cellular level an integral component of pathogenesis is adherence. A measure of this adherence facility is in the final assay for neuraminidase. This is an assay for an enzyme which is active towards the types of polysaccharides found on outside-cell-surfaces and in mucous. Organisms which produce neuraminidase are better able to bind to such surfaces and invade the host. This assay has particular relevance to invasion via respiratory and gastro-intestinal surfaces.

STATUS OF CULTURES AND SCREEN PROGRESSION

Rose bengal Chloramphenicol medium seemed highly selective for fungi over yeasts. All but 4 cultures isolated from this medium have been discarded. All but one or two isolates from crystal-violet marine agar are in pure culture and screening has commenced on these.

Some cultures from defined media have yet to be picked (methanol stationary enrichment), all others have been grown on respective carbon sources on broths and incubated after purity streaking. A large proportion of this latter group, particularly those grown on glucose need to be reprocessed to purity. Screening will commence on this group when *ca* 80% of cultures are pure.

A time estimate for purification and screening has been made (section 7.).

TAXONOMY OF THE CULTURES

The new bacteria and yeast cultures were identified by Dr S Nearhos, when he was employed by NSW Fisheries.

ORIGIN AND NUMBER OF CULTURE ISOLATES

			N° of Isolates
Shaking Enrichments:			
- Defined Medium with:	Methanol		66
	Acetate		57
	Glucose		89
		Total	212
Stationary Enrichments:			
- Defined Medium with :	Methanol		
Colonies yet to be picked ~ possible			75
Direct Isolations:			
- Defined Medium with methanol			44
- Complex medium (crystal-violet marine agar)			49
- Complex medium (Rose-Bengal Chloramphenicol Medium)			12
Total Potential Estimated Isolates (Subject to further culture picking and purification)			392

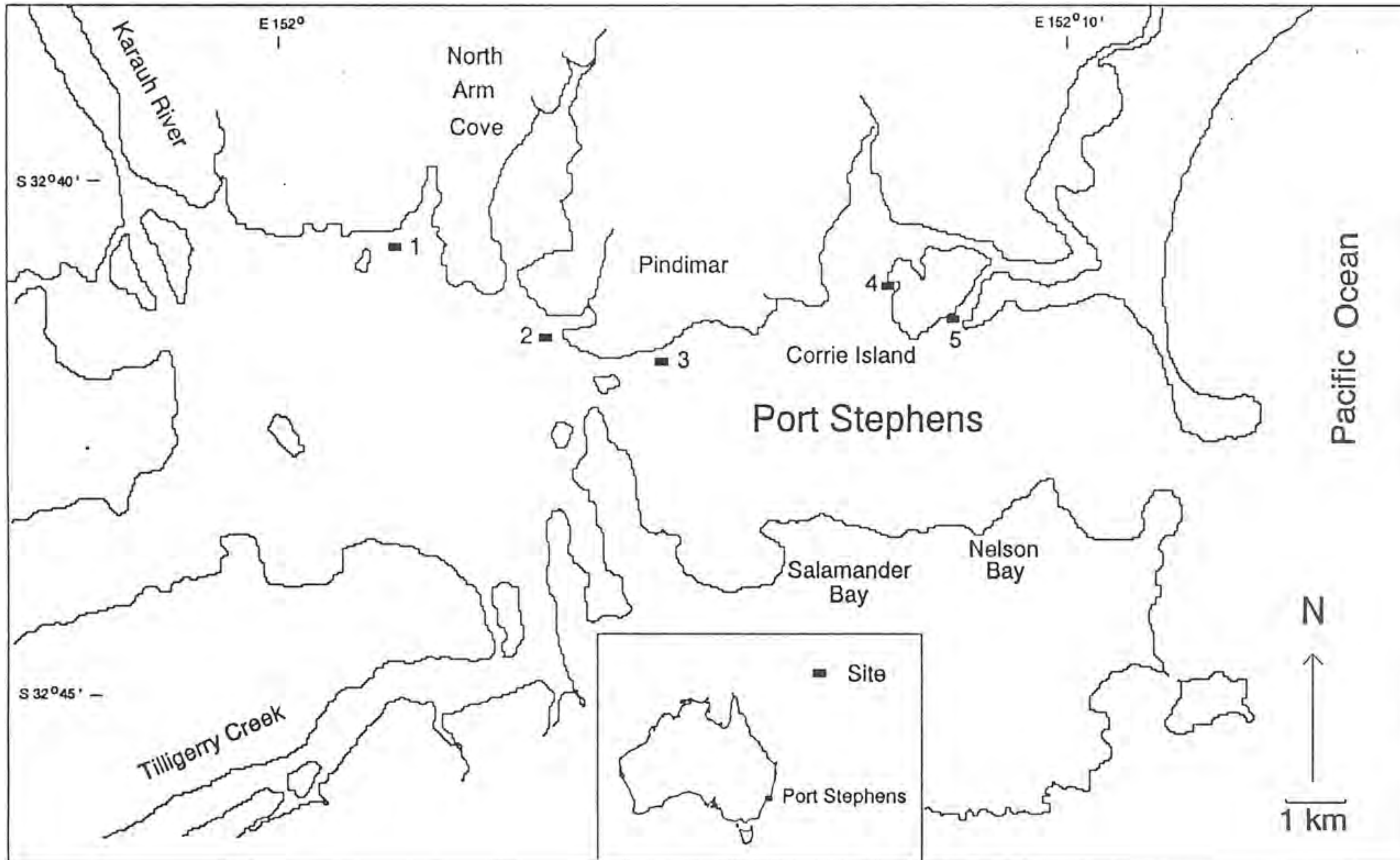
TIME ESTIMATES FOR RESIDUAL CULTURE ISOLATION, PURIFICATION AND SCREENING (DAYS)

Task	Days
Colony picking	1
Media preparation and culture purification	5
Colony descriptions	4
Cellular descriptions	10
Biochemical	6
Pathogenicity	11
Toxicity	7
Storage slope inoculation	2
Result tabulation	4
Strain selection	2
Reporting	3
Total estimated days	55

ACKNOWLEDGEMENT

S. McOrrie is thanked for his navigation through the sampling expedition.

(Map 1) Inner and Outer Port Stephens sampling sites: Site 1. Carrington; Site 2. Fame Point; Site 3. Pig Station; Sites 4 & 5 Inner and Outer Corrie Island (resp.).



b Cytotoxicity assays on samples from NSW Fisheries, 1993

CYTOTOXICITY ASSAYS ON SAMPLES FROM NSW FISHERIES, 1993

1. Background

The Bioanalytical Research Group was approached by Dr. Steven Nearhos to carry out cell cytotoxicity assays on bacteria and other organisms being developed by the NSW Fisheries as possible food sources for oysters. The NSW Fisheries wished to determine if any of these organisms were producing toxic substances which would be able to act on mammalian cells. The *in vitro* assays were seen as an alternative to assays on live animals.

2. *In vitro* Assays

(a) Cell Culture: Cytotoxicity assays on cells in tissue culture were carried out using the membrane filter procedure described by Lye and Dufour (Journal of Applied Bacteriology, 1991, Vol.70 pp.89-94).

(b) Haemolysis Assay

Analysis of the haemolytic activity of supernatants of broth cultures of the microorganisms supplied was determined using a standard haemolysis assay in use in our laboratory. In brief, this assay was carried out by preparing three times washed suspensions of red blood cells from each of the species sheep, rabbit, horse and human and then incubating these red blood cells with aliquots of culture supernatants. Haemolysis was determined by centrifugation of the incubation tubes and measurement at 530 nanometers using as reference, a standard curve generated by the release of haemoglobin by lysis of the appropriate red cells with water. Greater than 10% haemolysis was considered positive.

(c) Organisms

Sixteen coded organisms were provided for analysis. The organisms were provided in the form of cultures growing on filter papers on agar medium in sterile petri dishes as well as broth cultures in 20ml glass vials.

(d) Five cell culture assays were carried out using the Hep2 cell line and the Yac cell line.

Two haemolytic assays were carried out on the broth cultures provided.

3. Results

Organisms	Cell Culture Assays	Haemolysis Assay
Methylophilus methylotrophus	Cytotoxic in all five assays	Positive
Candida utilus	Weak cytotoxicity in one assay	Negative
Saccharomyces cerevisiae	Vey weakly cytotoxic	Negative
670A	Very weakly cytotoxic	Negative
760	Weakly cytotoxic	Negative
810	Strongly cytotoxic	Positive
1212	Strongly cytotoxic	Negative
1553	Cytotoxic on glucose medium	Negative
1620	Weakly cytotoxic	Negative
1930	Weakly cytotoxic	Negative
1950	Strongly cytotoxic	Positive
2040	Cytotoxic on glucose medium	Negative
2360	Cytotoxic	Negative
2750A	Weakly cytotoxic	Positive
3007	Weakly cytotoxic	Negative
4570	Strongly cytotoxic	Positive

Conclusions

The following organisms should be considered to be toxin producers. *Methylophilus methylotrophus*, 810, 1950 and 4570.

The following organisms show some toxicity: - 2040 and 2360.

Only trace levels of cytotoxic activity have been found in the following organisms and it would appear that they are of relative insignificance in terms of toxin production using our assays. These are Candida utilus, Saccharomyces cerevisiae, 670A, 760, 1553, 1620, 1930, 2750A and 3007.

Associate Professor T.K. Roberts
22 November 1993

THE University OF Newcastle

Bioanalytical Research Group
Phone (049) 215630
Fax (049) 216923

Dr John A Nell
Senior Research Scientist
NSW Fisheries
Post Office
Salamander Bay
NSW 2301

19/01/95

Dear John,
Re: Cytotoxicity assay on samples from NSW Fisheries, 1993

In reply to your request for more information on the experiments carried out in my laboratory I am pleased to answer as follows:

Q 1. What suite of assays were done on which microbes by whom?
There were two types of assay carried out on each of the 16 organisms listed in the Results table of the original report. The assays were to detect the ability of the organism to release products which were capable of causing cell death and detachment in the case of the cell culture assay, or haemolysis of red blood cells in the case of the haemolysis assay. In the case of the cell culture assay we used the membrane filter procedure involving growth of solid colonies of the organisms on agar, and in the case of the haemolysis assays we used the broth cultures provided.
All the assays were carried out by Associate Professor Tim Roberts.

Q 2 What is the difference between and significance of the three assays
In fact as I have alluded above there were only two types of assays done in this study. The description given in the method section of my report has lead to the confusion and I am sorry for this. The first assay was the cell culture assay and that was done using the bacteria grown on the agar discs being tested for their ability to kill target cells of two types (HEP2 and Yac) grown in plastic tissue culture plates. The second assay was the red cell haemolysis assay and that was done by testing the ability of broth culture supernatants of the 16 organisms to lyse sheep, rabbit, horse and human red cells in liquid suspension.
The significance of the cell culture assay is that it detects toxins released by living organisms. Such toxins are detected by their ability to cause cells in tissue culture to die and to loose their adherent capability. It is possible that a diverse range of mechanisms could result in loss of adherence. The haemolysis test on the other hand detects membrane active molecules that disrupt the stability of the membranes leading to cell lysis.

Please contact me if you require any further information.

Yours sincerely,

Assoc. Prof. T.K. Roberts.

The University of Newcastle, University Drive, Callaghan, Newcastle, NSW 2308, Australia. Telephone (049) 215000

c Taxonomic designations and corresponding Australian Collection of Microorganism (ACM) numbers (University of Queensland, St Lucia, Qld)

BWFCRS Microbial Food Cultures.

NSW Fisheries Number. Bacteria.	Australian Collection of Micro-Organisms Number*.	Name	Authors	Synonyms /proposed
631	ACM 4767	*Pseudomonas testosteroni*	Marcus and Talalay, 1956	*Acidovorax testosteroni (comb. nov.)* Nearhos & Nell.
670A	ACM 4768	*Pseudomonas testosteroni*	Marcus and Talalay, 1956	*Acidovorax testosteroni (comb. nov.)* Nearhos & Nell.
760	ACM 4769	*Pseudomonas testosteroni*	Marcus and Talalay, 1956	*Acidovorax testosteroni (comb. nov.)* Nearhos & Nell.
810	ACM 4770	*Pseudomonas sp.*		
2360	ACM 4771	*Aeromonas sp.*		Relaxation of genus criteria. Nearhos & Nell.
2750A	ACM 4772	*Derxia sp.*		Description Nearhos & Nell.
3007	ACM 4773	*Derxia sp.*		Description Nearhos & Nell.
Yeasts.				
1620	ACM 4779	*Dipodascus capitatus*	de Hoog et al. 1986.	*Geotrichium or Trichosporon capitatum.*
1930	ACM 4780	*Dipodascus sp.*	Description.	*Geotrichium or Trichosporon* spp.
1950	ACM 4781	*Dipodascus sp.*	Description.	*Geotrichium or Trichosporon* spp.
2040	ACM 4782	*Dipodascus sp.*		*Geotrichium or Trichosporon* spp.
1553	ACM 4784	*Debaryomyces hansenii*	Lodder & Kreger-van Rij, 1952.	*(Candida famata)*
1201	ACM 4776	*Dipodascus sp.*	Description.	*Geootrichium or Trichosporon* spp.
1210	ACM 4777	*Dipodascus sp.*		*Geotrichium or Trichosporon* spp.
1212	ACM 4778	*Dipodascus sp.*		*Geotrichium or Trichosporon* spp.
1490	ACM 4783	*Issatchenkia sp.*		*Pichia* sp.
UM 468	ACM 4774	*Candida utilus*	(Henneberg) Lodder et Kreger-van Rij (1952), or *Pichia jadinii* (Sartory et al. Kurtzman, 1984).	
AWRI Y77	ACM 4775	*Saccharomyces cerevisiae*	Meyen ex Hansen (1883).	

* University of Queensland.

7 EVALUATION OF FOOD VALUE OF NEW MICROBES

a Evaluation of new microbial foods as partial substitutes for microalgae in a diet for Sydney rock oyster *Saccostrea commercialis* larvae and spat

John A. Nell[a], David G. MacLennan[b], Geoff L. Allan[a], Steven P. Nearhos[a,1] and Jane Frances[a]

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[b] Agritechnology Pty Ltd, St Ives, NSW, 2075, Australia

[1] Present address: Baseline Pty Ltd, 85 Laipraik, St Albion, Queensland, Australia 4010

ABSTRACT

Newly isolated cultures of live marine bacteria were used to partially replace (86% of dry weight) a mixed diet of three species of microalgae for Sydney rock oyster *Saccostrea commercialis* larvae. The new diet of bacteria and algae produced increases in shell width of up to 67.2% of those obtained by the fully algal fed controls. Newly isolated live marine yeast cultures were also used to partially replace (86% of dry weight) the mixed microalgae diet for oyster spat. The new yeast and algae diet produced whole dry spat weight increases of up to 81.4% of those obtained by the fully algal fed controls. The use of live yeast cultures as partial replacements for live algae in diets for both juvenile (spat) and adult oysters has commercial potential.

