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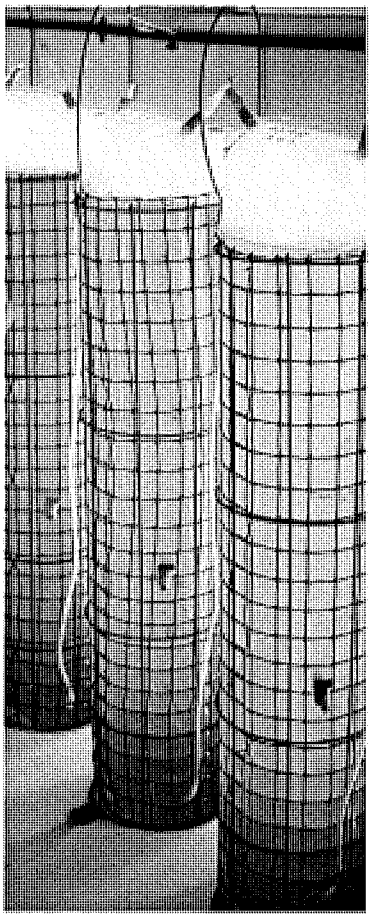
Increased Production of Juvenile Pacific Oysters (*Crassostrea gigas*) through Supplementary Feeding



M. R. Brown and M. A. McCausland

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FRDC Project 94/83

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   I. McCausland, M. A. (Malcolm A.).
   II. Fisheries Research and Development Corporation (Australia).
   III. CSIRO. Division of Marine Research.
   IV. Shellfish Culture Ltd.
   V. Title.

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1. NON-TECHNICAL SUMMARY

94/83	**Increased production of juvenile Pacific oysters (*Crassostrea gigas*) through supplementary feeding**

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OBJECTIVES:

1. To document changes in the water quality and in the growth rates of juvenile oyster (*Crassostrea gigas*) at two commercial nurseries.
2. To develop supplementary feeding techniques for increasing the productivity of juvenile oysters (*Crassostrea gigas*).
3. To test new Australian cold-water microalgal species as supplementary feed for juvenile oysters (*Crassostrea gigas*).

NON TECHNICAL SUMMARY:

The standard method for growing the early stages of juvenile Pacific oysters is to hold them in systems called upwellers in land-based nurseries. Seawater is pumped through to provide the oysters with food particles. Growth rates of oysters cultured using this method were highly variable at Pipe Clay Lagoon, one of the major oyster nurseries sites in Tasmania. Growth rates during the 1996/97 production season were less than one-third of that seen in the five previous seasons, and were also significantly less than at another oyster nursery – Little Swanport.

We conducted 15 trials at Pipe Clay Lagoon to assess whether the oysters' growth rates could by improved by "supplementing" their natural diet with additional feed sources. These supplementary diets included cultured microalgae, dried or concentrated microalgae and a yeast-based artificial diet. The results were variable, depending on the diet, its concentration, and the season – though across all trials we found that supplementary feeding (on average) increased the oysters' growth rate by 60%. Diets that were most effective included the microalgae *Isochrysis* sp. (T.ISO), *Chaetoceros calcitrans, Dunaliella tertiolecta, Rhodomonas salina* and microalgal concentrates of *Chaetoceros calcitrans* and *Skeletonema costatum.* Two commercial "off-the-shelf" products – Microfeast® MB-30 and Algamac 2000 – were also effective, though not as good as microalgae. Nevertheless the cheaper cost of these commercial products (≈AUS $80-100 kg^{-1} dry weight) compared to microalgae (eg. AUS $ 375 kg^{-1}) makes them viable alternatives to microalgae for supplementary feeding.

Supplementary feeding was most effective when natural levels of food (especially microalgae) in the inflowing seawater were low. For example, during one such 7 week period, supplementary feeding improved the growth rate of oysters by 3-fold.

Preliminary cost/benefit analysis of supplementary feeding was undertaken. Major factors include the cost of producing microalgae (dependent on scale and productions rates of the microalgae which vary from site to site, and seasonally) and the growth rates of non-supplementary fed oysters. The latter have a major influence on whether significant growth increases are possible through supplementary feeding, and were shown to vary significantly seasonally and from site-to-site. However, based on "average" microalgal production costs and the growth rates seen with supplementary feeding, we estimate that the direct additional feed costs would amount to $0.35 per thousand oysters to grow them from 0.5 to 3.0 mm. This compares to the total production cost of $\approx$ $15 per thousand oysters of 5 mm size. We believe the increased feeding costs for the nurseries would be more than offset by savings due to a reduced nursery time for the spat (less labour).

Supplementary feeding is probably restricted to use at sites like Pipe Clay Lagoon where oyster growth rates are reduced as a result of low or variable availability of food particles. There may be little benefit with supplementary feeding at Little Swanport, where the natural growth rates of oysters exceeded those of supplementary-fed oysters at Pipe Clay Lagoon. Nevertheless, we have demonstrated that supplementary feeding is an effective method for significantly enhancing growth rates of oysters at sites when natural productivity is otherwise low, providing the ability to have better control over juvenile oyster production. As a result, Shellfish Culture now plan to incorporate supplementary feeding as part of the routine production for juvenile oysters at Pipe Clay Lagoon.

2. BACKGROUND

In 1994/95, oyster culture had an annual value of $300 million and accounted for around 65% of total aquaculture production in Australia (O'Sullivan, 1996; Austasia Aquaculture). The three most important species were the pearl oyster *Pinctada maxima* ($252 million), the Sydney rock oyster *Saccostrea commercialis* ($28 million) and the Pacific oyster *Crassostrea gigas* ($20 million).

The outlook for the Pacific oyster industry was bright, with annual production increasing rapidly from $ 3.1 million in 1984 to $ 20 million in 1995. Predictions were that this industry could double by the year 2001 because of the development of new lease sites in both Tasmania and South Australia and the recent opening-up of the lucrative Sydney market to Pacific oysters. However, a further expansion of the industry depended on a corresponding increase in juvenile oyster (= seed) production. At the time, production of Pacific oyster seed (valued at $ 1.5 million p.a.) occurred only in Tasmania and was based on hatchery production of larvae. These were predominantly reared during the early juvenile stages (0.5 to 2 mm) in land-based nurseries using upweller systems where local seawater was pumped directly through to provide food particles (seston) for consumption by the oysters.

This technique had been used by Tasmanian nurseries for over a decade because it was effective, simple and low-cost. However, the variability in the quality and quantity of the seston in the water was a major limitation, producing a corresponding variability in the growth rates of the juvenile oysters. This made it difficult for seed companies and farmers to expand their production or make accurate projections and business plans.

In October 1993 we undertook a preliminary 2-week feeding trial at one of the largest oyster nursery sites in Tasmania (Pipe Clay Lagoon) to assess whether the growth of oysters was limited by an inadequate food supply. We showed that the growth rates of juvenile oysters were increased by 40% through supplementing their diet with cultured microalgae (i.e. supplementary feeding). This encouraging result prompted us to develop the 3 year FRDC project reported here, to evaluate whether supplementary feeding was a cost-effective option to enhance the production of oyster seed. The project specifically aimed to evaluate whether similar or greater increases in growth rates from supplementary feeding were possible throughout the growing season, for all sizes of oysters, at other nursery sites and using other nutritional sources.

