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The Retention of Picoplankton by the Pacific Oyster, *Crassostrea gigas*, and Implications for Oyster Culture

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Abstract

Pacific oyster (*Crassostrea gigas*) farming in New Zealand has reached a point where the pressures on resources appear likely to constrain current, and future, development. To maintain industry growth, security of juvenile oyster (spat) supply and productivity gains within the existing farm leases, are becoming industry imperatives. The use of hatchery technology could achieve both of these requirements, but it is expensive to establish and maintain. The additional expense of a hatchery could be offset by the establishment of, for example, a selective breeding program to enhance oyster productivity and/or marketability. Consequently a pilot-scale oyster hatchery facility was constructed to investigate the potential for establishing hatchery production of Pacific oysters in New Zealand.

This study showed that in the pilot-scale hatchery, oysters could be successfully spawned from in-season broodstock, the eggs fertilised and the resultant larvae reared through to settlement for on-growing to adult size. This process was successful for both oysters selected for morphological characteristics and those which were not. On-growing of the resultant stock indicated growth rate could be normal relative to wild caught oysters spat, although data was limited by the small scale of the experiment. However, an investigation of broodstock conditioning, to achieve out-of-season spawning, was less successful. Disease occurred and condition was lost in some broodstock, suggesting they were enduring stress within the conditioning system. The microalgal food supply was examined but the clearance rate of the microalgal species suggested they were an acceptable feed supply which agreed with previous reports of successful conditioning techniques. Comparing the pilot-scale facility in this study with descriptions of facilities which reported successful broodstock conditioning suggested that the use of a microfiltered recirculating water supply, as opposed to the more common flow-through, natural seawater systems containing a range of small size particles, limited necessary nutrient and/or maturation factors and may have had a significant impact on conditioning.

The nanoplanktonic (< 10 µm), food resource, which includes key microalgal species such as *Chaetoceros* spp. and *Isochrysis* spp., is generally considered the primary food resource for suspension feeding bivalves, including *C. gigas*. However, the picoplanktonic fraction (< 3 µm) can provide the largest proportion of this food resource in the water column in terms of abundance and biomass. Consequently, an investigation of the *in situ* retention of picoplankton populations (picoeukaryotes, *Synechococcus*-type cyanobacteria and heterotrophic bacteria) by oysters was undertaken. Flow cytometry was used to quantify the picoplankton populations in water samples taken from the inhalant and exhalant feeding currents of individual oysters, allowing retention efficiency of the particles to be calculated. Five picoplankton populations were identifiable by flow cytometry (picoeukaryotes, heterotrophic bacteria and 3 populations tentatively identified as cyanobacteria) and accounted for a large proportion (up to 97 %) of the estimated available carbon (picoplankton + microalgae) in Kerikeri Inlet water. Generally the heterotrophic bacteria accounted for the largest proportion of the biomass with up to 564 ± 51 ng C ml^{-1}.

Retention of each picoplankton population was found to be variable and not directly related to particle concentration. Cyanobacteria (Cy2 population) were retained with the greatest efficiency (up to 42 ± 4.4 %), followed by heterotrophic bacteria (up to 38 ± 4.5 %) and picoeukaryotes (up to 12 ± 3.8 %). Overall more picoplankton biomass was retained during the summer months, of which the heterotrophic bacteria made the largest contribution in either cell number or estimated carbon retained. Tracking of the condition and constituent fractions (glycogen, lipid and protein) of the subject oysters showed that in the summer months, post-spawn, these levels were lowest, indicting a period of nutritional stress. This appeared to suggest that *C. gigas* was able to alter its retention efficiency to expand the range of particles captured, and consequently the available nutrient pool. The retention of greater quantities of heterotrophic bacteria may allow for the acquisition of essential nutrients required for growth and later gametogenesis (such as B vitamins). However, it is also possible that the heterotrophic bacteria mediate access to otherwise inaccessible, or inefficiently accessed, nutrient resources through their degradation of, for example, crystalline cellulose.

Consequently, the microbial flora associated with the oyster gut was investigated. An initial investigation, cultivating bacteria from gut contents, showed considerable variability in the numbers of colonies present within and between samples, but was inconclusive for identifying differences in species diversity. Using culture independent histological and 16S rDNA PCR/RFLP techniques to investigate the oyster gut microflora a spirochaete flora, commonly associated with bivalve crystalline styles, was clearly present. Molecular analyses provided evidence of other bacterial in the gut. A signature RFLP band pattern was found in oysters at low tide and this generally reoccurred in oysters that had been immersed for varying lengths of time up to high tide. However, the signature RFLP pattern became more dilute as immersion time/potential feeding time extended.

The isolation of culturable bacteria from the oyster gut allowed characterisation and identification of a subset of the oyster gut microflora. 16S rDNA sequence analysis from selected isolates showed a predominance of *Vibrio* spp. These bacteria had previously been associated with marine molluscs, including as symbionts. Characterisation of these and other isolates from oyster gut showed a diversity of attributes including the ability to degrade cellulose. This suggests the bacterial production of enzymes, such as cellulases, which have been reported by other researchers as being present in ineffectual or low native levels in oysters. Consequently the bacterial presence in the oyster gut may be essential to efficient nutrient acquisition.

The results of these investigations have highlighted the potential importance of the heterotrophic bacteria to *C. gigas*. To date, bacteria have received relatively little attention in terms of their potential nutritive contribution to oysters, primarily due to observations that they are retained with low efficiency. However, even at low retention efficiencies the potential nutritive contribution is large due to the available abundances of heterotrophic bacteria. While the mechanisms and controls of bivalve suspension feeding have yet to be fully elucidated, the published literature indicates that selective mechanisms are available to bivalves including *C. gigas* and this current research

suggests that even pico-sized particles, retained with apparently low efficiency, can be subject to selection. The importance of the heterotrophic bacteria to *C. gigas* requires further investigation as it will have implications for not only hatchery production, but also farm management, public health and environmental impact monitoring.

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Abbreviations

S.I. (Systéme International d'unités) abbreviations for units and standard notations for chemical elements, formulae, and chemical abbreviations are used in this work. Other abbreviations used are listed below.

A_{xxx}	absorbance at xxx nm
bp	base pairs
cc	cubic centimetres
C.I.	colour index
dH_2O	sterile, glass distilled water
dNTP	deoxyribonucleoside triphosphate
EDTA	ethylenediamine-tetra-acetic-acid (disodium salt)
Exh	exhalant
g	grams
g	acceleration due to gravity
h	hour
Inh	inhalant
kb	kilobase pairs (1000 base pairs)
kDa	kiloDaltons
l	litre(s)
M	molarity (moles $litre^{-1}$)
MW	molecular weight
min	minute(s)
ml	millilitre(s)
N	normal (concentration)
PCR	Polymerase Chain Reaction
PEG	polyethylene glycol
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
s.e.	standard error (sample)
TBE	Tris-borate-EDTA-buffer
TE	Tris-EDTA buffer
TEMED	Tetramethlethylenediamine
Tris	[2-amino-2(hydroxymethyl) propane-1,3-diol, (tris)]
Tris-HCl	Tris solution pH adjusted with HCl
U	units of enzyme (manufacturer defined)
UV	ultraviolet light
v/v	volume:volume
w/v	weight:volume