INTRODUCTION

Bivalve hatcheries depend almost entirely upon the use of various strains of live mircoalgae for the hatchery and nursery phases (Coutteau and Sorgeloos, 1992) and the cost of algal production is a major limitation to the commercial development of hatchery-based oyster culture (Epifanio, 1975; Claus, 1981). The high cost and unpredictable supply of algae has prompted a search for non-algal food materials, however, to date no satisfactory non-algal diet is available for commercial bivalve culture (Urban and Langdon, 1984). Recent advances have been made in the development of encapsulated diets (Langdon, 1989; Langdon and DeBevoise, 1990; Numaguchi and Nell, 1991; Southgate et al., 1992) and although these should allow a more precise determination of the nutrient requirements of oysters than has been possible so far (Nell, 1992, 1993), they are not yet a viable alternative to live algae.

For bivalve larvae, supplementation of algal diets with pure or mixed cultures of both live bacteria (Martin and Mengus, 1977; Douillet and Langdon, 1993) and dry

bacteria (Nell and Wisely, 1983, 1984) has shown the potential to significantly reduce the cost of traditional diets for oysters. Dry yeast *Candida utilis* has been used successfully as the protein source in a starch based `fattening' diet for Sydney rock oysters *Saccostrea commercialis* (Nell, 1985) but live yeast or bacteria have not been evaluated for this species. Partial substitution of diets of live algal cells with 25 to 50% dry yeast *C. utilis* cells produced weight increases ranging from 60 to 90% of those obtained by fully algal fed juvenile American oyster *Crassostrea virginica* spat (Epifanio, 1979; Urban and Langdon, 1984). When live algal cells were substituted by either live *C. utilis* cells or *C. utilis* with cell walls removed by enzyme digestion, weight increases of Pacific oyster *C. gigas* spat were 74 and 89% of that of the fully algal fed controls respectively (Coutteau et al., 1993). This showed that the food value of yeast cells for oysters can be improved by enzymatically removing their cell walls.

In the wild, bacteria contribute a significant proportion of oysters' metabolic requirements for carbon and nitrogen (Crosby and Newell, 1990; Langdon and Newell, 1990). Despite this, and the aforementioned encouraging results with bacteria and yeasts, no attempts have been made to evaluate the nutritional quality of live bacteria or yeasts isolated from marine or estuarine areas.

In this study bacteria and yeasts from estuarine oyster growing areas which were capable of growing on simple, defined media were isolated. Those cultures evaluated as having low potential pathogenicity to humans (S. P. Nearhos and T. K. Roberts, unpublished data, 1994) were further evaluated as partial substitutes for live algae in diets for *S. commercialis* oyster larvae and spat.

MATERIALS AND METHODS

New microbial foods

Isolations of bacteria and yeasts were made from water and sediment collected in oyster growing areas and other locations in Port Stephens, NSW (32° 45'S, 152° 10'E) in February 1991. Microbes which grew on inexpensive media based on glucose, acetate or methanol were selected. 378 cultures were isolated and purified, 124 on glucose based media, 89 on acetate and 165 on methanol. Pure cultures were screened to exclude those with primary identification characteristics of problem groups of microbes which includes typical `spore formers', *Enterobacteriaceae* and *Vibrionaceae*. Apart from these taxonomic tests the potential pathogenicity of the new microbes relative to a known safe organism *Saccharomyces cerevisiae* was determined.

The chemically defined medium adopted from Aiking and Tempest (1976) was supplemented with a vitamin solution (Barnett et al., 1983), 0.1% yeast extract (Oxoid L21) and 0.85% sodium chloride. This medium was used for all cultures except for the *Methylophilus methylotrophus* media which did not contain sodium chloride. Media were sterilised in 0.9 l portions in 2 l conical flasks fitted with

bubblers and 0.2 μm inlet and exhaust vent-filters (Gelman ACRO TF 4464; Gelman Sciences Inc., Ann Arbor, Michigan). Media for glucose and acetate cultures were supplemented with 100 ml sterile 10% (w/v) carbon source while methanol utilising microbes were supplemented with a total of 11.5 ml of 0.2 μm filter sterilised methanol incrementally delivered by micropipette over 3 d. Bacteria were grown to stationary phase and yeasts to log phase.

Microbe cultures were harvested by centrifugation at approximately 1000 g for 15 min, resuspended in culture medium lacking yeast extract and trace metals and centrifuged at 1000 g for an additional 15 min. The washed cells were resuspended in about 30 ml of fresh culture medium. Dry cell weight densities for each microbe were determined using calibration curves for turbidity against measured dry cell weights from cultures of the same species of the same age. For Experiment 3, yeast cell walls were digested using a commercial preparation of lyticase (Sigma, St Louis, MO) in a procedure adapted from Wolska-Mitaszko et al. (1981). Dosage was determined by microscopic observation of Gram stained and bright field preparations. Digestions were conducted by resuspending washed cell pellets in 1.2 M pre-warmed sorbitol and supplementing with 19 μl enzyme mg^{-1} equivalent dry cell weight. After 1 h digestion was stopped by cooling the reaction vessel in iced water.

Algal culture techniques

The algal control diet and supplement consisted of *Isochrysis* aff. *galbana* (clone T-iso; termed Tahitian *Isochrysis*), *Pavlova lutheri* and *Chaetoceros calcitrans* on an equal dry weight basis. These algal cultures were produced axenically in 5 l borosilicate glass flasks or 20 l polycarbonate carboys. Culture conditions, cell count determinations and dry weights used were based on those of Nell and O'Connor (1991). Larvae and spat were fed daily.

Feeding/screening trials

Four large-scale feeding/screening trials were conducted with Sydney rock oyster larvae. A miniaturised experimental oyster larval rearing system was developed to test approximately 149 bacterial and 33 yeast cultures which passed through the pathogenicity and identification screens. The system consisted of 250 ml flasks held in one of two orbital-shaker incubators (Model 013422; Paton Scientific; Victor Harbor, South Australia) which could hold 54 flasks and which rotated at 100 revolutions/min. Each flask held 100 ml seawater containing approximately 500 two-day-old, D-stage larvae.

Each feeding/screening trial included an unfed treatment, a treatment fed a full algal ration (0.5 mg dry weight algae ml^{-1}) and a treatment fed an algal supplement only (0.1 mg dry weight algae ml^{-1}). The other treatments were monocultures of bacteria or yeast (0.5 mg dry weight ml^{-1}) fed in combination with the algal supplement. For each treatment, duplicate flasks were used, one in each incubator. Water in the flasks was maintained at 25.0±0.5°C and changed every 48 h with larvae being

retained on a 45 μm (diagonal) screen. Larvae were fed daily. These procedures were used to produce a short-list of the most promising cultures for larval food. Seven bacterial cultures were selected on the basis of larval or spat growth and were used for further evaluation. With larvae, bacteria were usually better feeds than yeasts, possibly because larvae did not have well enough developed guts to digest the thick yeast cell walls.

Feeding/screening trials with spat were conducted in 8 l aquaria each with 30 or 40 spat held in a 90 x 110 mm plastic mesh bag (1.8 mm [diagonal] mesh). Initial dry weight of spat for all these trials ranged from 2.2-8.0 mg. For the first feeding/screening trial with spat, the two bacterial and two yeast cultures which gave the best growth of larvae were used. Other treatments included an unfed control, spat fed a full algal ration, spat fed an algal supplement only and spat fed monocultures of yeast or bacteria plus the algal supplement. Daily feed rations were determined accordingly to the equation $Q_R = 0.01\ W^{-0.33}$, where Q_R is dry weight (g) of ration/live weight of oyster (g) and W is the live weight of bivalve (g) (Epifanio, 1979). The supplement was 14% of the full algal ration and the dry weight of yeast or bacteria was 86%. Four replicate aquaria, randomly allocated, were used for each treatment.

Growth of spat fed bacteria plus the algal supplement was no different from growth of spat fed the supplement alone. Although larvae could clearly utilise bacteria this was not evident with spat. Oyster larvae are capable of filtering smaller particles than spat (Wilson, 1980) and the spat used here may not have been able to retain the bacteria. Conversely, the yeast cultures showed promise.

Subsequently all 33 yeast cultures were screened using the procedures described above except that only two aquaria per treatment were used. A short-list of nine yeast cultures, selected on the basis of spat growth, were chosen for further evaluation.

Oyster larvae (Experiment 1)

Oyster larvae with initial shell widths ranging from 74.4-75.5 μm (mean ± SD of four samples of 50 larvae each was 75.0±0.45 μm) were obtained from oysters spawned according to techniques described by Holliday (1985). Two-day-old D-stage oyster larvae were stocked in 8 l aquaria with four replicates per treatment at a density of 5 larvae ml^{-1}. Temperature and water exchange was as described for the Feeding/Screening Trials. Growth of oyster larvae fed the following 12 diets was compared: full algal ration, 14% algal supplement ([dry weight basis] of the full algal ration), bacterial isolate ACM 4773, algal supplement plus *M. methylotrophus* (AS1=NRRC B539=NCIB 10515) and seven other diets consisting of the algal supplement plus one of seven newly isolated bacterial cultures (Table 1). Except for the algal supplement only diet, all diets had the same dry weight.

Feeding rates were as recommended by Frankish et al. (1991). The experiment was terminated after 16 days and larvae were preserved in seawater containing 10% buffered formalin. The width (greatest distance parallel to the hinge) of >50 preserved larvae per aquarium was measured (±0.5 μm) using a microscope and a calibrated micrometer slide.

Oyster spat

Two 16-day experiments were carried out with four replicate 8 l aquaria per treatment. In each aquaria, 30 spat were contained in a plastic mesh (1.8 mm [diagonal]) bag. The yeast cultures were used to substitute 86% of the dry weight of the algal diet.

For both spat feeding experiments (Experiments 2 and 3) the daily feed rations were determined as for the Feeding/Screening Trials for spat. The live weight of spat fed each diet was determined weekly and the feed rates for spat were adjusted as described. At the end of each experiment, spat were dried in a force draught oven at 110°C for 24 h and weighed. Average individual weight gain was calculated on a whole (meat and shell) dry spat basis.

Experiment 2

Growth of oyster spat fed the following 14 diets or feeding regimes was compared; unfed, full algal ration, algal supplement (14% of the full algal ration) and another 11 diets comprising *C. utilis* (ACM 4774), *Saccharomyces cerevisiae* (ACM 4775) or one of nine newly isolated marine yeasts (Table 2) plus the algal supplement. Initial dry weight of spat (six samples of 30 spat each) was 4.5±0.24 mg.

Experiment 3

This experiment was conducted to evaluate the most promising yeasts identified in Experiment 2 as complete replacements for algae and to investigate whether enzyme digestion improved the nutritional value of the most promising culture.

Growth of oyster spat fed the following 15 diets (Table 3) or feeding regimes was compared: unfed, full algal ration, algal supplement (14% of the full algal ration), and another 12 diets comprising *C. utilis* (same strain as above), *S. cerevisiae* (same strain as above) or one of three newly isolated yeast cultures (one of which was also pre-digested with an enzyme). All yeasts were fed with and without the algal supplement. Initial dry weight of spat (six samples of 30 spat each) was 6.3±0.52 mg.

Statistical analysis

The data were analysed by ANOVA, homogeneity of variance was confirmed using the Cochran test (Winer, 1971) and the Student-Newman-Keuls test was used to compare means (Sokal and Rohlf, 1981).

RESULTS

Experiment 1

By far the greatest shell width increase was recorded for fully algal fed larvae (Table 1). However, when larvae were fed the bacteria cultures plus a 14% algal supplement the increases in shell width were equal to or better than for larvae fed only the algal supplement.

Complete or partial (86% by dry weight) substitution of the live algal diet with the newly isolated bacteria culture ACM 4773, produced increases in larval shell width of 24.6 or 67.2% respectively of that observed for the fully algal fed control (Table 1). Increases in shell width of oyster larvae fed any of the five other new bacteria cultures (plus the algal supplement) ranged from 52.8 to 61.3%, whereas *M. methylotrophus* (plus the algal supplement) produced 46.5% which was virtually no improvement compared with that obtained with the algal supplement (43.7%; Table 1).

Experiment 2

The spat fed the full algal ration had the greatest weight increase (Table 2), however, the best new yeast, plus the algal supplement, produced 81.4% of this increase. The weight increases in spat fed any of the other eight newly isolated yeast cultures (plus the algal supplement) ranged from 42.6 to 77.5% of that observed for the fully algal fed control, whereas *C. utilis* and *S. cerevisiae* (plus supplements) produce weight increases of 63.0 and 66.1% respectively. The weight increases obtained with the best of the new yeast cultures (ACM 4779, 4780, 4784, 4778, 4782 and 4781) were similar ($P>0.05$) to those obtained with *C. utilis* and *S. cerevisiae* and significantly higher ($P<0.05$) than those obtained with the algal supplement alone (Table 2).

Experiment 3

The spat fed the full algal ration had the greatest weight increase. However, when combined with the algal supplement, growth of spat fed any of the yeasts (except the one which had been digested with enzymes) was greater ($P<0.05$) than that from spat fed the supplement alone. Growth of spat fed any of the yeasts without the algal supplement was significantly less ($P<0.05$) than for spat fed the algal supplement alone (Table 3).

Growth of spat fed enzyme digested yeast culture ACM 4782 was similar ($P>0.05$) to growth of unfed controls, regardless of whether an algal supplement was used, and was significantly ($P<0.05$) less than growth of spat fed undigested cultures of the same yeast (Table 3).

DISCUSSION

The increase in growth of Sydney rock oyster larvae fed bacteria plus an algal supplement, compared with the increase for spat fed the algal supplement alone, demonstrates that the larvae are capable of digesting and utilising bacteria. This confirms the findings of Martin and Mengus (1977) and Douillet and Langdon (1993) for other bivalve larvae. Similarly, the beneficial effects of adding yeasts plus an algal supplement, compared with algal supplements alone, demonstrates that spat are capable of digesting and utilising yeasts.

For hatchery culture of Sydney rock oysters, replacing 86% of a very successful algal diet with a monoculture of bacteria or yeast produced up to 67.2% or 81.4% of the growth produced by the full algal ration. Mixing several species of bacteria together, as has been found beneficial for algae (Nell, 1992), may promote even better growth than when monocultures are used. A biochemical profile of the cultures tested during this study may indicate nutritional deficiencies with some of the bacteria or yeasts which may be addressed by formulating cocktails of different cultures. Martin and Mengus (1977), for the mussel *Mytilus galloprovincialis*, and Douillet and Langdon (1993), for the oyster *C. gigas*, found that adding bacteria to a full algal ration increased growth rates. This was not tested with Sydney rock oysters during the present study.

The partial substitution of the algal diet for Sydney rock oyster spat produced very similar results in the present study to those obtained for *C. gigas* spat (Coutteau et al., 1993). In Coutteau's study, enzyme digestion of live *C. utilis* cell walls improved spat growth, whereas in our study enzyme digestion of our most promising yeast greatly reduced spat growth. We incubated a yeast with lyticase until cell wall degradation could be observed under the microscope. It is possible that cells may have lysed, releasing their contents, rendering the material unavailable to the spat. Spat did not grow when the enzyme treated yeast was fed, regardless of whether an algal supplement was added. The enzyme treated yeast may have interfered with normal feeding behaviour.

The advantage of using enzymes to partially digest thick yeast cell walls are probably related to the thickness of the yeast wall. Careful enzyme digestion of *C. utilis* and other yeast cultures with thick cell walls should still be considered an option and requires further evaluation.