3. NEED

At the commencement of this project, it was predicted that the Pacific oyster industry had considerable scope for expansion. This is now a reality, with the available zones for aquaculture in Tasmania (the major producer) likely to increase by more than 100% between 1997 and 1999. Such expansion is only possible if there is a corresponding increase in the production of oyster seed. This either requires 1) capital development of existing hatcheries and nurseries, or the building of new ones and/or 2) increasing the production of existing nurseries by developing more efficient and cost-effective feeding. We believed that a significant part (20–40%) of this expansion in seed production could be effected through improved feeding methods.

4. OBJECTIVES

1. To document changes in the water quality and in the growth rates of juvenile oyster (*Crassostrea gigas*) at two commercial nurseries.
2. To develop supplementary feeding techniques for increasing the productivity of juvenile oysters (*C. gigas*).
3. To test new Australian cold-water microalgal species as supplementary feed for juvenile oysters (*C. gigas*).

5. METHODS

5.1. SUPPLEMENTARY FEEDING OF OYSTERS

5.1.1. Oyster culture – experimental-scale trials

Fifteen experimental-scale trials were completed between the 1994/95 to 1996/97 nursery seasons. Protocols differed with respect to diet type, ration and presentation, oyster stocking density and size, and duration. The specific information relating to individual trials is summarised in Table 1, and also given (together with aims) in more detail in the reports of individual experiments presented in the Results. The general methods used are given below.

Shellfish Culture Ltd. provided hatchery-reared juvenile Pacific oysters (*C. gigas*) for feeding trials undertaken at their nursery at Pipe Clay Lagoon, 30 km Southeast of Hobart, Australia (42° 58' S, 147° 32' E) (Fig. 1). Juvenile *C. gigas* (from 500 to 1500 µm) were grown in experimental upweller systems, 1/20th the scale of commercially used upwellers (Figs 2 and 3). They consisted of a 10 L bucket with a 110 mm diameter, mesh-bottomed upweller chamber suspended inside, on which the oysters were retained (Fig. 3c). Seawater from Pipe Clay Lagoon (salinity 32-35 p.p.t) was continuously pumped into the upwellers at a rate of 700 mL.min^{-1} (1000 L d^{-1}). The water flowed up through the upweller chamber and the bed of oysters and out through an exit pipe. Oysters and chambers were removed from the upweller for 1 h each day and cleaned with a spray of fresh water to remove faeces and other particles. Chambers were also rinsed weekly with 1% sodium hypochlorite.

Most of the trials commenced on a Monday. An initial volume of 10 to 20 mL of oysters was dispensed into each upweller. Oysters were acclimated in flowing seawater overnight before commencing supplementary feeding the next day. A pre-conditioning period to allow the oysters to equilibrate to the experimental diets was not considered to be necessary. Whilst such a period is essential in studies with formulated feeds where palatability and acceptance of a new diet may be an issue (D'Abramo and Castell, 1997), it has generally not been applied in bivalve mollusc studies feeding on microalgae (Urban et al., 1983; Enright et al., 1986a; O'Connor et al., 1992; Wikfors et al., 1996). Also, all diets tested had previously been shown to be readily accepted by oysters (Enright et al., 1986a; Nell et al., 1996; Boeing, 1997; Brown et al., 1998).

The photoperiod under which the oysters were grown ranged from 6:18 h to 8:16 h light:dark (L:D). The positions of the upwellers were randomly altered 3 to 4 times throughout each experiment (Figure 4B). Control oysters ($n \geq 2$ buckets) in all trials received a diet of naturally occurring seston provided by the continuously flowing seawater. Treatment oysters ($n \geq 3$ buckets) also received this; in addition they received supplementary feed delivered by one of two standard methods. During the first 2 years of the project, feed was usually dispensed twice each day over 2 h periods (at noon and midnight) from Monday to Friday. Diets (i.e. 0.3 to 5 L of algal culture, or rations of artificial feeds or pastes resuspended in seawater or freshwater) were dispensed into 10 L buckets and diluted to 9 L with seawater. The suspension was pumped into each treatment upweller at 75 mL.min^{-1} with a small submersible aquarium pump (Aquarium Powerhead 480, Second Nature, NJ, USA) (Figure 3B). During the latter half of the project, supplementary feed was delivered continuously using a peristaltic pump (Cole-Palmer, Masterflex model 7519-05) to pump algal culture from the bags (at ≈ 1 to 2 L h^{-1}; depending on culture density and the ration required). The cultured algae were continuously mixed and diluted with seawater (≈ 1:50 to 1:100), also pumped using a Cole-Palmer peristaltic pump (model 7018-20). This diluted mixture was dripped into individual upweller buckets by pumping through Nylex irrigation drippers, delivering 4 L h^{-1} per upweller bucket.

The duration of trials was between 16 to 26 days. Initial experiments established that a) this was sufficient to have a high probability of discriminating 20% differences in growth rates between treaments (*a posteriori* power analyses), and b) supplementary-fed treatments increased in biomass by ≈300%, a value which is normally considered sufficient to detect any deficiencies within diets (D'Abramo and Castell, 1997).

Oyster survival was estimated by microscopic examination and count of live oysters of replicate sub-samples of 100 oysters at the start and end of each experiment. Oyster growth rates were determined from measurements made at the start and end of each experiment using three different methods: (a) volumetric; measuring the packed volume of the oyster population within each upweller, (b) dry weight (DW); a subsample of 200 oysters from each upweller was collected, rinsed with distilled water, dried at 100°C for 72 h, then weighed, (c) organic weight (OW); the subsample of 200 oysters used for DW analysis was heated in a muffle furnace (450°C; 24 h), then reweighed to determine the OW by weight loss. In each of these methods the formula used to determine the instantaneous growth rate (k) in d^{-1} was:

$$k = \ln (M_t / M_0) / t$$

where, M_t = measurement at day t, M_0 = measurement at day zero.

5.1.2. Oyster culture – commercial scale trials

Four commercial-scale trials were undertaken; two involved supplementary feeding, and two compared control growth rates at different sites. The commercial-scale upweller comprised a 45 cm diameter nylon-meshed chamber retained within 150 L plastic tubs (Figure 2A). Unfiltered seawater was pumped into each upweller (between inner wall of tub and outer wall of inner chamber) at 14 L min^{-1}. Oysters (700 to 1300 µm) were stocked at commercial densities (250 to 520 mL). Duplicate upwellers were maintained for control and treatment groups. Oysters and upwellers were cleaned daily to remove faeces and adherent particles according to standard methods used in the nursery.

Supplementary feeding of oysters was undertaken in similar fashion (apart from scale) to that of the experimental-scale trials above. Methods used to measure growth rates were also identical.

Details of the trials are summarised in Table 1, with additional detail as follows:

Trial A: Supplementary feeding with P. pinguis
Oysters (1300 µm) were stocked in upwellers at an initial volume of 520 mL. Treatment oysters were supplementary fed on weekdays in 2 rations, fed over 1 h at ≈ noon and midnight. The ration (1.7 g $weekday^{-1}$) was held constant throughout.

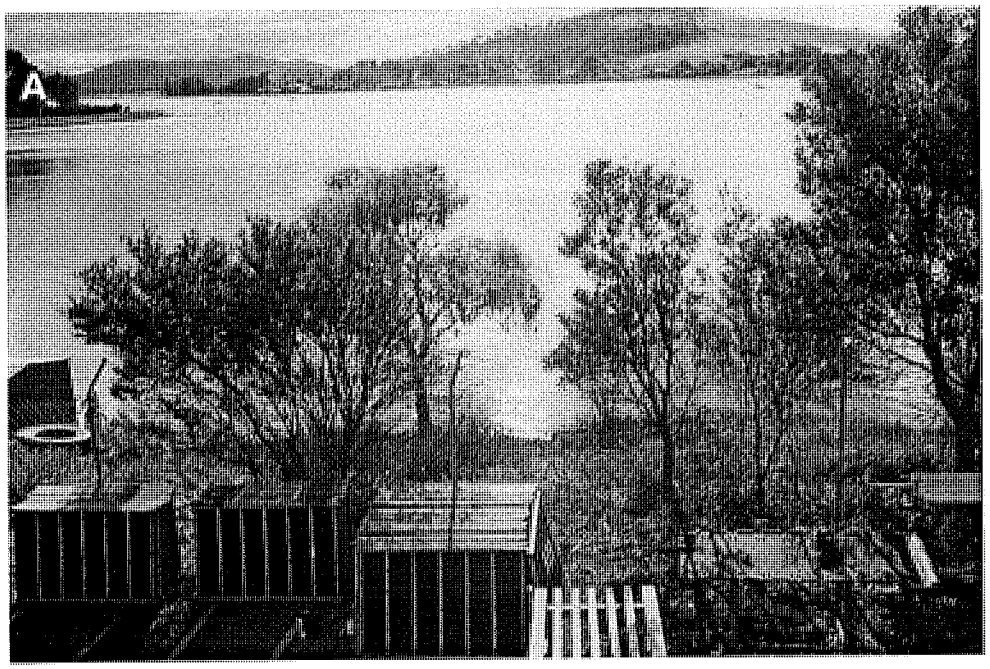
Trial B: Supplementary feeding with P. pinguis
Oysters (700 µm) were stocked in upwellers at an initial volume of 250 mL. Treatment oysters were supplementary fed on weekdays in 2 rations, fed over 2 h at ≈ noon and midnight. The ration (1.3 g $weekday^{-1}$) was held constant throughout.

Trials C and D: Control growth rates at Pipe Clay Lagoon versus Little Swanport
Prior to the site comparison studies, a volume of 1.5 L of oysters (900 µm) maintained at the Pipe Clay Lagoon site was divided; half of the stock was translocated to the Little Swanport site and the other half retained at Pipe Clay Lagoon. After 2 days of acclimation to normal nursery conditions, duplicate upwellers were stocked at 300 mL of oysters. Oysters were then subjected to normal nursery maintenance until the completion of the experiment.

Table 1. Details of the experimental- and commercial-scale trials undertaken in the project.

Trial number/ code	Date	Initial oyster stocking density	Initial oyster size (µm)	Supplementary diets	Duration (d)	Feeding days	Average water T (°C) (min-max)	Average chl a in nursery water (µg L^{-1})	Weekday algal ration (mg weekday^{-1} upweller^{-1})	Mean daily algal ration (mg day^{-1} upweller^{-1})	Mean daily algal ration (mg day^{-1} mL^{-1} oysters)
Experimental scale trials:											
1	28/11 to 16/12/'94	≈ 20 mL*	700	*P. pinguis* (12:12 h L:D) at various rations	18	13	16 to 22	0.82	53 to 212	38 to 153	1.9 to 7.7
2	23/1 to 10/2/'95	≈ 20 mL*	700	*P. pinguis* (12:12 h L:D); by drip and recirculation methods	18	13	17 to 20	0.70	103	74	3.7
3	27/2 to 17/3/'95	≈ 20 mL*	700	*P. pinguis* (12:12 h L:D); continuously and intermittently	18	13	16 to 21	0.69	98	70	3.5
4	3 to 21/4/'95	≈ 20-25 mL*	900, 1300	*P. pinguis* (12:12 h L:D); oysters of different sizes	18	13	12 to 15	1.47	106	76	2.8 to 3.2
5	29/5 to 16/6/'95	27 mL	1300	*P. pinguis* (12:12 h L:D); 1 vs 2 feeds/day	18	13	9 to 12	2.90	97	69	2.6
6	6 to 24/11/'95	19 mL	700	*P. pinguis* (12:12 h L:D), *Pavlova* sp. CS-63 (12:12 h L:D)	18	13	14 to 18	0.51	90 to180	65 to 130	3.4 to 6.8
7	4 to 22/12/'95	13 mL	700	*P. pinguis* (12:12 h L:D), *P. pinguis*	18	13	16 to 19	0.95	140	103	7.9
8	8 to 26/1/'96	19 mL	700	*P. pinguis*, *Rhodomonas salina*,1:1 mixture of the two	18	13	18 to 22	0.69	190	137	7.2
9	19/2 to 8/3/'96	19 mL	700	*P. pinguis*, *Chaetoceros calcitrans* (18:6h L:D) *C. calcitrans*, *Skeletonema* sp. pastes	18	13	17 to 21	0.63	180	130	6.8
10	18/3 to 4/4/'96	19 mL	700	*P. pinguis*, *Isochrysis* sp. (T ISO), *Dunaliella tertiolecta*	17	12	15 to 18	0.98	190	134	7.1
11	15/4 to 3/5/'96	18 mL	1100	not applicable (control oysters only)	18	n/a	13 to 15	1.30	n/a	n/a	n/a
12	6/11 to 2/12/'96	9 mL	500	*Isochrysis* sp. (T ISO), Algamac 2000, Microfeast MB-30	26	17	14 to 17	0.55	135#	88#	9.8#
13	2 to 19/12/'96	12 mL	700 to 1300	*Isochrysis* sp. (T ISO) at various rations	17	17	16 to 19	0.70	58 to 175#	58 to 175#	4.8 to 14.6#
14	10 to 26/2/'97	10, 20, 30 mL	500	*Isochrysis* sp. (T ISO) to oysters stocked at different levels	16	16	19 to 21	0.30	224#	224#	7.5 to 22.4#
15	10 to 26/3/'97	20 mL	700	*Isochrysis* sp. (T ISO) to oysters at different flow rates	16	16	16 to 19	0.36	196#	196#	9.8#
Commercial scale trials:											
A	29/5 to 9/6/'95	520 mL	1300	*P. pinguis* (12:12 h L:D)	11	8	9 to 12	2.20	1670	1215	2.3
B	4 to 22/12/'95	250 mL	700	*P. pinguis* (12:12 h L:D)	18	13	16 to 19	0.95	1300	939	3.8
C	14 to 24/2/97	300 mL	900	n/a (control oysters; comparison of L.S. and P.L. sites)	10	n/a	19 to 20 (P.L.) 20 to 24 (L. S.)	0.30 (P.L.) 2.5 (L.S.)	n/a	n/a	n/a
D	22/4 to 5/5/'97	300 mL	900	n/a (control oysters; comparison of L.S. and P.L. sites)	13	n/a	12 (avge; P.L.) 13 (avge; L. S.)	0.68 (P.L.) 2.1 (L.S.)	n/a	n/a	n/a

* measured as 25 g wet wt; # initial rations; increased weekly relative to oyster growth. Microalgae grown under 24:0 h L:D unless otherwise specified.
Abbreviations: n/a = not applicable; P.L. = Pipeclay Lagoon; L.S. -= Little Swanport; avge. = average; chl a = chlorophyll a; T = temperature.