The results obtained with partial substitution of live algal diets with yeasts for spat were better than similar substitutions with bacteria for larvae. The much greater amounts of food required by spat than larvae greatly enhances the commercial application for the use of yeast in oyster diets. Growth of spat fed diets largely based (86%) on live yeasts as partial algal substitutes were less than that for spat on the fully algal fed control diet (mixture of three species). However, comparisons of yeasts with algal species of lower food value for Sydney rock oysters (Nell and O'Connor, 1991; O'Connor et al., 1992), some algal species concentrated to a paste

(Nell and O'Connor, 1991) or dried algae (Laing and Verdugo, 1991) are likely to produce more favourable results. Photosynthetically grown algae (as were used here) rarely attain maximum culture cell densities in excess of 5 g l^{-1} whereas industrial fermentation of heterotrophic yeasts and bacteria can produce cultures with densities of 40-140 g l^{-1} (Gladue, 1991). Heterotrophic production of algae can yield similar densities to those for industrially fermented yeasts or bacteria although the food value of heterotrophically grown algae has not been evaluated for Sydney rock oysters. Ultimately, the selection of a commercial diet is based on economic considerations. Optimum production conditions and production costs for the yeasts or bacteria used in the present study have not yet been determined but, especially with yeasts for oyster spat, may make the use of yeasts or bacteria as partial substitute for algae a cost-effective alternative to using algae as the total ration. Perhaps slight increases in the proportion of algae in the diet (>14%) may increase the food value of the yeast/algal mix.

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TABLE 1

Evaluation of bacteria as partial substitutes in diets for Sydney rock oyster *Saccostrea commercialis* larvae (Experiment 1).

Diets	Carbon source for bacteria culture	Size (μm)		16 day shell width[1] increase (μm)	Shell width increase[2] (%)
		Length	Breadth		
Algal fed control	-	4.0-8.5	3.1-5.4	142 ± 22^{d}	100.0
14% algal fed control	-	4.0-8.5	3.1-5.4	62 ± 4^{b}	43.7
Derxia sp. (ACM 4773)[3] without algae	glucose	1.2-1.5	0.2-0.3	35 ± 7^{a}	24.6
Derxia sp. (ACM 4773)[3]	"	1.2-1.5	0.2-0.3	95 ± 8^{c}	67.2
Derxia sp. (ACM 4772)[3]	"	1.0-1.2	0.3-0.5	87 ± 10^{bc}	61.3
Pseudomonas testosteroni (ACM 4767)[3]	methanol	0.9-1.2	0.3-0.5	62 ± 22^{b}	43.7
Pseudomonas testosteroni (ACM 4768)[3]	"	1.2-1.5	0.5-0.5	75 ± 6^{bc}	52.8
Pseudomonas testosteroni (ACM 4769)[3]	"	0.8-1.2	0.5-0.5	86 ± 14^{bc}	60.6
Pseudomonas sp. (ACM 4770)[3]	"	0.8-1.2	0.3-0.3	85 ± 9^{bc}	60.0
Aeromonas sp. (ACM 4771)[3]	"	0.6-0.9	0.3-0.3	84 ± 8^{bc}	59.2
Methylophilus methylotrophus, AS1, NRRL B5359 (NCIB 10515)	"	0.8-1.2	0.3-0.3	66 ± 14^{b}	46.5

[1] Means ± SD. Means with a common letter do not significantly (P<0.05). Initial shell width of larvae (four samples of 50 larvae each) was 75.0±0.45 μm.

[2] Expressed as a percentage of that observed for the algal fed control.

[3] Australian Collection of Micro-organisms Number, University of Queensland, St. Lucia, Qld 4072.

TABLE 2

Evaluation of yeasts as partial algal substitutes in diets for Sydney rock oyster *Saccostrea commercialis* spat (Experiment 2).

Diets	Carbon Source for yeast culture	Size (μm)		16 days dry weight[1] Weight increase (mg)	increase[2] (%)
		Length	Breadth		
Unfed control	-	-	-	5.0±0.3[a]	27.4
Algal fed control	-	4-9	3-5	18.2±2.5[f]	100.0
14% algal fed control	-	4-9	3-5	8.4±0.5[b]	46.1
Dipodascus capitatus (ACM 4779)[3]	Acetate	4-6	2-2	13.6±0.7[de]	74.8
Dipodascus sp. (ACM 4780)[3]	"	6-8	2-3	13.6±1.1[de]	74.6
Dipodascus sp. (ACM 4781)[3]	"	6-8	2-3	14.1±2.4[de]	77.5
Dipodascus sp. (ACM 4782)[3]	"	6-6	2-6	14.8±1.6[de]	81.4
Debaryomyces hansenii (ACM 4784)[3]	"	3-3	2-2	13.4±1.2[de]	73.8
Dipodascus sp. (ACM 4776)[3]	Glucose	6-8	2-4	7.8±0.7[b]	42.6
Dipodascus sp. (ACM 4777)[3]	"	6-8	2-4	9.3±0.7[bc]	51.2
Dipodascus sp. (ACM 4778)[3]	"	7-8	2-3	13.3±1.0[de]	73.3
Issatchenkia sp. (ACM 4783)[3]	"	2-4	1-2	9.3±0.7[bc]	51.3
Candida utilis (ACM 4774)[3]	"	5-7	3-3	11.5±0.4[cd]	63.0
Saccharomyces cerevisiae (ACM 4775)[3]	"	4-5	2-2	12.0±1.5[d]	66.1

[1] Means ± SD. Means with a common letter do not differ significantly ($P<0.05$). Initial dry weight of spat (six samples of 30 spat each) was 4.5±0.24 mg.

[2] Expressed as a percentage of that observed for the algal fed control

[3] Australian Collection of Micro-organisms Number, University of Queensland, St. Lucia, Qld 4072.

TABLE 3

Evaluation of yeast as complete algal substitutes in diets for Sydney rock oyster *Saccostrea commercialis* spat (Experiment 3).

Diets	16 day dry weight[1] increase (mg)	
	With algae[2]	Without algae
Unfed control	7.3±0.4[a]	-
Algal fed control	27.7±2.1[g]	-
14% algal fed control	12.4±0.6[d]	-
Dipodascus capitatus (ACM 4779)[3]	18.2±1.1[f]	10.8±0.3[c]
Dipodascus sp. (ACM 4781)[3]	15.4±0.8[e]	8.4±0.8[ab]
Dipodascus sp. (ACM 4782)[3]	16.3±1.0[e]	9.9±1.5[bc]
Dipodascus sp. (ACM 4782)[3] enzyme[4]	7.8±0.9[ab]	7.5±1.0[a]
Candida utilis (ACM 4774)[5]	17.3±1.2[ef]	9.3±1.3[abc]
Saccharomyces cerevisiae (ACM 4775)[6]	16.1±0.5[e]	8.6±1.1[ab]

[1] Means ± SD. Means with a common letter do not differ significantly ($P<0.05$). Initial dry weight of spat (six samples of 30 spat each) was 6.3±0.52 mg.

[2] 14% (by dry weight) algal supplement.

[3] Australian Collection of Micro-organisms Number, University of Queensland, St. Lucia, Qld 4072.

[4] Enzyme digested cell walls.

b Nutritional composition of new yeast and bacteria with potential as food for bivalve mariculture

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ABSTRACT

The biochemical composition of newly isolated marine bacteria (7 strains) and yeast (6 strains), together with 2 conventional yeast species, were evaluated to assess their potential for bivalve mariculture. Protein was a major constituent in all yeast and bacteria, ranging from 24.9% to 48.6% of the dry weight. Yeast contained from 15.2% to 26.6% carbohydrate, bacteria from 1.2% to 8.6%. Levels of lipid in yeast (2.5-7.7%) were negatively correlated to their previously reported value as supplementary food for oyster (*Saccostrea commercialis*) spat. Bacteria contained similar percentages of lipid (4.7-9.0%). Ash ranged from 4.7 to 14.4% in 11 of the microorganisms; the remaining species, all bacteria, had high levels (28.8-39.8%). All microorganisms had significant levels of nucleic acid (3.3-8.4%).

The compositions of the amino acids were similar in the yeast and bacteria species, and closely matched that of *Saccostrea commercialis* oyster larvae and spat, indicating a high protein quality. Yeast and bacteria both lacked the polyunsaturated fatty acids recognised as being important for bivalve molluscs, i. e. 20:5(n-3) and 22:6(n3), and this feature restricts their use in mariculture to a partial replacement for live algae. However, their high levels and good quality of protein indicates their potential to provide important nutrients in a mixed diet. Further studies are required to assess the digestibility and availability of the nutrients in the yeast and bacteria.

INTRODUCTION

The culture of bivalve mollusc spat in commercial hatcheries and nurseries depends almost entirely upon the production of large amounts of live microalgae as food (Couteau and Sorgeloos, 1992). The continuous mass production of microalgae, however, is recognised as a constraint to bivalve rearing, because it is costly, labour intensive and requires specialised facilities. This has led to the development and examination of alternatives to on-site algal production including non-algal diets

(reviewed by Couteau and Sorgeloos, 1992), but to date no satisfactory non-algal diet is available as a complete food for commercial bivalve culture (Urban and Langdon, 1984).

Partial replacement of algal diets for bivalves by bacteria (Martin and Mengus, 1977, Nell and Wisely, 1983, 1984, Nell et al., 1994) and yeast (Epifanio, 1979; Urban and Langdon, 1984; Nell et al., 1994) have shown the potential to significantly reduce animal production costs. Recently, we evaluated the value of bacteria and yeast isolated from oyster growing areas as partial substitutes (86% of dry weight) for live algae in the diet of *Saccostrea commercialis* oyster larvae and spat (Nell et al., 1994). Bacteria and algae mixtures produced increases in larval shell width of up to 67% of those obtained by the fully algal-fed controls. Yeast and algae mixtures produced increases in spat dry weight of up to 81% of those obtained by the fully algal-fed controls. In the present study, we compare the nutrient composition of some of the yeast and bacteria species used in our feeding trials and relate our findings to the reported food values. The composition of the yeast and bacteria is also compared with those of microalgae commonly used in mariculture (Volkman et al., 1989, 1993; Brown, 1991; Brown and Jeffrey, 1992, Dunstan et al., 1992).

MATERIALS AND METHODS

Microbe isolation and culture

Seven bacteria and six yeast strains isolated from water and sediment collected in oyster growing areas and other locations in Port Stephens, NSW (32° 45'S, 152° 12'E) in February 1991, were examined in this study. The microbes were non-spore formers, evaluated as having low potential pathogenicity to humans (S. P. Nearhos and T. K. Roberts, unpublished data, 1994) and were previously assessed as food for Sydney rock oyster (*Saccostrea commercialis*) larvae and spat (Nell et al., 1994). Bacteria were in the size range 0.9-1.5 x 0.2-0.5 μm, yeast were 2-9 x 1-5 μm. Two other yeast species were examined: *Candida utilis* (ACM 4774) from the University of Queensland, St Lucia, Queensland, and *Saccharomyces cerevisiae* (ACM 4775).

Cultures were grown in chemically defined media (2 l flask cultures) (Nell et al., 1994). Bacteria were grown to stationary phase and yeast to log phase. Cultures were harvested by centrifugation (1000 g, 15 min), freeze-dried and stored at -20 °C prior to analysis.

Sydney rock oyster larvae and spat

Sydney rock oyster (*Saccostrea commercialis*) larvae and spat were reared using standard hatchery and nursery techniques at the NSW Fisheries, Brackish Water Fish Culture Research Station (Holliday, 1985; Frankish et al., 1991). Freeze-dried samples were stored at -20°C prior to analysis of fatty acid and amino acid composition.

Biochemical analysis

Analytical procedures for amino acids, protein and lipid were identical to those described in detail for microalgae (Volkman et al., 1989; Brown, 1991).

Crude protein was estimated by the Kjeldahl method with ninhydrin finish (Strickland and Parsons, 1972). The ash content was determined after combusting samples at 450°C for 16 h. Carbohydrate was estimated using the cysteine-sulphuric acid assay (Chaplin, 1986). DNA and RNA were estimated by colorimetric assays with diphenylamine and orcinol, respectively (Kochert, 1978).

RESULTS

Gross composition

There were some significant differences in the gross composition between the individual yeast and bacteria species (Tables 1 and 2). Yeast, as a group, contained less protein and ash, but more carbohydrate than yeast (t-test, $P \leq 0.05$; Fig. 1).

Protein (as determined from the sum of anyhydroamino acids) was the major constituent in all yeast and bacteria (except *Aeromonas* sp.) In yeast, values ranged from 24.9% (*Debaromyces hansenii*) to 37.2% (*Candida utilis*). In bacteria, protein ranged from 29.2% (*Aeromonas* sp.) to 48.6% (*Methylophilus methylotrophus*).

Values for crude protein closely matched protein determined from summation of anhydroamino acids in yeast, but were significantly higher in bacteria (e.g. 50% more in *Derxia* sp. [ACM 4773]).

Yeast species were also rich in carbohydrate, from 15.2% (*C. utilis*) to 26.6% (*Saccharomyces cerevisiae*). Bacteria contained from 1.2% (*Aeromonas* sp.) to 8.6% (*M. methylotrophus*).

Yeast and bacteria contained similar concentrations of lipid, typically between 4-6%. *M. methylotrophus* had most lipid (9%) and *Dipodascus* sp. (ACM 4782) the least (2.5%).

Ash ranged from 4.7 to 13.1% in yeast. Four of the bacteria, *Aeromonas* sp. and all three *Pseudomonas* strains, had high levels of ash (28.8-39.8%); other species contained 10.1-14.4%.

RNA accounted for 80-99% of total nucleic acid in the microbes. Values were similar for yeast and bacteria, typically in the range 4-7%. DNA levels were higher in yeast (0.26-0.70%) than in bacteria (0.06-0.32%).

Amino acid composition

The compositions of both the essential (as defined for mussel by Harrison, 1975) and non-essential amino acids were similar in the 8 yeast and 7 bacteria species (Table 3 and 4, Fig. 2). Aspartate and glutamate were present in the highest concentrations (8.1-20%), whereas cystine, methionine, tryptophan and histidine were present in the lowest concentrations (0.4-3.6%). Other amino acids ranged from 3.5 to 9.6%. Bacteria contained slightly more of the essential amino acids arginine and methionine than yeast (t-test $P \leq 0.02$; Fig. 2).

The levels of essential amino acids in both yeast and bacteria were, in most instances, equivalent to the levels in the *Saccostrea commercialis* oyster larvae and spat, indicating high protein quality (Fig. 2). The proportion of lysine, however, was less in all microbes (3.5-6.1%) than in *S. commercialis* larvae (6.9%). An overall assessment of the protein quality is also provided by the essential amino acid index (EAAI), which factors in the ratios of the individual amino acid composition of the feed substance to the body amino acid composition of the feeding animal (Dy-Penaflorida, 1989). EAAIs for all yeast and bacteria were high (89-95), based on the composition of *S. commercialis* larvae, indicating good protein quality (Tables 3 & 4).

Fatty acid composition

Dominant fatty acids in the yeasts were 16:0 (13.3-19.9% of total fatty acids) and 18:1(n-9) (12.3-53.8%) (Table 5). Seven of the eight species were also rich in 18:2(n-6) (21.7-64.1%) and contained 18:3(n-3) (0.9-9.3%); the exception was *S. cerevisiae* which lacked these acids but was unique in its high content of 16:1(n-7) (43.9%).