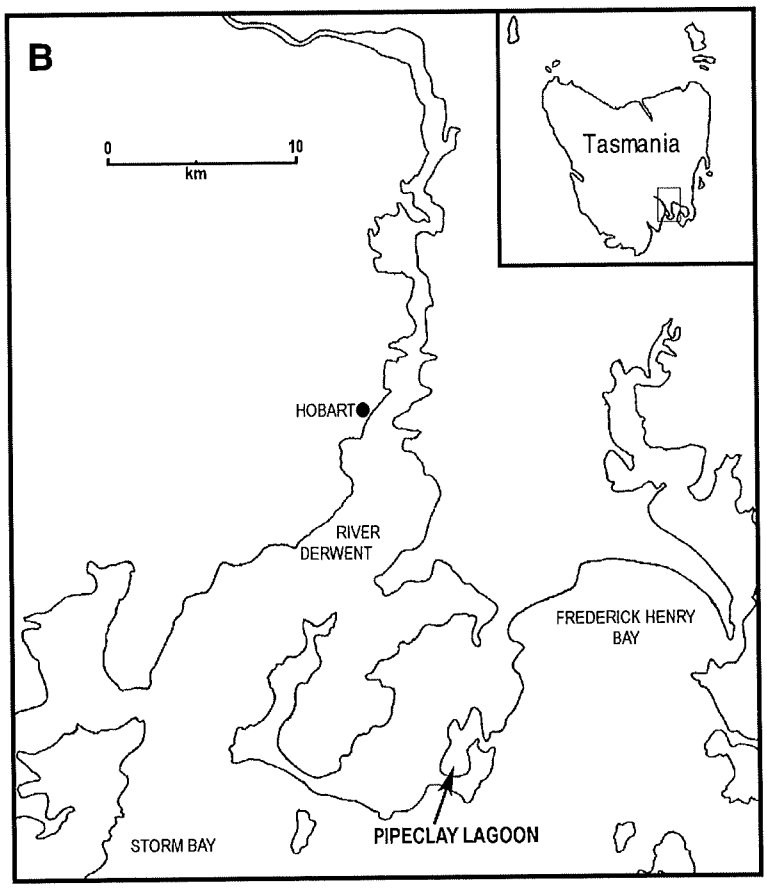


Fig. 1. A. View of Pipe Clay Lagoon from the Shellfish Culture nursery.
B. Regional map indicating the location of Pipe Clay Lagoon.

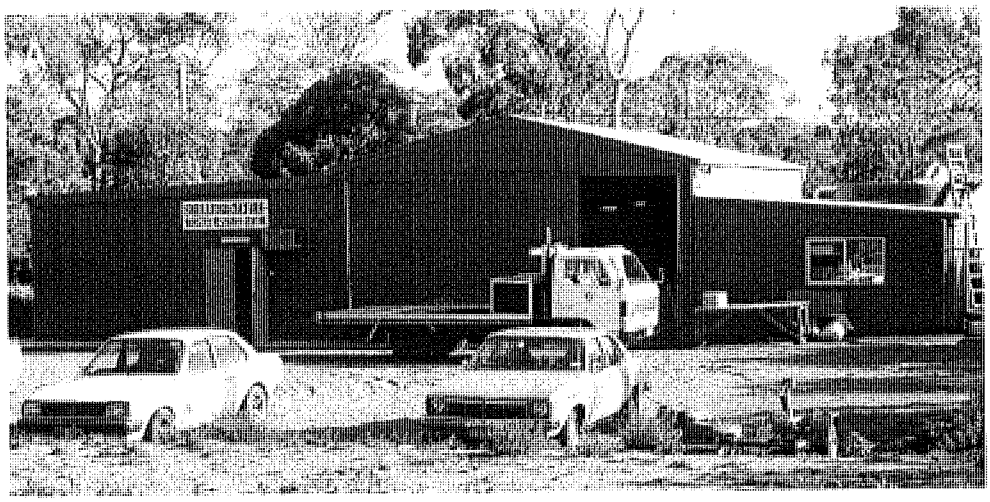


Fig. 2. A. Shellfish Culture's Pipe Clay Lagoon oyster nursery and research facility.
B. Commercial upwellers containing oysters.