Bacteria were characterised by high proportions of n-7 fatty acids (>50% total). *Aeromonas* sp., *Derxia* spp. (ACM 4772 and 4773) and *Pseudomonas* sp. (ACM 4770) contained 18:1(n-7) as their dominant fatty acid (61.3-83.6%) and *M. methylotrophus* contained 56.3% of 16:1(n-7). The two strains of *Pseudomonas testosteroni* (ACM 4768 and 4769) contained equal amounts of 16:1(n-7) and 18:1(n-7) (24-26%), as well as a significant amount of a fatty acid tentatively identified as 17:1(n-7) (14-17%).

Yeast and bacteria both lacked the polyunsaturated fatty acids recognised as being important for bivalve molluscs, i. e. 20:5(n-3) and 22:6(n-3).

DISCUSSION

The major nutrient fractions (protein, carbohydrate, lipid, ash and nucleic acids) accounted for between 62-87% of the total dry weight in the yeast and bacteria species. A significant proportion of the unaccounted dry weight may comprise of fibre (i.e. non-hydrolysable carbohydrate such as cellulose or similar

polysaccharides). Also, bacterial cell walls contain peptidoglycans rich in N-acetylglucosamine and N-acetylmuramic acid (White et al., 1979) - sugars that would be undetected by assays used in this study. The presence of significant levels of these N-containing sugars in the bacteria could also explain why the crude protein (based on total N) was significantly higher than protein values obtained from summation of anhydroamino acids. In microalgae, about 10% of the crude protein may consist of non-protein N (Becker, 1986).

Yeast analysed in the study were harvested during logarithmic phase and bacteria during stationary phase. Some of the differences in composition between the bacteria and yeast (and also between the bacteria and microalgae) may be attributed to this difference in the stage of harvest. The composition of microalgae can differ significantly between these two growth stages (Whyte, 1987, Brown et al., 1993).

Individual species of yeast and bacteria showed significant differences in their gross composition and fatty acid composition, but only minor differences in amino acid composition. These differences could account for some nutritional differences between the species.

The nutritional value of any food organisms will depend on the nutritional requirements of the animal to which it is fed. Balanced algal diets containing high levels of carbohydrate are reported to produce the best growth for juvenile oyster *Ostrea edulis* (Enright et al., 1986) and larval scallop *Patinopecten yessoensis* (Whyte et al., 1989), while high dietary protein provides best growth for juvenile mussel *Mytilus trossulus* (Kreeger and Landgon, 1993). In the current study, the bacteria *Methylophilus methylotrophus* and *Pseudomonas testosteroni* 760 were richest in protein, and all yeast had high levels carbohydrate, with *Saccharomyces cerevisiae* and *Dipodascus* sp. 1212 the richest. Compared to microalgae commonly used in mariculture, yeast and bacteria had similar levels of protein and less lipid (Fig. 1). Microalgae had levels of carbohydrate intermediate to those of yeast and bacteria (Fig. 1).

Both yeast and bacteria contained significant proportions of nucleic acid (about 4 to 6% of dry weight), which was more than reported for microalgae by Ricketts (1970) (about 1-3%) (Fig. 1). Another report has suggested that algae contain 4-6% nucleic acid, yeast 8-12% and bacteria up to 20%, and that the high levels in yeast and bacteria may limit their use as a food source for humans, which lack the uricase enzyme (Becker, 1986). Bivalve molluscs have been shown to possess this enzyme and have efficient pathways for catabolism of nucleic acids (reviewed by Bishop et al., 1983), so high levels of nucleic acids in yeasts and bacteria fed to these animals should not have toxicological effects.

The relatively high protein content in all yeast and bacteria, together with their essential amino acid profiles compared with oyster *S. commercialis* tissue, indicates that they would provide a rich and well-balanced source of the essential amino acids

for bivalve culture. Their amino acid composition also closely matched microalgae used in mariculture, and Pacific oyster *Crassostrea gigas* larvae (Fig. 2)

The lack of the polyunsaturated fatty acid important for bivalve nutrition, i.e. 20:5(n-3) and 22:6(n-3) in the yeast and bacteria makes them unsuitable as a complete diet in mariculture. However, our earlier study has shown the potential of some of the species as diet supplements, when mixed with microalgae rich in the polyunsaturated fatty acids (Nell et al., 1994).

All the yeast species examined in the present study improved the growth rate of *S. commercialis* spat when added as a supplement to a basal, low-ration algal diet (final diet concentration, 86% yeast, 14% algae) (Nell et al., 1994). This clearly indicated an ability of the oysters to utilise the yeast for nutrition. Of particular note, growth rates produced by the yeast used as supplement were negatively correlated to their percentage of lipid (as determined in this study) ($p<0.01$; $r2=0.71$); in other words, yeast with the lowest lipid gave the best growth as supplementary food for *S. commercialis* spat. Growth rates were up to 81% of a control "high-ration" complete algal diet (equivalent in dry weight to the yeast plus low-ration algal diet). The bacteria examined here, were less effective as a supplementary food for *S. commercialis* larvae when included at a similar ration of 86% of dry weight (Nell et al., 1994). Supplementation with Derxia sp. (ACM 4773) improved growth; supplementation with other bacteria showed apparently small (but not statistically significant) improvements in growth. Further studies are required to determine whether inclusion of the bacteria at a lower ration (eg. 50%) might improve their usefulness as a partial food replacement.

The biochemical data presented here provides indicative nutritional information of yeast and bacteria with potential for mariculture. The nutritional value of these species will also depend on the concentrations of other micronutrients (e.g. vitamins and minerals) and their digestibility and assimilation. Microalgae are efficiently digested by molluscs (e.g. 80% of C assimilated by the bay scallop *Argopecten irradians concentricus*; Peirson, 1983). Other studies have shown bacterial carbon is digested and assimilated by the juvenile oyster *Crassostrea virginica* (efficiency of 52%; Crosby et al., 1990) and oyster larvae *Crassostrea gigas* (efficiency > 25%; Doillet, 1993). Quantitative data on assimilation is lacking for yeast. Future studies are required to assess the digestibility of the new yeast and bacteria, and their value as supplementary food for other molluscs and at other algal replacement rations.

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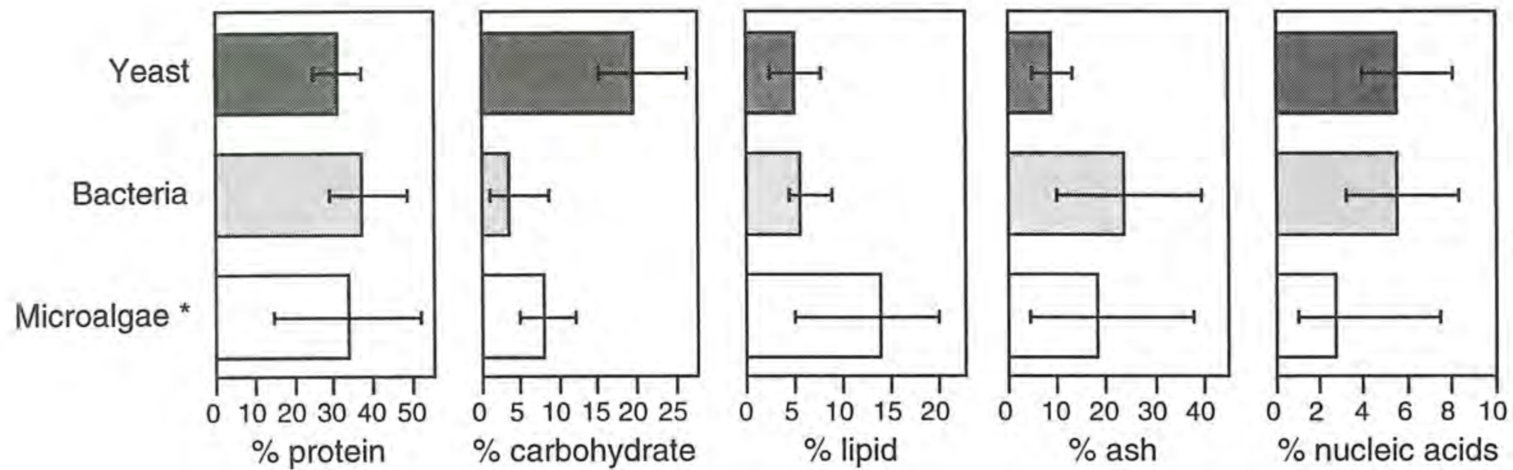

Fig. 1 The average composition (% of dry weight) of the major nutrients in yeast, bacteria (present study) and microalgae (% protein, carbohydrate and lipid, Brown et al., 1993, M. R. Brown and S. W. Jeffrey, unpublished; % ash, Whyte, 1987; Ben-Amotz et al., 1985; % nucleic acids, Ricketts, 1966). The range of values are indicated by range bars.

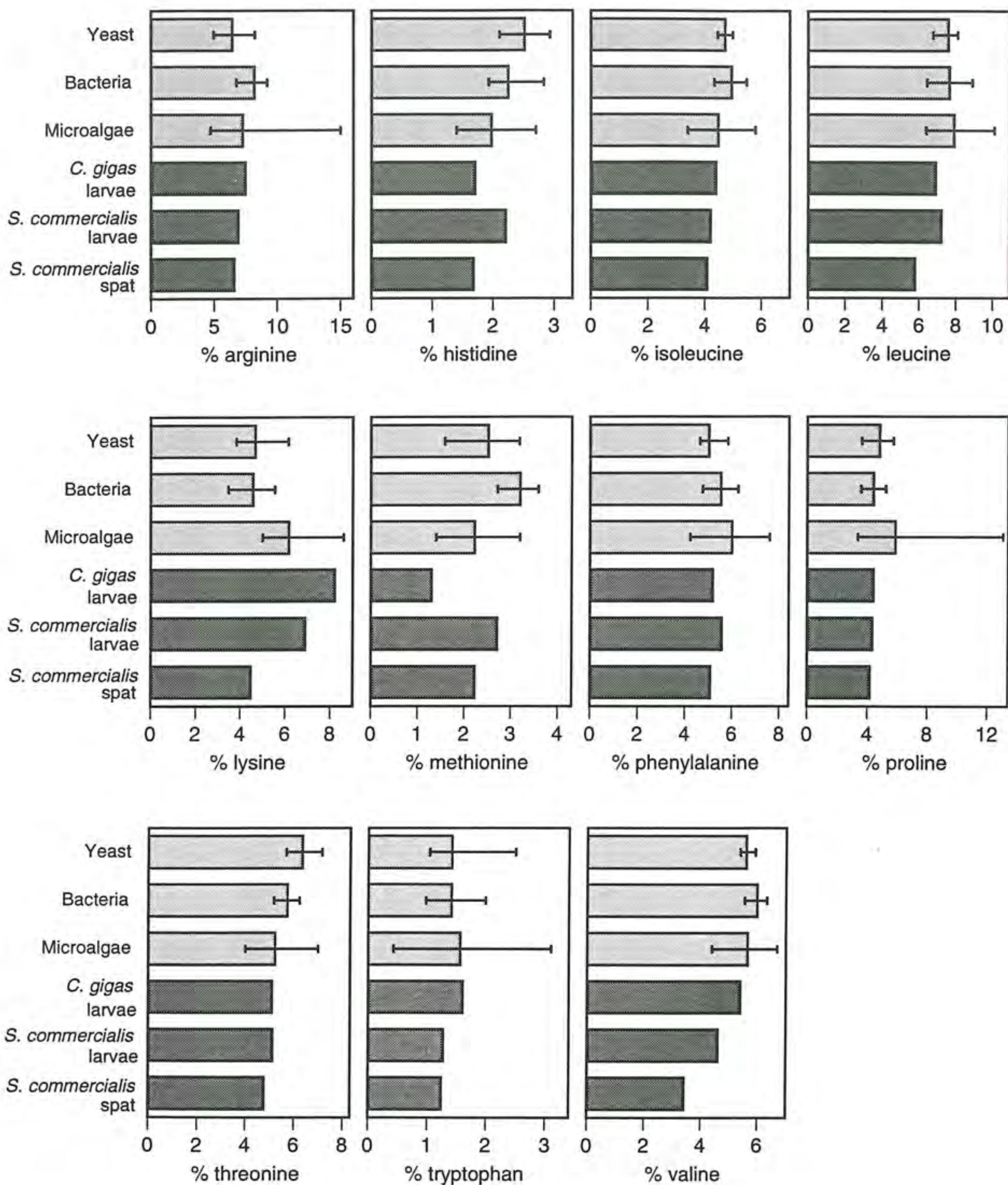

Fig. 2 The average composition of essential amino acid in yeast, bacteria (present study) and microalgae (Brown, 1991; Volkman et al., 1993; Brown et al., 1993; Brown and Jeffrey, 1992 and unpublished) compared to the composition in whole tissue of Saccostrea commercialis larvae and spat (present study) and Crassostrea gigas larvae (Brown, 1991). The range of values are indicated by range bars.

Table 1 Gross composition (% of dry weight) of marine yeast

	Candida utilis	Debaryomyces hansenii 1553	Dipodascus capitatus 1620	Dipodascus sp. 1930	Dipodascus sp. 1212	Dipodascus sp. 1950	Dipodascus sp. 2040	Saccharomyces cerevisiae
Protein								
sum anhydroamino acids	37.2	24.9	36.3	30.4	32.4	29.4	29.5	29.7
crude (N x 6.25)	42.3	23.0	32.9	36.2	34.5	31.5	32.3	28.7
Carbohydrate	15.2	19.4	16.5	17.2	24.7	18.6	17.5	26.6
Lipid	6.1	5.9	4.4	4.3	4.5	4.6	2.5	7.7
Ash	13.1	11	9.3	6.8	4.7	9.4	7.9	6.4
RNA	7.7	3.8	4.5	6.8	4.6	4.9	4.6	5.8
DNA	0.32	0.08	0.15	0.05	0.11	0.08	0.06	0.14
Sum total *	79.6	65.1	71.1	65.5	71.0	67.0	62.0	76.4

* Protein value from sum anhydroamino acids used in this calculation

Table 2 Gross composition (% of dry weight) of marine bacteria

	Aeromonas sp. 2360	Derxia sp. 2750A	Derxia sp. 3007	Methylophilus methylotrophus	Pseudomonas testosteroni 670A	Pseudomonas testosteroni 760	Pseudomonas sp. 810
Protein							
sum anhydroamino acids	29.2	37.0	36.2	48.6	33.4	43.5	32.9
crude (N x 6.25)	36.1	53.8	55.6	55.4	47.5	44.4	43.5
Carbohydrate	1.2	4.4	3.9	8.6	2.6	2.8	1.1
Lipid	4.7	5.0	5.6	9.0	4.8	5.9	5.4
Ash	39.8	10.1	11.2	14.4	31.5	28.8	30.2
RNA	2.6	7.7	8.1	5.9	3.2	4.4	4.4
DNA	0.67	0.33	0.26	0.51	0.29	0.29	0.7
Sum total *	78.2	64.5	65.3	87.0	75.8	85.7	74.7

* Protein value from sum of anhydroamino acids used in this calculation

Table 3 Amino acid composition (weight %) of marine yeast

	Candida utilis	Debaryomyces hansenii 1553	Dipodascus capitatus 1620	Dipodascus sp. 1930	Dipodascus sp. 1212	Dipodascus sp. 1950	Dipodascus sp. 2040	Saccharomyces cerevisiae
Non-essential								
Alanine	6.7	7.6	6.8	7.9	8.4	6.1	6.5	6.6
Aspartate	10.6	9.4	9.0	9.6	9.6	8.5	8.2	11.7
Cystine	1.0	1.1	0.7	1.0	0.7	1.5	1.4	0.7
Glutamate	20.0	11.7	11.1	12.0	11.4	10.9	10.7	14.6
Glycine	5.2	6.4	6.3	6.8	6.7	5.5	6.3	5.6
Serine	6.0	8.1	7.4	6.8	7.2	6.8	6.7	6.8
Tyrosine	4.7	4.9	4.3	4.9	4.3	4.9	5.8	4.6
Essential								
Arginine	5.0	6.0	7.2	6.5	5.6	7.6	8.2	5.3
Histidine	2.2	2.7	2.7	2.4	2.4	2.8	2.9	2.1
Isoleucine	4.6	4.5	5.0	4.5	4.7	5.0	4.6	4.9
Leucine	7.8	7.5	7.5	7.9	8.0	6.8	7.1	8.1
Lysine	3.8	4.8	4.6	3.9	4.9	6.1	4.2	5.0
Methionine	1.6	2.1	3.2	2.6	2.7	3.2	2.8	2.1
Phenylalanine	4.6	4.7	4.7	5.1	4.7	5.5	5.8	5.0
Proline	3.6	4.4	5.7	5.3	5.2	5.1	5.2	3.8
Threonine	5.8	7.1	6.8	6.2	5.7	6.7	6.4	6.1
Tryptophan	1.5	1.3	1.0	1.3	2.5	1.3	1.1	1.5
Valine	5.4	5.6	5.9	5.4	5.4	5.9	5.8	5.4
EAAI	89	92	91	93	93	95	92	92

EAAI = Essential amino acid index (Dy-Penaflorida. 1989; SEAFDEC Asian Aquaculture, Vol XI(4) pp. 6-9)
Values of ≈ 90 = good quality; ≈ 80 = useful; ≤ 70 inadequate

Table 4 Amino acid (weight %) of marine bacteria

	Aeromonas sp. 2360	Derxia sp. 2750A	Derxia sp. 3007	Methylophilus methylotrophus	Pseudomonas testosteroni 670A	Pseudomonas testosteroni 760	Pseudomonas sp. 810
Non-essential							
Alanine	7.1	6.0	6.2	8.3	9.6	9.0	6.9
Aspartate	10.1	8.2	8.1	11.0	10.5	10.5	8.7
Cystine	1.3	1.1	1.0	0.7	1.3	0.4	1.0
Glutamate	11.4	9.3	9.6	12.5	12.8	12.7	10.0
Glycine	6.1	5.5	5.5	6.5	5.9	6.5	6.0
Serine	5.0	6.6	5.9	4.5	4.4	4.7	5.4
Tyrosine	5.2	5.9	6.7	6.9	3.9	5.0	5.4
Essential							
Arginine	8.0	9.2	9.1	6.8	7.7	7.8	8.7
Histidine	2.1	2.8	2.8	2.0	1.9	2.1	2.0
Isoleucine	5.3	5.1	5.5	4.7	4.3	4.3	5.5
Leucine	7.3	6.4	6.8	8.5	8.9	8.6	7.1
Lysine	4.7	5.5	4.5	3.5	4.6	4.2	4.9
Methionine	3.2	3.6	3.6	2.8	2.7	3.3	3.2
Phenylalanine	5.6	6.2	6.3	4.9	4.9	4.8	6.2
Proline	4.5	4.9	5.2	3.6	3.9	4.1	4.6
Threonine	5.7	6.2	6.2	5.3	5.4	5.2	6.2
Tryptophan	1.1	1.4	1.2	2.0	1.3	1.0	1.9
Valine	6.4	6.0	5.8	5.6	5.9	5.9	6.3
EAAI *	93	94	93	91	93	91	93

* EAAI = Essential amino acid index (Dy-Penaflorida. 1989; SEAFDEC Asian Aquaculture, Vol XI(4) pp. 6-9)
Values of ≈ 90 = good quality; ≈ 80 = useful; ≤ 70 inadequate

Table 5. Fatty acid composition (% of total fatty acids) of the marine yeast

	Candida utilis	Debaryomyces hansenii 1553	Dipodascus capitatus 1620	Dipodascus sp. 1930	Dipodascus sp. 1212	Dipodascus sp. 1950	Dipodascus sp. 2040	Saccharomyces cerevisiae	Range
Saturated fatty acids									
14:0	-	-	-	-	-	-	-	-	0.0
15:0	-	-	-	-	-	-	-	-	0.0
16:0	16.3	17.8	16.5	13.9	18.0	15.4	13.3	19.9	13.3–19.9
17:0	0.6	-	-	-	-	0.4	0.7	-	0–0.7
18:0	2.9	9.4	3.4	3.4	2.6	2.8	2.0	5.3	2.0–9.4
20:0	0.1	-	0.2	-	-	-	-	-	0–0.2
22:0	-	-	-	-	-	-	-	-	0.0
Branched-chain fatty acids									
4,8,12 TMTD	-	-	-	-	-	-	-	-	0.0
i15:0	-	-	-	-	-	-	-	-	0.0
a15:0	-	-	-	-	-	-	-	-	0.0
i16:0	-	-	-	-	-	-	-	-	0.0
i17:0	-	-	-	-	-	-	-	-	0.0
a17:0	-	-	-	-	-	-	-	-	0.0
19:1	-	-	-	-	-	-	-	-	0.0
Monoenoic fatty acids									
14:1	-	-	-	-	-	-	-	-	0.0
16:1(n-7)	0.5	4.9	0.5	4.2	0.8	0.6	0.5	43.9	0.5–43.9
16:1(n-5)	-	-	-	-	-	-	-	-	0.0
17:1(n-9)	-	-	-	-	-	-	0.8	-	0–0.8
17:1(n-7?)	-	-	-	-	-	-	-	-	0.0
18:1(n-9)	13.5	46.0	12.3	28.2	51.0	17.1	53.8	28.8	12.3–53.8
18:1(n-7)	1.1	1.0	1.6	0.3	0.9	1.4	0.2	2.1	0.2–2.1
18:1(n-5)	-	-	-	-	-	-	-	-	0.0
19:1(n-?)	-	1.5	-	-	-	-	-	-	0–1.5
20:1(n-13)	0.2	-	-	0.2	0.1	-	-	-	0–0.2
20:1(n-9)	-	-	-	-	-	-	-	-	0.0
20:1(n-7)	-	-	-	-	-	-	-	-	0.0
22:1(n-9)	-	-	-	-	-	-	-	-	0.0

Polyunsaturated fatty acids									
18:2(n-6)	62.7	16.1	64.1	40.4	26.0	60.9	21.7	-	0–64.1
18:3(n-3)	1.5	3.4	0.9	9.3	0.5	1.0	7.0	-	0–9.3
18:3(n-6)	-	-	-	-	-	-	-	-	0.0
18:4(n-3)	-	-	-	-	-	-	-	-	0.0
20:2(n-6)	0.6	-	0.5	-	0.1	0.4	-	-	0.6
20:3(n-6)	-	-	-	-	-	-	-	-	0.0
20:4(n-6)	-	-	-	-	-	-	-	-	0.0
20:4(n-3)	-	-	-	-	-	-	-	-	0.0
20:5(n-3)	-	-	-	-	-	-	-	-	0.0
21:5(n-3)	-	-	-	-	-	-	-	-	0.0
22:4(n-6)	-	-	-	-	-	-	-	-	0.0
22:5(n-3)	-	-	-	-	-	-	-	-	0.0
22:5(n-6)	-	-	-	-	-	-	-	-	0.0
22:6(n-3)	-	-	-	-	-	-	-	-	0.0
Non-methylene Interrupted (NMI)									
20:2(5,11)	-	-	-	-	-	-	-	-	0.0
20:2(5,13)	-	-	-	-	-	-	-	-	0.0
22:2(7,13)	-	-	-	-	-	-	-	-	0.0
22:2(7,15)	-	-	-	-	-	-	-	-	0.0
Total fatty acids (µg mg^{-1})	20	3.6	25	20	16	18	17	6.6	3.6–25

Table 6. Fatty acid composition (% of total fatty acids) of the marine bacteria compared to *Saccostrea commercialis* larvae and spat.

	Aeromonas sp. 2360	*Derxia* sp. 2750	*Derxia* sp. 3007	*Methylophilus methylotrophus*	*Pseudomonas testosteroni* 670A	*Pseudomonas testosteroni* 760	*Pseudomonas* sp. 810	Range	*Saccostrea commercialis* larvae	*Saccostrea commercialis* spat
Saturated Fatty Acids										
14:0	-	-	-	1.3	1.0	0.7	-	0 – 1.3	3.2	1.1
15:0	-	-	-	0.2	0.3	0.3	-	0 – 0.3	0.9	0.8
16:0	2.3	2.2	1.8	33.9	27.2	27.8	3.0	1.8 – 33.9	25.5	23.2
17:0	-	-	-	-	0.5	0.6	-	0 – 0.6	2.0	3.8
18:0	6.8	9.8	7.0	0.4	1.1	1.5	6.1	0.4 – 9.9	7.0	11.7
20:0	-	-	-	-	-	-	-	0.0	0.3	-
22:0	-	-	-	-	-	-	-	0.0	0.2	-
								0.0		
Branched-chain fatty acids										
4,8,12 TMTD	-	-	-	-	-	-	-	0.0	0.2	-
i15:0	-	-	-	-	-	-	-	0.0	0.4	0.3
a15:0	-	-	-	-	-	-	-	0.0	0.1	0.6
i16:0	-	-	-	-	-	-	-	0.0	0.5	0.5
i17:0	-	-	-	-	-	-	-	0.0	1.0	1.0
a17:0	-	-	-	-	-	-	-	0.0	0.6	0.9
19:1	16.6	2.3	1.8	-	-	-	12.3	0 – 16.6	-	-
Monoenoic fatty acids										
14:1	-	-	-	0.2	-	-	-	0 – 0.2	-	-
16:1(n-7)	0.7	0.5	0.5	56.3	26.0	24.3	0.5	0.5 – 56.3	3.0	0.9
16:1(n-5)	-	-	-	-	-	-	-	0.0	0.3	-
17:1(n-9)	-	-	-	-	-	-	-	0.0	-	-
17:1(n-7?)	-	-	-	5.5	14.2	17.0	-	0 – 17.0	-	-
18:1(n-9)	0.5	0.5	0.2	0.1	2.1	0.5	0.4	0.1 – 2.1	3.8	2.5
18:1(n-7)	61.3	83.6	87.4	1.6	23.8	23.6	68.8	1.6 – 87.4	3.2	3.1
18:1(n-5)	-	-	-	-	-	-	-	0.0 – 0.0	0.3	-
19:1(n-?)	11.8	0.8	1.1	-	3.5	3.3	8.5	0 – 11.8	-	-
20:1(n-13)	-	-	-	-	-	-	-	0.0	2.6	3.7
20:1(n-9)	-	-	-	-	-	-	-	0.0	0.5	1.2
20:1(n-7)	-	-	-	-	-	-	-	0.0	2.9	4.3
22:1(n-9)	-	-	-	-	-	-	-	0.0	0.4	-

Polyunsaturated fatty acids										
18:2(n-6)	-	0.3	-	0.6	0.4	-	0.4	0 – 0.6	2.6	1.0
18:3(n-3)	-	-	-	-	-	-	-	0.0	3.3	1.5
18:3(n-6)	-	-	0.2	-	-	0.4	-	0 – 0.4	-	-
18:4(n-3)	-	-	-	-	-	-	-	0.0	3.3	1.2
20:2(n-6)	-	-	-	-	-	-	-	0.0	-	-
20:3(n-6)	-	-	-	-	-	-	-	0.0	0.3	-
20:4(n-6)	-	-	-	-	-	-	-	0.0	3.2	3.0
20:4(n-3)	-	-	-	-	-	-	-	0.0	0.8	0.4
20:5(n-3)	-	-	-	-	-	-	-	0.0	7.1	4.4
21:5(n-3)	-	-	-	-	-	-	-	0.0	0.5	-
22:4(n-6)	-	-	-	-	-	-	-	0.0	0.4	0.4
22:5(n-3)	-	-	-	-	-	-	-	0.0	1.2	3.1
22:5(n-6)	-	-	-	-	-	-	-	0.0	0.6	1.4
22:6(n-3)	-	-	-	-	-	-	-	0.0	10.7	8.7
Non-methylene Interrupted (NMI)										
20:2(5,11)	-	-	-	-	-	-	-	0.0	-	-
20:2(5,13)	-	-	-	-	-	-	-	0.0	0.9	5.3
22:2(7,13)	-	-	-	-	-	-	-	0.0	1.3	3.1
22:2(7,15)	-	-	-	-	-	-	-	0.0	4.9	7.0
Total fatty acids (μg mg^{-1})	21	32	57	57	21	18	17	17 – 57	9.0	0.3

8 APPENDICES

a Nell, J. A. The development of oyster diets. Australian Journal of Agricultural Research, 44: 557-566.

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The Development of Oyster Diets

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Abstract

Although oyster larvae and spat have been reared successfully on algal diets for many decades, these are expensive to produce and do not always synchronize with hatchery requirements; considerable research has been conducted to determine which and why certain algal species are most appropriate and to investigate substitutes to algal feeds. Algal concentrates, commonly used in the United States, have overcome some problems associated with fresh algal feeds. However, work continues to develop cheaper non-algal alternatives. Recent promising advances have been in the formulation and preparation of experimental microencapsulated diets. However, further work upon capsules is required before the process can be commercialized. The possibility of rearing and substituting marine yeasts and bacteria for algae is an exciting and cost competitive alternative even to the microencapsulation approach.

Keywords: nutrient requirements, algae, heterotroph, bacteria, yeast, microcapsules.

Introduction

Commercially important oysters are farmed under natural conditions (Nell 1993), and grow on diverse natural diets, which vary depending on season, location and environmental conditions (Nell 1992). However, in hatcheries algal diets have been used since the 1940s (Bruce *et al.* 1940). The dependence upon the various algal species for feed during the hatchery and nursery phases has caused problems due to high cost and unpredictable supply. This has prompted a search for non-algal food materials; however, to date no satisfactory non-algal diet is available for commercial bivalve culture (Urban and Langdon 1984). Similarly, food cost has been a major limitation to the commercial development of intensive controlled oyster culture (Epifanio 1975; Claus 1981).

Early attempts to find algal substitutes involved the development of commercial oyster fattening diets based on cereal starch (Haven 1965; Turgeon and Haven 1978; Nell and Wisely 1983, 1984); however, limitations in the understanding of oyster nutritional requirements have hampered efforts. Recent advancements in the development of encapsulated diets (Langdon 1989; Langdon and DeBevoise 1990; Southgate *et al.* 1992) should allow a more precise determination of the nutrient requirements of oysters than has been possible so far (Nell 1992).