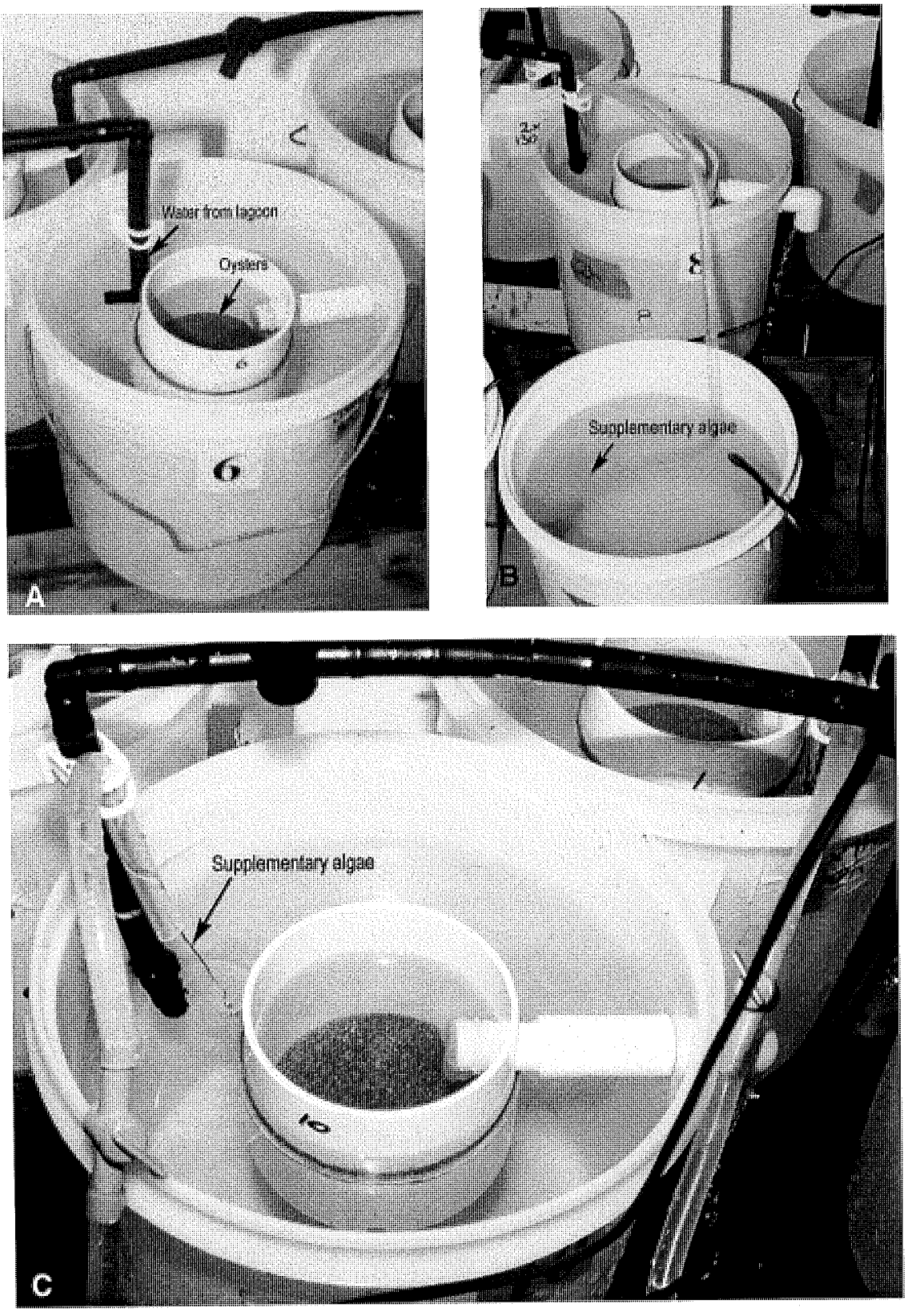


Fig. 3. A. Control upweller receiving unfiltered seawater pumped from Pipe Clay Lagoon.
B. Bucket of supplementary algal feed being pumped into an upweller.
C. Supplementary feeding of oysters, showing the flow-rate at which food was added (60-80 mL min^{-1}).

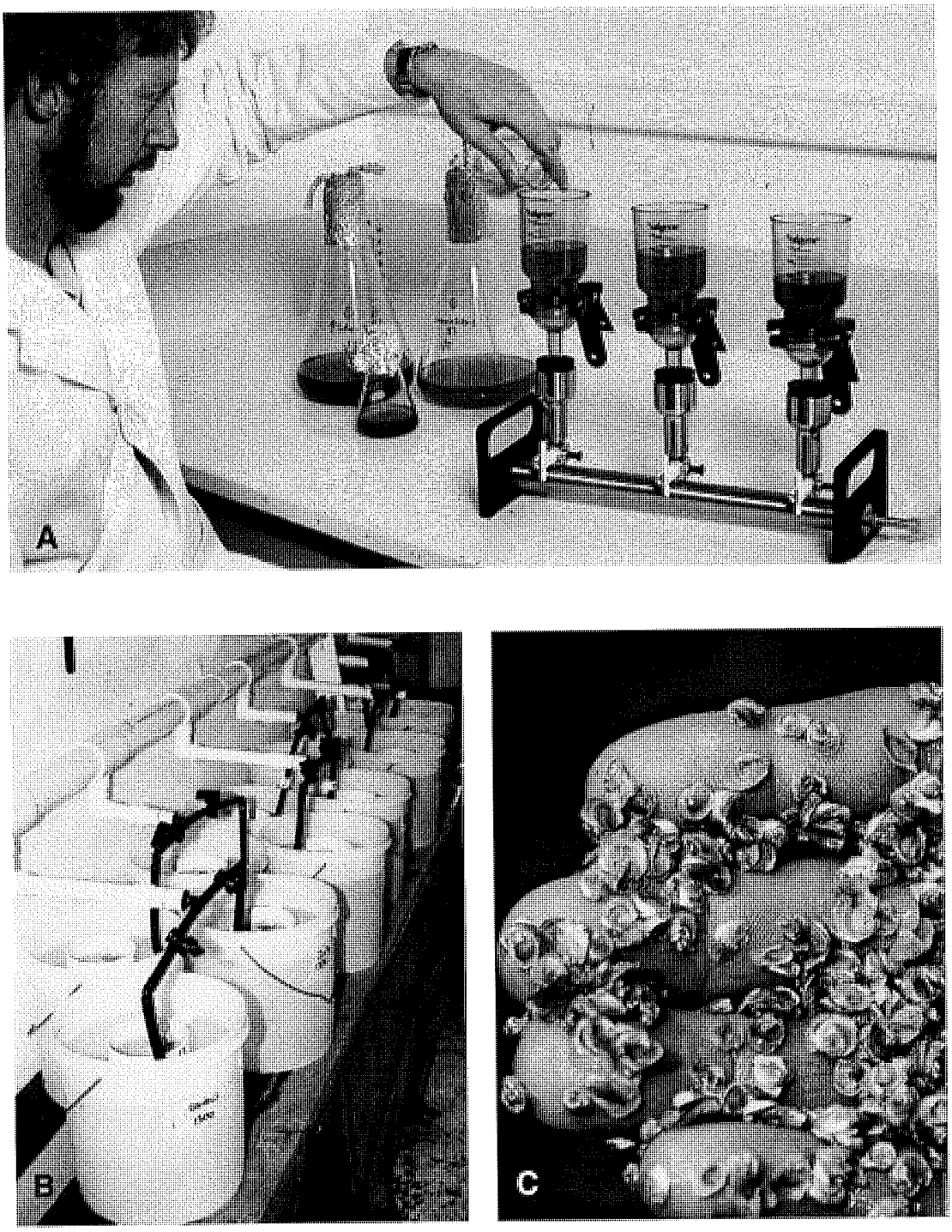


Fig. 4. A. Filtering of algal cultures onto glass-fibre filters, prior to biochemical analysis.
B. Layout of experimental upwellers. Typically, experiments utilised 16-23 buckets, and their positions were randomly altered 3 to 4 times during each experiment.
C. The size of oyster spat at the completion of a feeding trial, i.e. 2 to 3 mm.

5.1.3. Algal cultures

Microalgae were obtained from the CSIRO Collection of Living Microalgae (Table 2). They were cultured in medium f_2 (Guillard and Ryther, 1962), except *R. salina*, which was cultured in medium f_E (Jeffrey, 1980). Starter cultures (150 mL in mid to logarithmic phase) were inoculated into 1.4 L of seawater enriched with nutrients in 2 L Erlenmeyer flasks (Figure 5A). Flasks were placed on glass shelves and illuminated with white fluorescent light (Philips daylight tubes) from beneath at 100 µmol photon m^{-2} s^{-1} at 20 ± 2°C. Cultures were bubbled with air enriched with 0.5 to 1% CO_2 at a flow-rate of 0.4 L min^{-1}. Most cultures received continuous illumination, except for several indicated in Table 1 that received 12:12 h or 18:6 h L:D. At mid to late logarithmic phase, the flask contents were transferred to 10 L polycarbonate carboys containing 8 L of seawater enriched with nutrients (Figure 5B). Carboy cultures were grown under identical conditions. At mid to late logarithmic phase, their contents were transferred to sterile polyethylene bags (except *C. calcitrans*) containing 85 L of 0.2 µm filtered seawater enriched with nutrients (Figure 5C). Bag cultures were grown under identical conditions to flask and carboy cultures; however the light intensity was 50 to 75 µmol photon m^{-2} s^{-1} for continuously illuminated cultures and 100 to 150 µmol photon m^{-2} s^{-1} for 12:12 h L:D cultures. Cultures were mixed with air enriched with 1 to 2% CO_2, at a flow rate of 20 L min^{-1}, maintaining pH between 7.0 and 7.5. *C. calcitrans* 10 L carboy cultures were transferred into glass carboys containing 20 L of 0.2 µm filtered seawater enriched with nutrients, and grown in otherwise identical conditions. Replicate (3 to 6) cultures (bags or carboys) of each species were used throughout the experiments. Cultures were maintained in late-logarithmic growth phase by removing 20 to 40% of the culture volume every 2 to 3 days and replenishing with fresh media.