Successful supplementation of algal diets with the inclusion of pure or mixed cultures of microbes (Martin and Mengus 1977; Douillet 1989) has shown the potential for significantly reducing the cost of live food diets for oysters.

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Table 1. Estimated nutrient requirements for oysters
Source: Adapted from Nell (1992)

Nutrients	Dry weight (%)	References
Protein	20	Flaak and Epifanio 1978; Nell and Wisely 1983
Lipids	5	Castell and Trider 1974
Fatty acids		
20:5*n*-3	small requirement	Chu and Webb 1984; Enright *et al.* 1986*b*
22:6*n*-3	small requirement	Langdon and Waldock 1981; Chu and Webb, 1984; Enright *et al.* 1986*a*, 1986*b*
Carbohydrate	required	Flaak and Epifanio 1978; Wikfors *et al.* 1984; Utting 1986; Whyte *et al.* 1990
Cellulose	poorly digested	Crosby *et al.* 1989; Langdon and Newell 1990
Vitamins		
Fat soluble	probable requirement	Urban and Langdon 1984
Water soluble	probable requirement	Davis and Chanley 1956; Langdon 1983; Urban and Langdon 1984; Ukeles and Wikfors 1988
Minor minerals	obtained from seawater	Nell 1992
Calcium	obtained from seawater	Nell 1992

Nutrient Requirements

The estimated nutrient requirements of oysters are summarized in Table 1. Difficulty has been encountered in determining the nutrient requirements of oysters because of the need to first meet the desirable physical characteristics of a filter-feeders diet (Nell 1992); however, the development of diets packaged in appropriately sized microcapsules has eased such oyster studies. The protein requirement of an oyster is approximately 20% of the dry weight of the diet and the total lipid requirement probably no more than 5% (Nell 1992). Oysters have a small but specific requirement for the long-chain fatty acids 20:5*n*-3 and 22:6*n*-3, with the latter being of more importance (Nell 1992). Other factors such as dietary carbohydrate contribute to the nutritional value of an alga to bivalves (Enright *et al.* 1986*b*; Whyte *et al.* 1990). Little is known about the essential and optimum amino acid requirements in food protein for oysters, nor about their vitamin requirements. However, mineral requirements of oysters are probably largely met by direct absorption from sea water (Nell 1992) and food.

Algal Diets

Algal Suspensions

A number of studies have been conducted which assess the value of algal species as a food source for oysters and their larvae. Davis and Guillard (1958) tested ten algal species with larvae of the American oyster *Crassostrea virginica* (Gmelin) and recommended a mixed diet consisting of the flagellates *Isochrysis galbana* (Green), *Pavlova lutheri* (Droop) Green, a *Tetraselmis* sp. and *Dunaliella euchlora* Lerche. After testing five algal species singly and in various combinations, Laing and Millican (1986) recommended the diatom *Chaetoceros calcitrans* (Paulsen) Takano as a single species diet for *C. virginica* spat. Walne (1970) tested 19 algal species as food sources for juvenile flat oysters (*Ostrea edulis* L.) and found *P. lutheri* and *Ch. calcitrans* to be among the best. The two

algal species *P. lutheri* and *Isochrysis* aff. *galbana* Green (clone T-iso; termed Tahitian *Isochrysis*) are widely used in overseas hatcheries (Holliday 1985) and have been used routinely for feeding Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) larvae (Numaguchi and Nell 1991). Tahitian *Isochrysis* has a high concentration of 22:6*n*-3 long-chain fatty acid, while *P. lutheri* has high concentrations of both the essential 20:5*n*-3 and the 22:6*n*-3 fatty acids (Brown *et al.* 1989). These two fatty acids individually or in combination have been found to be important for growth in *O. edulis* spat (Enright *et al.* 1986*a*, 1986*b*), Pacific oyster *Crassostrea gigas* (Thunberg) spat (Langdon and Waldock 1981) and *C. virginica* larvae (Chu and Webb 1984).

The algal species, *P. lutheri*, Tahitian *Isochrysis* and *Ch. calcitrans*, produced the greatest shell length increase in *S. commercialis* larvae whether fed singly, or in separate combinations with Tahitian *Isochrysis* and *Ch. calcitrans* (Nell and O'Connor 1991). *S. commercialis* spat, however, produced the greatest length increase when fed the diatom *Skeletonema costatum* (Greville) Cleve (O'Connor *et al.* 1992). These four algal species all contain high levels of one or both of the 20:5*n*-3 and 22:6*n*-3 fatty acids (Brown *et al.* 1989). Similarities in protein levels (Enright *et al.* 1986*a*) and protein amino acid compositions of the algal species (Webb and Chu 1983) suggests that protein compositions alone are unlikely to be important factors in determining the food value of algal species to oysters.

Algal Concentrates

Algae concentrated into pastes or slurries, often by centrifugation have been used to feed oyster larvae in laboratories (Ukeles 1975) and in commercial oyster hatcheries (Watson 1986; Watson *et al.* 1986; Donaldson 1989,1991; Nell and O'Connor 1991). This technique allows algae to be stored for several days in the dark at 10°C (Ukeles 1975), or for up to 2 weeks at 4°C (Watson *et al.* 1986) before the food value declines.

Such concentrated storage of algae is having an impact upon aquaculture research, and commercial hatcheries in the United States. In Australia centralized algal production units using this technology could supply a number of hatcheries and research institutions with a consistently high quality product of uniform nutritional value (O'Connor and Nell 1992). In addition, hatcheries could store rather than discard excess algal production either for their own use or to supply aquaculturists who wish to settle larvae purchased from hatcheries (Holliday *et al.* 1991).

Heterotrophic Algae

Microalgae are generally considered to be strictly photosynthetic organisms; however, there are a significant number of species with heterotrophic or mixotrophic capability (Gladue 1991). While photosynthetic or autotrophic algal production systems are often inefficient and expensive (Gladue 1991), alternative culture methods may improve yields. Some algae can be grown heterotrophically and reach productivities similar to those obtained with industrial fermentations of heterotrophic yeasts and bacteria (Soong 1980; Gladue 1991). Good growth rates were obtained by juvenile Pacific and flat oysters fed spray-dried *Tetraselmis*

suecica (Kylin) (Celsys, Cambridge, England) grown heterotrophically (Laing and Verdugo 1991). Many other species that can be grown heterotrophically (Gladue 1991) remain to be evaluated as foods for oysters.

Artificial Diets

As hatchery oyster spat production has usually been dependant on the expensive and sometimes unreliable production of microalgae (Bolton 1982), efforts have been made to develop artificial dietary supplements as substitutes. In order to be successful the non-algae substitute should meet a number of criteria. Primarily the substitute should be neutrally buoyant and stable in aqueous suspension. In the case of encapsulated diets for bivalve larvae and spat, which ingest their food whole, a capsule wall that is impermeable and stable in seawater but readily broken down by a change in pH or by the action of digestive enzymes is required (Jones *et al.* 1974). Gabbott *et al.* (1976) investigated the feeding of microencapsulated diets to blue mussel *Mytilus edulis* (L.) and Pacific oyster *C. gigas* spat, and confirmed the importance of capsule size and digestibility.

The use of microparticulate diets for oysters was reviewed by Langdon (1983) and for aquaculture generally by Langdon *et al.* (1985). A comprehensive artificial diet consisting of microgel particles, lipid-walled microcapsules and kaolin showed promise when fed to *C. virginica* spat (Langdon and Siegfried 1984). This kind of diet, however, tends to encourage blooms of water fouling and potentially pathogenic bacteria (Langdon and Bolton 1984), and is therefore less suitable for larvae. Gelatin-acacia (protein-polysaccharide) and lipid-walled microcapsules, including some based on cod liver oil, have been used as the sole diet to rear *C. virginica* larvae to the 'eyed' stage, although growth rates were considerably lower than on a live microalgal diet (Chu *et al.* 1987). Gelatin-acacia microcapsules fed at a concentration of $0 \cdot 05$ mg L^{-1} ($1 \cdot 33 \times 10^6$ microcapsules mL^{-1}), considerably more than the 5×10^2 microcapsules mL^{-1} recommended by Chu *et al.* (1982), were an effective supplement for algal diets fed to *S. commercialis* larvae (Numaguchi and Nell 1991) at doses between $0 \cdot 1$ and $0 \cdot 4$ mg L^{-1}. Algal meal (Betatene Ltd, Cheltenham, Vic.), a dried extract of *Dunaliella salina* (Teodoresco), fed at $0 \cdot 123$ mg L^{-1} performed as well as microcapsules fed at $0 \cdot 05$ mg L^{-1} as a dietary supplement (Numaguchi and Nell 1991). More recently cod liver oil encapsulated in acacia has been used successfully as a source of essential long-chain fatty acids for *S. commercialis* larvae (J. A. Nell, unpublished data, 1991).

Lipid-walled microcapsules containing an aqueous core may be used to deliver water-soluble nutrients to oysters (Langdon and Siegfried 1984; Chu *et al.* 1987). Protein-walled microcapsules have been used successfully to deliver protein to *C. gigas* (Langdon 1989; Langdon and DeBevoise 1990). The proportion of protein assimilated from these capsules by *C. gigas* spat was 39%, greater than the 29% for glyceride-coated, nylon protein-walled microcapsules (Langdon and DeBevoise 1990). Good growth rates were obtained with *S. commercialis* larvae fed wholly on protein-walled capsules, consisting of 68% protein, 16% carbohydrate and 4% lipids (Southgate *et al.* 1992). Supplementation of the alga *Ch. calcitrans* with 40% by dry weight of a commercially prepared microencapsulated diet (Frippak Feeds, Basingstoke, England), gave similar growth in spat of the three oyster species *O. edulis*, *C. gigas* and *C. virginica*, compared to those fed an algal control diet (Laing 1987).

Non-encapsulated diets have been successful in fattening (gonad maturation) adult oysters. These diets were shown to be unsuitable for oyster larvae, because of the risks associated with high numbers of bacteria growing on the particles in the larval rearing tanks (K. Numaguchi, personal communication, 1988). Corn starch has been used successfully for the fattening of adult American oysters *C. virginica* (Haven 1965; Turgeon and Haven 1978); however, a more comprehensive diet of corn starch, casein and cod liver oil was found to meet more closely the approximate nutritional requirements of *C. virginica* (Castell and Trider 1974; Trider and Castell 1980). Adult Sydney rock oysters were successfully fattened with diets based on wheat starch, bacterial protein Pruteen®(ICI, Ltd, Billingham, England) and cod liver oil alone and in combination (Nell and Wisely 1983, 1984; Nell 1985).

Marine Yeast and Bacteria

Various studies have shown that the algal content of adult diets for scallops, mussels, clams and oysters can be reduced by up to half by using compressed and dry yeasts. The yeast *Candida utilis* (Henneberg) Lodder & Kreger-van Rij has been used successfully to replace up to 50% of the algae in the diet of the American oyster *Crassostrea virginica* spat (Epifanio 1979; Urban and Langdon 1984). In addition, Nell (1985) has shown that adult *S. commercialis* can be variably fattened solely on diets containing protein from two types of microbe, the yeast *Candida utilis* and the bacterium *Methylophilus methylotrophus* (MacLennan, Ousby, Owen & Steer) Jenkins, Byrom & Jones as its commercial spray dried preparation Pruteen®(Jenkins *et al.* 1987).

On the basis that such heterotrophic organisms can be used as oyster feeds at different stages of their life history, the major attraction is that yeasts and bacteria can be produced much more rapidly, efficiently and economically, i.e. with a much higher 'productivity' than photosynthetic algae because of shorter generation times, higher cell densities and inexpensive growth substrates. Yeasts and bacteria have much faster growth rates than algae (doubling times typically 0·5–4 h compared with 1–4 days for algae). A comparison of the productivities of a widely used aquaculture feed and typical marine alga Tahitian *Isochrysis* reported by Jeffrey and Garland (1988) and a marine yeast *C.guilliermondii* (Castellani) Langeron & Guerra reported by Higashihara *et al.* (1983) with growth characteristics typical of many yeasts (including *C.utilis*), indicated that yeast is some 2000-fold more productive than photosynthetic algae (D. G. MacLennan, unpublished data, 1990). Bacteria are known to have similar growth performance characteristics to those of yeasts (MacLennan *et al.* 1976) and hence similar production 'productivities', i.e. in the order of some 2000-fold higher than photosynthetic algae. Much higher culture cell concentrations (D. G. MacLennan, unpublished data, 1990) can be achieved with yeast and bacteria (30–50 g dry wt L^{-1}) compared with 0·3–0·5 g dry wt L^{-1} for commonly used marine algae, e.g. Tahitian *Isochrysis* at $13\text{–}22 \times 10^9$ cells L^{-1}.

The technology required to isolate and produce these novel yeast and bacterial aquaculture feeds at such high levels of productivity differs from that required for the culture of photosynthetic algae. In these processes, the yeast or bacterium is grown non-photosynthetically on high concentrations of cheap organic substrates under carefully controlled conditions.

Another problem with autotrophic production is that photosynthetic algae are susceptible to microbial contamination when grown on a large scale under commercial conditions, resulting in unreliable quality of feeds and often total batch rejection. In contrast, a very wide range of yeast and bacteria can be grown on simple sugar minerals salts media on a large scale very reliably and economically and in a totally uncontaminated form.

The potential problem in feeding microbes stems from their potential either to cause disease or fouling in aquaculture situations. However, not all microbes are potentially pathogenic, either to oysters or humans, who consume their fermentation products. The nutritious but non-pathogenic organisms need to be found and evaluated in controlled conditions. Good growth rates of $\geq$80% of that achieved with algal fed controls have been obtained when new marine yeast and bacterial cultures were used to replace 80% of the algal diet (J. A. Nell, unpublished data, 1992).

As there are likely many thousands as yet undescribed species of yeast and bacteria in nature, and the above promising results have been obtained from studies on only two or three traditionally reared species, there could clearly be rich rewards from the isolation and evaluation of new yeasts, particularly of marine origin, as aquaculture feeds.

The potential application is not limited to oysters. Other heat-killed and processed bacterial heterotrophs (Martin and Mengus 1977; Nell and Wisely 1983,1984; Nell 1985) and unresolved live bacteria (Martin and Mengus 1977; Douillet 1989) have been used incidentally or by design as non-algal feeds or feed supplements in aquaculture.

So this dependence of many Australian (and world) aquaculture ventures on algal production may be reduced greatly or eliminated if suitable microbes were found. These procedures could revolutionize the Australian aquaculture industry, not only in the hatchery and nursery phases, but also in the growth and/or fattening phases for bivalves by enabling intensively fed, highly controlled (i.e. optimized) environmental production techniques to be used for a much larger proportion of the life cycle. Such feeds/production techniques could not only lead to major gains in efficiency, and hence substantially reduced costs of production, but more importantly could enable year round reliable supply of large quantities of the top quality bivalve product necessary to establish and sustain major export markets. For example, despite demand, there is no significant export of the gourmet Sydney rock oyster as the above requirements cannot be fulfilled. Bivalve 'feedlots', analogous to the well-established intensive production procedures for pigs, poultry and cattle, could therefore become economically viable, to underpin local and potential export markets.