5.1.4. Alternative supplementary diets

Algal pastes were supplied from the NSW Fisheries, Port Stephens Research Centre. *S. costatum* and *C. calcitrans* were grown in f_2 media in 1000 L tubs at 23 ± 1°C and were aerated with CO_2-enriched air. *S. costatum* was grown under 24:0 h L:D illumination and *C. calcitrans* under 18:6 h L:D. The algae were harvested at late logarithmic phase using a continuous super-centrifuge. The pastes were collected, transferred to 250 mL polycarbonate sample jars and placed in a 4 L insulated container with frozen ice-packs. This was sealed, dispatched by overnight courier to the CSIRO Hobart laboratory, and the sample jars were refrigerated at 4°C upon arrival. Pastes were received 3 d before the commencement of trial 8 and a second batch was received 1 week later, so that the pastes used within the trial were between 4 and 11 d old on any particular day. The pastes (2 g wet weight) were resuspended in 1 L of seawater by mixing for 30 sec with a hand-held blender prior to dispensing the rations into the individual feed buckets.

Two "off-the-shelf" dried products were evaluated in Trial 13: 1) AlgaMac 2000, spray-dried cells of *Schizochytrium* sp. grown heterotrophically, supplied by Aquafauna Bio-Marine Inc. and 2) Microfeast® MB-30, a yeast-based microdiet supplemented with fish oils, from Microfeast. These products were provided as free samples from the respective companies, and normally cost $US 50-55 (1996 prices). They were resuspended (0.5 g in 2L seawater for AlgaMac 2000; 2L of freshwater for MB-30) by mixing for 30 sec with a hand-held blender prior to dispensing the rations into the individual feed buckets.

Table 2. Microalgae used as supplementary diets for juvenile Pacific oysters.

Algal Class and Species	CSIRO Culture No.	Deposition or origin code(s)	Axenic	Culture medium	Cell size (µm)	Australian isolate
Bacillariophyceae						
Chaetoceros calcitrans (Paulsen) Takano[a]	CS-178	C.CAL, CCMP1315	Yes	f_2	3-6	No
Skeletonema costatum (Greville) Cleve[a]	CS-181	SKEL, CCMP1332	Yes	f_2	5 x 10	No
Chlorophyceae						
Dunaliella tertiolecta Butcher[a]	CS-175	WHOI1, DUN, CCMP1320	Yes	f_2	10-12	No
Cryptophyceae						
Rhodomonas salina [b]	CS-24	—	No	f_E	5 x 12	Yes
Prynesiophyceae						
Isochrysis aff. *galbana* Parke (T. ISO)[a]	CS-177	T. ISO, CCMP1324	Yes	f_2	3 x 5	No
Pavlova sp.[c]	CS-63	SPECK 16.3	No	f_2	5	Yes
Pavlova pinguis Green[d]	CS-375	PRPL01	No	f_2	5	Yes

[a] R. R. L. Guillard, Bigelow Laboratory for Ocean Science, West Boothbay Harbor, Maine, USA
[b] CSIRO Collection of Living Microalgae, isolated from Port Hacking, New South Wales, Australia
[c] D. Frood, isolated from Port Philip Bay, Victoria, Australia
[d] CSIRO Collection of Living Microalgae, isolated from Pipe Clay Lagoon, Tasmania, Australia

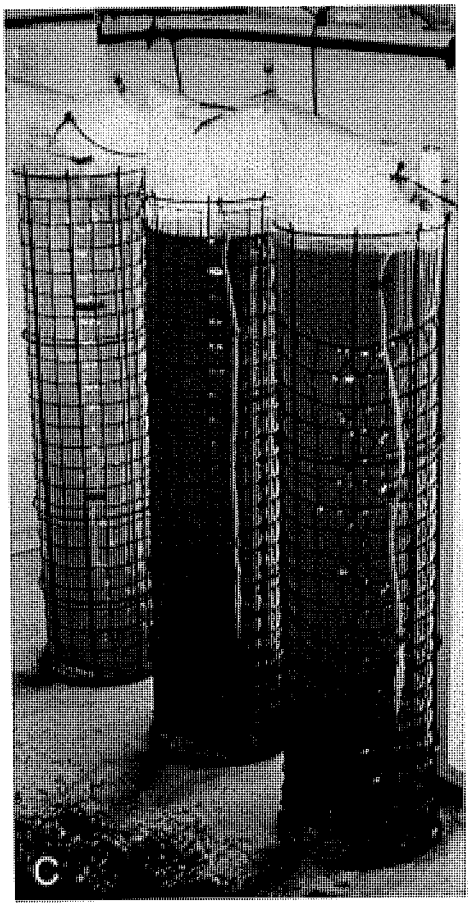


Fig. 5. Various scales of algal culture:
A. Two litre flask culture.
B. Ten litre carboy culture.
C. Eighty-five litre bag culture.

5.1.5. Biochemical analysis of microalgae and oysters

For experimental-scale trials, aliquots (1 L) were removed from microalgal cultures every 2 to 4 days for biochemical analyses. For carbohydrate and protein determination, subsamples (10 to 80 mL) were filtered through 25 mm glass-fibre filters (Whatman GF/C). For total lipid determination, subsamples (150 to 600 mL) were filtered through 47 mm glass-fibre filters (Whatman GF/C). All filters were stored at -20°C and analysed within 6 months. For fatty acid analysis (trial 11), subsamples (80 to 300 mL) were filtered through 47 mm filters and stored in liquid nitrogen until analysed (Figure 4A). Subsamples (50 to 400 mL) were also filtered through precombusted (450°C; 24 h), preweighed, 47 mm filters for dry and ash weight. The filters were washed with 30 mL of 0.5 M ammonium formate to remove residual salts, dried at 80° C overnight, then reweighed to determine the dry weight. Ash weight was determined by subsequently heating the filters in a muffle furnace (450°C; 24 h) and reweighing. Pastes (0.21 to 0.42 g) were resuspended in 200 mL of 1 µm filtered seawater, and subsamples filtered for later analysis of carbohydrate and protein (5 mL), lipid (80 to 100 mL), dry and ash weight (35 to 60 mL) in the same way.

Carbohydrate was analysed after hydrolysing filters with 3.9 mL of 0.5 M H_2SO_4 at 100°C for 4 h in polypropylene centrifuge tubes by the phenol-sulphuric acid method (Dubois et al. 1956). Protein was analysed after homogenising filters with 6% TCA, with a modified Lowry et al. (1951) technique (Clayton et al. 1988). Lipid was determined gravimetrically; filters placed into 10 mL Mini-vials were repeatedly extracted in chloroform-methanol-water (2:4:1, v/v/v; 7 to 8 x 5 mL) (Whyte, 1987). The supernatants were combined, and chloroform and water added to bring the ratio to 1:1:0.9 for phase separation and extraction of lipids in the lower chloroform phase. The chloroform layer was concentrated under vacuum and weighed to determine total lipid.

In trial 11, we compared the fatty acid compositions of the supplementary diets with those of the oysters fed on the respective diets. Samples of microalgae were extracted overnight with chloroform-methanol-water (1:2:0.8, v/v/v) (Bligh and Dyer, 1959). Chloroform and water were added to bring the ratio to 1:1:0.9 for phase separation and extraction of lipids in the lower chloroform phase. The lipid extracts were saponified and the liberated fatty acids were acidified, then extracted (Volkman et al., 1989). Fatty acids were transesterified to methyl esters and analysed by capillary gas-chromatography (GC) using polar and non-polar capillary columns and GC-mass spectrometry. Oysters were collected at the completion of trial 9 and ground with a mortar and pestle then freeze dried before analysing for fatty acids in the same way.

5.1.6. Apparent growth efficiency

Apparent growth efficiency (%AGE) is a measure of the efficiency with which food was used and was determined for each diet by the equation:

$$\%AGE = [(\Delta OW_{sf} - \Delta OW_c) / DW_{feed}] \times 100$$

where ΔOW_{sf} was the change in OW of supplement-fed oysters, ΔOW_c was the increase in the OW of control oysters, and DW_{feed} was the dry weight of supplementary food. The OW of a known volume (1 to 4 ml) of oysters was measured at the start and end of an experiment to calculate the OW per upweller. This was used to calculate ΔOW_{sf} and ΔOW_c.

5.1.7. Analysis of seawater flowing through upwellers

Daily temperature maxima and minima of seawater flowing into upwellers were recorded using a maxima/minima thermometer. Water samples (filtered through a 20 µm nylon screen) were taken weekly from Pipe Clay Lagoon, and analysed for salinity, chlorophyll *a*, total particulate matter (TPM) and particulate organic matter (POM). Fatty acids were determined during trial 9. Water (1.5 to 4.0 L) was filtered through 47 mm filters. Filter samples for fatty acids were stored and analysed as

previously described; filter samples for chlorophyll *a* analysis were stored at -20° C for 1 to 2 months and later extracted in 90% acetone (Jeffrey and Humphrey, 1975). The concentration of chlorophyll *a* was determined spectrophotometrically using the equations from Jeffrey and Humphrey (1975). TPM filters were washed with 30 mL of distilled water to remove residual salts, dried overnight at 80° C, and reweighed to determine the mass of the TPM. The mass of POM was determined by heating these filters in a muffle furnace (450°C, 24 h) then reweighing to determine by weight loss.

5.1.8. Statistical analysis of growth trials and biochemical composition

The different measures (dry weight, organic weight, volumetric) of oyster growth rate (k) were compared by linear regression analysis. Growth rates from individual trials were compared using analysis of variance (ANOVA), with significant analyses followed by Fisher's protected least significant difference (PLSD) test for pairwise comparisons.

Differences in the gross composition (lipid, carbohydrate, protein) of diets were examined by ANOVA, and significant tests analysed by Fisher's PLSD for pairwise comparisons. Linear regression of oyster growth increase against the gross composition of diets was performed to determine their influence in a supplementary diet. All statistical tests were at a significance level of $P < 0.05$.

A posteriori power analyses were undertaken to determine the minimum difference in growth rates of oysters required to give a significant effect ($P < 0.05$), with a power value of 0.8, based on 4 replicates/treatment group and 4 treatments (Searcy-Bernal, 1994).

5.2. MONITORING PROGRAM AT THE OYSTER NURSERIES

5.2.1. Prelude

Prior to this project, we undertook a monitoring program of the water entering upwellers at the Shellfish Culture Ltd. nursery at Pipe Clay Lagoon for 2 years to gain a better understanding of the factors affecting animal growth rates in the commercial situation. This data are included in this report. Water parameters measured included temperature, salinity, inorganic nutrients, total and organic particulate matter, and chlorophyll *a*. As part of the FRDC project, this monitoring was continued and enhanced to describe dissolved organic carbon, and the polyunsaturated fatty acid and pigment content of particulate matter in the water entering upwellers. Data were compared to the weekly growth rates of oysters at the site.

Monitoring initiated at the Little Swanport oyster nursery site was also continued. Because of logistics and cost, the sampling was less frequent (fortnightly) and less comprehensive (temperature, inorganic nutrients, salinity, chlorophyll *a*) than at Pipe Clay Lagoon. Oyster growth rates were not assessed routinely at the Little Swanport site – though we twice compared oyster growth rates at Pipe Clay Lagoon and Little Swanport (commercial scale trials C and D, in previous section).

5.2.2. Monitoring of animal growth in the Pipe Clay Lagoon nursery

At the start of each week, a nursery upweller (Fig 2A) containing newly graded juvenile oysters (1 L of 1600-1800 µm size class) was set aside for the growth trial. A sub-sample of 200 oysters was removed from the upweller for the analysis of dry and organic (meat) weight of oysters. For these analyses, the sub-sample was rinsed with distilled water, dried at 100°C for 24 h, then weighed to determine dry weight; the sample was then heated in a muffle furnace (450°C; 24 h), and reweighed to determine the organic weight by weight loss.

The upweller was left ungraded for the following week. Unfiltered seawater was pumped continuously into the upweller (i.e. between inner wall of tub and outer wall of inner chamber) at 14 L min^{-1}. Oysters and the upweller were cleaned daily to remove faeces and adherent particles according to standard methods used in the nursery. After 7 days, a further sub-sample of 200 oysters was collected for the same analyses of dry and organic weight. The differences between the weights of the oysters between day 7 and day 0 gave a measure of their weekly growth.

5.2.3. Collection of water

Water was collected weekly from the sites at 10 a.m. each Thursday. Temperature was recorded and 20 L was initially filtered through a 250 µm nylon screen to exclude larger zooplankton. Most of the water (15 L) was then passed through an additional 20 µm nylon screen. Water passing through this screen was regarded as containing particles that were capable of being ingested by the oyster spat.

Water leaving upwellers used in the animal growth rates trials was also collected (after filtering through a 20 µm screen) for measurement of chlorophyll *a*. All water samples were transported to CSIRO for processing.

5.2.4. Chlorophyll *a* and other pigments

Water samples (2.5 L of both 20 µm-filtered and 250 µm-filtered) were filtered under vacuum through glass fibre filters (Whatman GFC; 47 mm), and the filters then stored at -20°C for < 2 months prior to analysis[#]. Filters were cut into 5 mm squares and transferred to 10 mL centrifuge tubes together with 4.5 mL of ice-cold acetone. Samples were then vortexed, sonicated, diluted with 0.5 mL water, and then left in the dark at 4°C for 30 min to complete extraction (Wright *et al.*, 1991). Chlorophyll *a* in the extracts were determined spectrophotometrically (Jeffrey and Humphrey, 1975).

[#] Jeffrey et al. (1997) reported an 82% recovery of chlorophyll a after storing a mixed microalgal samples at -20°C for 2 months.