Conclusions and Future Developments

Much progress has been made in the development of oyster diets over the past two decades. The importance of the $20:5n$-3 and $22:6n$-3 fatty acids for oysters is now well recognized (Langdon and Waldock 1981; Chu and Webb 1984; Enright *et al.* 1986*a*, 1986*b*) and hatcheries are now using combinations of algal species which meet the fatty acid requirements of oyster larvae. Algal concentrates are being used to store excess production for use during peak demand (Watson 1986; Watson *et al.* 1986; Donaldson, 1989; Nell and O'Connor 1991). Gelatin-acacia

capsules have been used to study fatty acid requirements (Langdon and Waldock 1981) and to supplement algal diets (Numaguchi and Nell 1991). These capsules are also an excellent source of energy and fatty acids for artificial diets. Such protein-polysaccharide walled-capsules containing protein, carbohydrate and lipids have produced encouraging growth rates in oyster larvae (Southgate *et al.* 1992), although further refinements of capsule production technology are required before application in commercial hatcheries.

Although bacteria are undoubtedly an important component of the natural diet of oysters (Nell 1992), live bacteria have not been consciously used in oyster diets until recently. Martin and Mengus (1977) and Douillet (1989) have used pure and mixed cultures respectively. The evaluation of marine yeasts and bacteria is a promising field of research, that may produce a dietary supplement or alternative to algal diets. Preliminary results at the BWFCRS have indicated that partial substitution of algae with live yeast and bacteria cultures has great promise, but much work remains to be done to assess the full potential of yeasts and bacteria in oyster diets.

It is likely that in future aquaculturists and hatchery operators will have a range of feeding and packaging options available such as live, and concentrated, and dried algae, yeast and bacteria as well as microcapsules which might be cost-effectively blended according to the nutritional requirements of the aquaculture species. These products may be produced on site, but most likely at least some of them would be purchased from commercial suppliers. The shelf-life of these products is likely to vary considerably, depending on storage temperature and moisture content.

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Oyster nutrition

Abstract
Oyster nutrition has generated much research over the past two decades. This paper is a review and discussion of this research.

In the wild, oysters feed on micro-algae, detritus, bacteria, protozoans, zooplankton and dissolved organic nutrients from which they derive different benefits. The composition of their diet depends largely on what is available in the water, which in turns depends on location, season, vegetation in and around the waterway, and prevailing winds and currents. Small quantities of silt in the diet may improve food retention in the digestive tract and increase growth rates.

For the highest gain in meat weight, cultured oysters require 10 mg dry weight L^{-1} of food, as particles of 3–12 µm. Juveniles require around 2.8% (in dry weight) their live weight per day, depending on their size, the environmental conditions, and feeding frequency.

An oyster requires approximately 20% protein in the diet. Its total lipid requirement is probably no more than 5% of the dry weight of the diet. Oysters have a small but definite requirement for the long-chain fatty acids 20:5*n*3 and 22:6*n*3, with the latter being more important. Diets should contain only a minimum of cellulose, as it is not readily digested by oysters. Little is known about the optimum amino acid composition of protein for oysters, or about their vitamin requirements; these gaps in our knowledge need to be addressed. The mineral requirements of oysters are probably largely met by direct absorption from sea water.

To obtain the best growth rates in a hatchery, the diet of oysters is best made up of a mixture of micro-algae such as *Isochrysis* aff. *galbana* Green (clone T-iso; termed Tahitian *Isochrysis*), *Pavlova lutheri* (Droop) Green and *Chaetoceros calcitrans* (Paulsen) Takano. Much progress in formulating and preparing artificial diets for oysters has been made, but more research should be carried out before such diets can be used commercially for oyster "fattening" or larval rearing.

Introduction

The search to understand the nutritional requirements of an oyster has benefited from studies in several areas. Investigations of the natural feeding habits of oysters, such as those of Thangavelu (1988), Kreeger (1986) and Crosby and Newell (1990), can be considered in conjunction with the information from mollusc hatcheries on the algal species that promote the highest growth rates in larvae (e.g., Davis and Guillard, 1958; Walne, 1970), and the reports on attempts to produce artificial diets, such as those of (e.g., Langdon and Siegfried, 1984; Chu et al., 1987). A clearer, but by no means complete, picture of oyster nutrition has emerged, but much more research is required. This review covers four aspects of oyster nutrition: physical aspects of nutrition; natural foods; artificial diets; and nutrient requirements.

Natural foods

The diet of oysters in the wild

The availability and composition of the food of oysters in the wild is of great interest to researchers and government regulators, as oyster farmers often ask: "What do my oysters feed on, and what is the maximum stocking density of my growing area?". The research discussed below provides some suggestions.

In Pulicat Lake, south India, the diet of the oyster *Crassostrea madrasensis* (Preston) was characterised by its stomach contents, which averaged 52.8% diatoms, 45.7% detritus and 1.5% zooplankton (Thangavelu, 1988). The proportion of detritus in the diet ranged from 28.0 to 64.6% over the two-year study. In the oyster breeds of Chesapeake Bay, Delaware, detritus forms 77% of the seston (suspended particles) (Van Valkenburg et al., 1978). The percentage of seston in subestuaries of the Choptank River, Maryland is highest in summer (Berg and Newell, 1986).

The amount of cellulose in the carbohydrate fraction of detritus depends on its origin (Langdon and Newell, 1990). In Canary Creek, Delaware, which drains extensive marshland dominated by the marshgrass *Spartina alterniflora* (Loisel.), the carbohydrate fraction ranged from 2 to 43% (Kreeger, 1986 as cited by Langdon and Newell, 1990) and may contain 85% cellulose (Newell and Langdon, 1986). The cellulose portion of the carbohydrate fraction was found to be largely unavailable to *Crassostrea virginica* (Gmelin) (Crosby et al., 1989; Langdon and Newell, 1990). Stuart et al. (1982) demonstrated that the ribbed mussel *Aulacomya ater* (Molina) could digest and assimilate up to 50% of detritus prepared from kelp. Detritus from such macroalgae is more readily digestible than detritus from the angiosperm *S. alterniflora* (Findlay and Tenore, 1982).

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Bacteria are another natural food source for oysters. They may be present free (unattached) or, more commonly, attached to detritus, microalgae or zooplankton. The oyster *C. virginica* filters free bacteria with an efficiency of only 5% (Langdon and Newell, 1990). However, it was estimated with the use of ^{14}C and ^{15}N labelled bacteria, that attached and unattached bacteria could contribute 5.5% of the metabolic carbon requirement and 27% of the nitrogen requirements of *C. virginica* in Canary Creek, Delaware (Langdon and Newell, 1990). The high concentrations of attached bacteria in Chesapeake Bay were found to contribute up to 19.2% of the total carbon and to make a significant contribution to the nitrogen requirement of *C. virginica* in this estuary (Crosby and Newell, 1990). Although few studies on protozoans as a food source for oysters have been conducted, it appears that they may also form an important component of the natural diet of oysters (Baldwin and Newell, 1991).

Clearly there is a lot more to an oyster diet than just microalgae, and more than a measurement of phytoplankton is required to determine the quantity of potential oyster food in water. The amount of food available in estuaries for bivalves may be assessed by such measurements of seston as particulate organic matter (POM) (Brown, 1988; Carver and Mallet, 1990), particulate organic carbon (POC) and particulate organic nitrogen (PON) (Wilson, 1987). The ratio of POM to particulate inorganic matter (PIM) in the seston may also be important, because a high PIM concentration could dilute ingested diet (Widdows et al., 1979). On the other hand, the presence of a PIM such as kaolin could improve ingestion and/or digestion rates and/or efficiencies (Langdon and Siegfried, 1984). A food index defined as the percentage of food (food = lipid + carbohydrate + protein) in the total seston was used as a measurement of available food by Soniat and Ray (1985). Chlorophyll *a* is another measurement often taken (Brown and Hartwick, 1988a, b), but it indicates the abundance of phytoplankton only (Jeffrey, 1981).

The role of dissolved organic nutrients

The role of dissolved organic nutrients in the diet of oysters has received a lot of attention. In filter-feeding activity, the large epithelial surface areas of the gill and mantle in oysters are exposed to a large water volume; consequently these surfaces are ideal for the direct absorption of nutrients. Péquignat (1973) demonstrated that in adult bivalves, the gill was the major organ responsible for the uptake of small organic molecules from sea water, whereas in veligers the velum is the major site of uptake for dissolved organic matter (Manahan and Crisp, 1982). Many other studies have focused on the role of dissolved amino acids in the nutrition of marine bivalves (see reviews by Stewart, 1979; Stephens, 1981,1983; Manahan and Crisp, 1982; Wright, 1982; Manahan, 1990).

Oysters have the ability to absorb amino acids (Rice et al., 1980; Manahan, 1983b, 1990; Nell et al., 1983; Nell and Dunkley, 1984; Nell and Gibbs, 1986), vitamins (Nell et al., 1983), fatty acids (Bunde and Fried, 1978) and glucose (Schulte et al., 1973; Bamford and Gingles, 1974; Nell et al., 1983; Melaouah, 1989; Welborn and Manahan, 1990) directly from sea water. Amino acids in particular are absorbed as early as the fertilised egg stage (Manahan, 1983a). Amino acids taken up from solution are rapidly distributed to the internal tissues (Rice and Stephens, 1987). Langdon (1983a) reported that oyster larvae grew on a mixture of amino acids and other dissolved nutrients for six days under axenic conditions.

It has been suggested that dissolved vitamins also have nutritional importance for oysters (Davis and Chanley, 1956; Urban and Langdon, 1984; Ukeles and Wikfors, 1988) and increase larval growth (Davis and Chanley, 1956; Urban and Langdon, 1984). To date, little is known about the benefits to oysters of the direct absorption of fatty acids (Stewart, 1979). The nutritional importance of glucose for bivalves has been well documented (Gillespie et al., 1964; Welborn and Manahan, 1990). Dissolved glucose in seawater may be utilised by bivalve larvae when there are insufficient algal cells available (Melaouah, 1989), and it has increased longevity in starved oysters (Gillespie et al., 1964).

Dissolved nutrients may be of vital importance to oyster larvae when particulate matter is scarce, since they are provided with minimal food reserves by the parent (Manahan and Crisp, 1982; Manahan, 1983a), and at settlement they rely totally on reserves. The concentrations of amino acids may range from 0.6 μM in surface waters to 6.0 μM in sediment waters (Henrichs and Farrington, 1979). At these concentrations glycine may contribute between 0.6 and 2.8% of the energy requirement (Manahan, 1983b) or between 2.0 and 9.5% of the protein requirement of oyster larvae (Manahan, 1983c).

The role of silt/kaolin

The blue mussel, *Mytilus edulis* L., clearly benefits from small quantities of suspended silt in the water (Winter, 1978), because silt assists in grinding up food in the digestive tract (Murken, 1976), and also because the organic matter component of natural silt is an important source of nutrients (Kiørboe et al., 1981).

The value of totally inorganic kaolin supplements in artificial diets for oyster spat is not so clear. Urban and Langdon (1984) reported increased growth in *C. virginica* when a diet of algae and yeast was supplemented with kaolin at 20 mg L^{-1}; growth was comparable to that of spat fed only algae. However, kaolin did not improve growth of spat fed artificial diets other than yeast. Kaolin suspensions have been recommended to improve the benefit of artificial diets to oysters (Langdon and Bolton, 1984; Langdon and Siegfried, 1984) and of diets with algal contents (Ewart and Carriker, 1983). The retention of particles in the digestive tract of Pacific oysters, *Crassostrea gigas* (Thunberg), was improved by the addition of kaolin particles of between 3 and 4 μm in size (Sornin et al., 1988). The benefits of kaolin in the diet of oysters come both from its assistance in grinding up food in the oyster's digestive tract and from the nutrients obtained from bacteria growing on kaolin.

Physical aspects of nutrition

Particle size

The feeding mechanism in oysters depends on the ciliary action of gills driving a current of water through the ostia (Ukeles, 1969). During passage through the gills, particulate matter is filtered out, trapped in mucus, and transported to the labial palps, where it is ingested, or, if it is large or spiny, rejected (Ukeles, 1969). Because oysters will ingest both algae and

non-nutritive particles such as graphite (Owen, 1966), selection appears to be made on particle size (Wisely and Reid, 1978) and possibly other factors such as chemical acceptability (Ukeles, 1969). Both filtration efficiency and ingestion are affected by particle size. The oyster *C. virginica* retains diatoms (4–24 μm), but allows 70–80% of *Escherichia coli* Castellani and Chalmers cells (1–2 μm) to pass (Owen, 1966). Similarly, adult *C. virginica* filter out particles in the range of 3–12 μm 30–50% more efficiently than those in the range of 1–3 μm (Haven and Morales-Alamo, 1970). However, is appears that at least in the case of the flat oyster *Ostrea edulis* L., larvae have a smaller optimum size than adults (Wilson, 1980). In the case of starch particles fed to *Saccostrea commercialis* (Iredale & Roughley), Wisely and Reid (1978) recommended a size range of 2–4 μm, which is much smaller than that reported in the previous study, possibly because the authors used aeration to keep the inert particles suspended, whereas Haven and Morales-Alamo (1970) used a more effective electric stirrer for the same purpose.

Maximum food concentration

Food concentration, the amount of food suspended per litre of seawater, is an important criterion to consider when formulating oyster diets, however there is little consistency in the available data. This is probably due to differences in experimental conditions. For example, based on maximum glycogen content, condition index and meat weight gain, Nell and Wisely (1984) recommended 9 mg of dietary particles L^{-1} of seawater for the fattening (gonad maturation) of Sydney rock oysters *S. commercialis*, considerably more than the 2 mg L^{-1} of starch, estimated by the production of a minimum ratio of pseudofaeces to faeces, as reported by Wisely and Reid (1978). This suggests that for rapid oyster fattening or maximum meat weight of *S. commercialis* some food may need to be wasted as pseudofaeces. Based on the weight gain and glycogen content of the American oyster *C. virginica* Turgeon and Haven (1978) recommended 5 mg L^{-1} of starch to improve meat condition in adult oysters. Based on pseudofaeces production, Epifanio and Ewart (1977) recommended a maximum concentration of live algae at 10 mg dry weight L^{-1}. The results of studies based on meat gain/condition will depend on the nutritional content of the experimental diets, while studies based on faeces/pseudofaeces production largely depend on the physical characteristics of the experimental food particles.

Although food concentration can affect the rate of filtration in molluscs, studies of the blue mussel *M. edulis* report that, despite decreasing filtration with increasing food concentrations, the actual ingestion rate is not affected; that is, a similar amount of food is consumed (Winter, 1973,1978).

Feeding rate

Feeding rate, the daily amount of food supplied per oyster, is an important management tool for conditioning (gonad maturation) of broodstock, for spawning, for "fattening" or increasing meat size for marketing, for rearing larvae in hatcheries and spat in nurseries. The suggested feeding rates below emphasise maximum food consumption and not the highest assimilation efficiency.

An empirical equation for the maximum daily ration for *C. virginica* of any size at 20°C was produced by Epifanio and Ewart (1977):

$Q_R = 0.01\ W^{-0.41}$

It was refined by Epifanio (1979) to:

$Q_R = 0.01\ W^{-0.33}$,

where Q_R is daily dry weight (g) of ration g^{-1} live weight of oyster, and W is live weight of oyster (g). The exponent in the latter equation is closer to the theoretical value used by Pruder et al. (1977). Both equations are suitable for both live algal diets and artificial diets, although they may underestimate maximum food consumption at high (28°C) temperatures (Epifanio, 1983).

A similar equation for *C. virginica* was produced by Pruder et al. (1976), using wild collected adults and laboratory-reared spat:

$Y = 5.3\ W^{-0.41}$.

Because laboratory-reared spat had a higher organic content than wild oysters it was later modified to:

$Y = 8.2\ W^{-0.21}$,

where Y is the daily ration of algal cells $\times 10^8\ g^{-1}$ live weight of oyster, and W is the live weight of oyster (g) (Pruder et al., 1977). Pruder et al. (1976, 1977) used a 50/50 mixture (by cell number) of *Thalassiosira pseudonana* Hasle & Heimdal and *Isochrysis galbana* Green. These equations could also be used for artificial diets by converting the daily ration of algal cell numbers to dry weight (g).

Urban et al. (1983), however, reported that all these formulae for calculating adequate rations for *C. virginica* may not be sufficient for maximum growth of juvenile oysters. They recommended an effective daily ration (dry weight) of 2.8% of the oyster live weight.

Whatever method is used for calculating the daily ration for small, rapidly growing oysters, it would have to be recalculated weekly. As daily ration depends on culture conditions, all methods for calculating should only be used as a guide by aquaculturists.

Frequency of feeding

A tidal rhythm for digestion in flat oysters *O. edulis* was reported by Morton (1971, 1977); however, Langton and Gabbott (1974) found this not to be endogenous but controlled by feeding activity. Although continuously fed oysters filter most of the time (Higgins, 1980a), the rates at which continuously and intermittently fed *C. virginica* ingested food (consumption per hour) is not different; consequently the daily ration cleared by intermittently fed *C. virginica* is much less than that of oysters fed continuously at the same algal concentration (Higgins, 1980b). Intertidal bivalves, which have a shorter time available for feeding, do not compensate by increasing their feeding rate during immersion (Bayne and Hawkins, 1988). However, Langton and McKay (1974, 1976) reported that *C. gigas* spat in the laboratory grow faster when fed intermittently (6 h fed/6 h unfed) than when fed continuously with the same number of algal cells per day, a phenomenon that could be put to good use in hatcheries. Possibly other oysters could also benefit from intermittent feeding, provided they are supplied with the same daily ration as continuously fed oysters.

Nutrient requirements

Protein, lipids, carbohydrates and vitamins

The protein requirement for greatest growth of *C. virginica* oyster spat fed on algae was 21% of the dry

weight of the algae (Flaak and Epifanio, 1978), whereas the protein requirement for greatest meat weight gain of adult *S. commercialis* fed a microparticulate diet based on wheat starch and bacterial protein (Pruteen®) was approximately 17% of the dry weight of the diet (Nell and Wisely, 1983). Although mixtures of algae can provide a diet balanced in amino acids, the essential amino acids for oysters have not yet been determined, but in the interim the essential amino acids (Harrison, 1976) of the mussel *Mytilus californianus* Conrad could be used a guide. Langdon and Siegfried (1984) showed that different protein sources in artificial diets produced different growth rates of *C. virginica* spat, which indicates that the protein sources varied in their ability to satisfy the amino acid requirements of the oysters. Therefore, although protein quality may not be an important factor in algal diets (due to similarities in their amino acids compositions), it is likely that protein source is important in artificial diets for bivalves.

The fatty acid metabolism of bivalves was reviewed by Ackman (1983). The total lipid requirement of oysters is probably no more than 5% of the dry weight of the diet (Castell and Trider, 1974). Data on growth and on the tissue composition of oysters imply that oysters have an essential fatty acid requirement for both the linolenic or (*n*3) and linoleic or (*n*6) series fatty acids (Trider and Castell, 1980). The quantity of these essential fatty acids in algal diets for bivalve larvae need not be high (Whyte et al., 1990), although lipids are an important energy source for oyster larvae (Chu and Webb, 1984).

Oyster diets should contain a high concentration of carbohydrates (Flaak and Epifanio, 1978; Wikfors et al., 1984; Utting, 1986; Whyte et al., 1990). The composition of carbohydrates fed to oyster larvae may be of importance (Chu et al., 1982a) and requires further investigation.

The carbohydrate portion of the diet should contain a minimum of cellulose, as little is digested by oysters (Crosby et al., 1989; Langdon and Newell, 1990). It is not clear whether oysters digest cellulose through the action of endogenous cellulase or with the help of bacteria in the digestive tract of oysters. A study of bacterial activity in *C. virginica* tissue indicated that bacteria play a role in the assimilation of cellulose by oysters (Crosby and Peele, 1987), whereas Newell and Langdon (1986) found no difference in the very low cellulose assimilation of *C. virginica* treated with antibiotics and of untreated controls. Brock et al. (1986) demonstrated that cellulase activity of the hepatopancreas is a common phenomenon in natural oyster populations and later demonstrated that the intake of food high in cellulose, increases cellulolytic activity in the hepatopancreas of *C. gigas*. Brock (1989) also showed that purified bacterial cellulases do digest cell walls of chlorophytes (green algae).

Although oysters can absorb vitamins directly from seawater (Nell et al., 1983), *C. virginica* spat fed microencapsulated vitamins grew faster than spat supplied with dissolved vitamins (Langdon, 1983b). Little is known of the specific vitamin requirements of oysters, although a mixture of vitamins is required for maximum growth (Davis and Chanley, 1956; Urban and Langdon, 1984; Ukeles and Wikfors, 1988).

Mineral requirements

As with vitamins, oysters can directly absorb from seawater all the calcium that they require (Bevelander, 1952; Jodrey, 1953; Sick et al., 1979), as well as soluble phosphorus (Bevelander, 1952; Pomeroy and Haskin, 1954) and trace metals (Windom and Smith, 1972; Kopfler and Mayer, 1973; Mackay et al., 1975; Nell and Livanos, 1988; Nell and Chvojka, 1992).

The role of calcium and the processes involved in shell formation and regeneration in molluscs have been reviewed by several authors (Wilbur, 1964, 1972; Timmermans, 1969; Sick and Siegfried, 1983). However, increases of growth in shell and meat in oysters do not always occur simultaneously (Brown and Hartwick, 1988a, b) great care should be taken when interpreting experimental results (Hilbish, 1986). Both food availability and temperature affect the patterns of shell deposition and meat growth in oysters (Epifanio, 1983). Under conditions of long-term low food availability, shell thickening is more energetically efficient than meat growth (Brown and Hartwick, 1988b). Marked reduction in the calcium concentration of sea water may reduce or prevent calcification in oysters (Kado, 1960; Conger et al., 1978). Shell growth of *C. virginica* stopped at calcium concentration of about 60 mg L^{-1} of sea water or less (Conger et al., 1978). Calcium concentrations in water can drop during periods of low salinity (Nell and Holliday, 1988), which could result in stress and in reductions in both meat weight gain and shell deposition in oysters. Kado (1960) reported that when the calcium concentration of sea water increased from about 124 mg L^{-1} to the normal concentration of about 400 mg L^{-1}, the rate of calcium deposition in *C. gigas* increased, but did not increase further at higher concentrations.

Algal diets

The value of algal species as a food source for oysters and oyster larvae has been extensively studied. Davis and Guillard (1958) tested ten algal species for larvae of *C. virginica* and recommended a mixed diet of the flagellates *I. galbana*, *Pavlova lutheri* (Droop) Green, a *Tetraselmis* sp. and *Dunaliella euchlora* Lerche. After testing five algal species singly and in various combinations, Laing and Millican (1986) recommended the diatom *Chaetoceros calcitrans* (Paulsen) Takano as a single-species diet for *C. virginica* spat. Walne (1970) tested 19 algal species as a food source for juvenile flat oysters (*O. edulis*) and found *P. lutheri* and *Ch. calcitrans* to be among the best.

Two algal species — *P. lutheri* and *Isochrysis* aff. *galbana* Green (clone T-iso; termed Tahitian *Isochrysis*) — are widely used in overseas hatcheries (Holliday, 1985) and have been used routinely for feeding Sydney rock oyster *S. commercialis* larvae (Numaguchi and Nell, 1991). Tahitian *Isochrysis* has a high concentration of the 22:6*n*3 long chain unsaturated fatty acids, while *P. lutheri* has high concentrations of both the 20:5*n*3 and the 22:6*n*3 fatty acids (Brown et al., 1989). These two fatty acids individually or in combination have been found to be important for growth of *O. edulis* spat (Enright et al., 1986a, b), *C. gigas* spat (Langdon and Waldock, 1981) and *C. virginica* larvae (Chu and Webb, 1984).

Three species — *P. lutheri*, Tahitian *Isochrysis* and *Ch. calcitrans* — produced the greatest increase in shell length of *S. commercialis* larvae, whether fed

singly, or the latter two in combination with *P. lutheri* compared to other algal diets tested (Nell and O'Connor, 1991). *S. commercialis* spat, however, produced the greatest length increase when fed the diatom *Skeletonema costatum* (Greville) Cleve (W. A. O'Connor and J. A. Nell, unpublished data, 1991). These four algal species all contain high levels of one or both of the long chain unsaturated fatty acids, 20:5*n*3 and 22:6*n*3 (Brown et al., 1989). Algal species are very similar in protein levels (Enright et al., 1986a) and protein amino acid compositions (Webb and Chu, 1983) and it is therefore unlikely that they are important factors in deciding the food value of algal species to oysters.

Algae excrete organic substances termed ectocrines, which include sugars, amino acids, fatty acids, vitamins, steroids and numerous other secondary metabolites (Lefevre, 1964; Stephens and Manahan, 1984). These ectocrines can affect filtration rates and particle selection in bivalves (Ward and Targett, 1989) and the nutritional value of algal diets (Manahan, 1983c). Algal diets have been improved by the inclusion of cultures of selected strains of bacteria (Martin and Mengus, 1977; Douillet, 1989). This new and exciting development in the feeding of live food to oysters should be explored further.

Artificial diets

As oyster spat production in hatcheries usually depends on the expensive and sometimes unreliable production of microalgae (Bolton, 1982), efforts have been made to develop artificial dietary supplements or algae substitutes. To be successful the substitute should be neutrally buoyant, and stable in aqueous suspension. In the case of encapsulated diets for bivalve larvae and spat, which ingest their food whole, a capsule wall that is impermeable and stable in seawater but readily broken down by a change in pH or by the action of digestive enzymes is required (Jones et al., 1974). Gabbott et al. (1976) demonstrated the importance of capsule size and digestibility in microencapsulated diets for blue mussel *M. edulis* and Pacific oyster *C. gigas* spat.

The use of microparticulate diets for aquaculture was reviewed by Langdon et al. (1985) and the use of these diets for oysters by Langdon (1983b). A comprehensive artificial diet consisting of microgel particles, lipid-walled microcapsules and kaolin showed promise when fed to *C. virginica* spat (Langdon and Siegfried, 1984). This kind of diet, however, tends to produce high bacterial numbers (Langdon and Bolton, 1984) and is therefore less suitable for larvae. Gelatin-acacia and lipid-walled microcapsules based on cod liver oil, or lipid-walled microcapsules alone, have been used as an artificial diet for *C. virginica* larvae. These were reared to the "eyed" stage on this diet alone, although growth rates were considerably lower than on a live microalgal diet (Chu et al., 1987). Gelatinacacia microcapsules fed at a concentration of 0.05 mg L^{-1} (1 330 microcapsules mL^{-1}), considerably more than the 500 microcapsules mL^{-1} recommended by Chu et al. (1982b), were an effective supplement for algal diets fed to *S. commercialis* larvae (Numaguchi and Nell, 1991). Algal meal (Betatene Ltd, Cheltenham, Vic., Australia), a dried extract of *Dunaliella salina* (Teodoresco), fed at 0.123 mg L^{-1} performed as well as microcapsules fed at 0.05 mg L^{-1} as a dietary supplement (Numaguchi and Nell, 1991). More recently cod liver oil encapsulated in acacia has been used successfully as a source of essential long chain fatty acids for *S. commercialis* larvae (J. A. Nell, unpublished data, 1991).

Lipid-walled microcapsules, which contain an aqueous core, may be used to deliver water-soluble nutrients to oysters (Langdon and Siegfried, 1984; Chu et al., 1987). Protein-walled microcapsules have been used successfully to deliver protein to *C. gigas* (Langdon, 1989; Langdon and DeBevoise, 1990). The proportion of protein assimilated from these capsules fed to *C. gigas* spat was 39%, whereas that for glyceride-coated, nylon-protein-walled microcapsules was 29% (Langdon and DeBevoise, 1990). Good growth rates were obtained with *S. commercialis* larvae fed wholly on protein-walled capsules, consisting of 68% protein, 16% carbohydrate and 4% lipids (Southgate et al., 1992). Spat of the three oyster species *O. edulis*, *C. gigas* and *C. virginica* fed on *Ch. calcitrans* grew equally well when 40% of the algae (by dry weight) was replaced with a commercially prepared microencapsulated diet (Frippak Feeds, Basingstoke, England) (Laing, 1987).

Non-encapsulated diets have been successful in fattening (gonad maturation) adult oysters. However, these diets are unsuitable for oyster larvae, because of the high numbers of bacteria growing on the particles in the larval rearing tanks (K. Numaguchi, personal communication, 1988). Corn starch has been used successfully for the fattening of adult American oysters *C. virginica* (Haven, 1965; Turgeon and Haven, 1978). A more comprehensive diet of corn starch, casein and cod liver oil was used to study the nutritional requirements of *C. virginica* (Castell and Trider, 1974; Trider and Castell, 1980). Adult Sydney rock oysters *S. commercialis* were successfully fattened with diets based on wheat starch, bacterial protein Pruteen® (ICI, Billingham, England) and cod liver oil (Nell and Wisely, 1983, 1984; Nell, 1985).

The yeast *Candida utilis* (Henneberg) Lodder & Kreger-van Rij has been used successfully to replace up to 50% of the algae in the diet of the American oyster *C. virginica* spat (Epifanio, 1979; Urban and Langdon, 1984). It has also been used successfully as the protein source in a fattening diet for adult Sydney rock oysters *S. commercialis*, although it was inferior to the bacterial protein Pruteen® (Nell, 1985).

Conclusion and future developments

Much progress has been made in the field of oyster nutrition over the past two decades, but although our knowledge of the feeding habits of oysters in the wild has increased and artificial diets have improved, knowledge of the specific nutrient requirements of oysters is scant and more research is required. An understanding of exactly what oysters feed on in the wild is still incomplete, e.g., it is not known whether, or under what circumstances, oysters can benefit from dietary cellulose. Further investigation of the role that bacteria play in oyster nutrition, and the use of cultured strains of bacteria to supplement both algal and artificial diets is warranted. Although many species of microalgae are available for feeding to oysters, there are probably many other suitable strains or species yet to be evaluated. Recent improvements to artificial diets and the development of suitable microcapsules for oysters will assist this research. Although oyster

broodstock can be conditioned successfully on algae in hatcheries (Lannan et al., 1980), the development of commercially viable oyster "fattening" techniques has eluded researchers. Further research on the manipulation of feeding behaviour is needed to assist the management of hatcheries, nurseries and oyster "feedlots".

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