For the analysis of pigments by HPLC, samples were collected in a similar fashion, with storage in liquid nitrogen for up to 2 years prior to analysis*. Most of the filters from the weekly samples within the same month and year were combined to form an "integrated" monthly sample. This sample was extracted using 15 mL of acetone, and the final extract filtered through a 0.45 μm nylon syringe-filter prior to HPLC. HPLC analysis was undertaken by reverse-phase HPLC with a C18 column (Wright et al., 1991) and the pigments were identified and quantified using a Water UV-Visable Diode-Array detector.

5.2.5. Particulate matter

Water samples (2.5 L) were filtered under vacuum through preweighed, precombusted glass fibre filters (47 mm). Filters were immediately rinsed with 10 mL of water to remove residual seawater, dried (100°C, 16 h) then weighed to determine the total particulate matter. Organic material on filters was combusted by heating at 450°C for 16 h. Filters were reweighed, and the change in weight calculated to estimate the particulate organic matter in the original sample.

5.2.6. Nutrients and salinity

Samples of 10 mL and 200 mL were collected and stored for the analysis of nutrients and salinity respectively. These analyses were performed by the Ocean Monitoring Service (OMS) group at CSIRO Marine Research: nutrients by segmented-flow analysis and salinity by inductive conductimetry.

5.2.7. Dissolved organic carbon

Water samples were filtered using an Amicon stirred-cell system to remove molecules of > 1000 molecular weight. Filtrates were collected and stored in acid-rinsed, pre-combusted bottles, then sent to the South Australian Water Laboratories for analysis.

5.2.8. Polyunsaturated fatty acid content of particulate matter

Water samples (2.5 L; prefiltered through 20 μm nylon screen) were filtered under vacuum through glass-fibre filters (Whatman GFC; 47 mm), and the filters then stored in liquid nitrogen prior to analysis. Samples were extracted for lipid, and the fatty acid composition determined by gas-chromatography (Volkman *et al.*, 1989).

5.2.9. Ingestion of microalgae by oysters

The difference in chlorophyll *a* between water entering and leaving upwellers was measured to provide a measure of microalgal uptake by oysters. Water samples (2.5 L) were filtered through glass fibre filters (Whatman GFC; 47 mm), and the filters then stored at -20°C for < 2 months prior to analysis of chlorophyll *a* by methods detailed above.

5.2.10. Statistical analysis

Data were analysed by regression modelling approaches, with growth rate of oysters as the dependent variable, and nutrient composition as explanatory variables. Effects were examined to see if they were linear or non-linear.

* Jeffrey et al. (1997) reported an average recovery of 93% for pigments after storing algal samples for 328 days in liquid nitrogen.

6. RESULTS

6.1 ACHIEVEMENTS AGAINST STATED OBJECTIVES OF PROJECT

Objective 1: To document changes in the water quality and in the growth rates of juvenile oyster (*C. gigas*) at two commercial nurseries

Concentrations of nitrate, phosphate, silicate, chlorophyll *a*, total particulate matter (TPM), particulate organic matter (POM), dissolved organic carbon (DOC), and salinity and temperature were measured in water samples from Pipe Clay Lagoon and Little Swanport weekly; fatty acid and pigment concentrations were measured monthly. Nutrient profiles differed between the sites, Little Swanport was characteristically estuarine with fluctuating salinities and significantly higher concentrations of silicate. Chlorophyll *a* concentrations at Little Swanport were approximately double that of Pipe Clay Lagoon.

Growth rates of oysters were assessed weekly at the Pipe Clay Lagoon site. Multiple regression analysis showed no relationship between oyster growth and water quality, although significantly lower growth rates occurred from January to June 1997 and this was associated with significantly lower concentration of chlorophyll *a* during the period.

Growth rates of oysters were assessed at Little Swanport on two occasions; during February 1997 and April 1997. Rates were significantly greater than those of oysters from Pipe Clay Lagoon during the same period. The difference in growth rate was attributed to a much higher level of phytoplankton (based on measured chlorophyll *a*) in the water during this time.

Objective 2: To develop supplementary feeding techniques for increasing the productivity of juvenile oysters (*C. gigas*).

Supplementary feeding techniques were developed that typically (depending on season, diet type and concentration) increased the growth rates of oysters at the Pipe Clay Lagoon site by 60%. The most effective diets were *Isochrysis* sp. (T.ISO), *D. tertiolecta*, *R. salina*, *C. calcitrans* (live culture or paste) and *S. costatum*. The technique was most effective (and cost-effective) when natural "background" levels of phytoplankton (based on measured chlorophyll *a)* in the water were low (eg. in the 1996/97 production season compared to previous seasons).

Objective 3: To test new Australian cold-water microalgal species as supplementary feed for juvenile oysters (*C. gigas*).

Three new Australian species were tested as supplementary feed: *P. pinguis* (isolated from Pipe Clay Lagoon); *Pavlova* sp. CS-63 (from Port Philip Bay) and *R. salina* (Port Hacking). *R. salina* was the most effective microalga and produced a similar enhancement of growth as the overseas strains tested. However, the cell division rate of this strain would need to be improved to make it as cost-effective a diet as some of the overseas strains (e.g. *Isochrysis* sp. (T.ISO), *D. tertiolecta).*

6.2 SUPPLEMENTARY FEEDING OF OYSTERS

The results from the 15 experimental-scale trials and 4 commercial-scale trials are not reported in chronological order, as listed in Table 1. Instead, related experiments are grouped and reported together to simplify the reporting. A discussion is given at the end of each subsection of related experiments, and further discussion is given in section 7 (i.e. Discussion).

6.2.1. The efficiency of uptake of microalgae over a 24 h cycle

Prior to undertaking experimental-scale supplementary feeding trials, we assessed whether oysters had a preference for night- or day-time feeding. Juvenile Pacific oysters (≈2 mm) were placed in chambers in duplicate 10-L experimental upweller units. The density of animals was similar to those used in the commercial nursery (≈ 40 g wet weight/85 cm^2 of chamber screen). The animals were maintained under a 9:15 h light:dark photoperiod. Water (from Pipe Clay Lagoon) was pumped through the upwellers at 700 mL min^{-1}. Animals were acclimated for 24 h prior to the experiment.

During the experiment, samples (2L) were taken every 90 min from water entering and exiting upwellers. Samples were filtered, frozen (-20°C), and later analysed for chlorophyll *a*. The percentage of chlorophyll *a* removed from the water was calculated, to estimate the uptake efficiency by oysters.

Percentages of chlorophyll *a* removed varied significantly, and (apparently) randomly over the experiment, from 10–60%, with an average of ≈40% (Fig. 6). There was no difference in removal between the light and dark phases. Because the water was pumped directly from Pipe Clay Lagoon, its physical properties (eg. food concentration and quality, nutrients, T) were not controlled. Temperature was relatively constant (9–11°C), chlorophyll *a* levels ranged from 1–2 μg L^{-1}; other water parameters were not measured. The variation in uptake of chlorophyll *a* by oysters was likely to be related (at least in part) to naturally occurring physical changes in the water.

In view of this result, and because of logistic constraint, it was decided that short-term supplementary-feeding of oysters proposed (at least in the initial phases of the project) would be undertaken during the day.

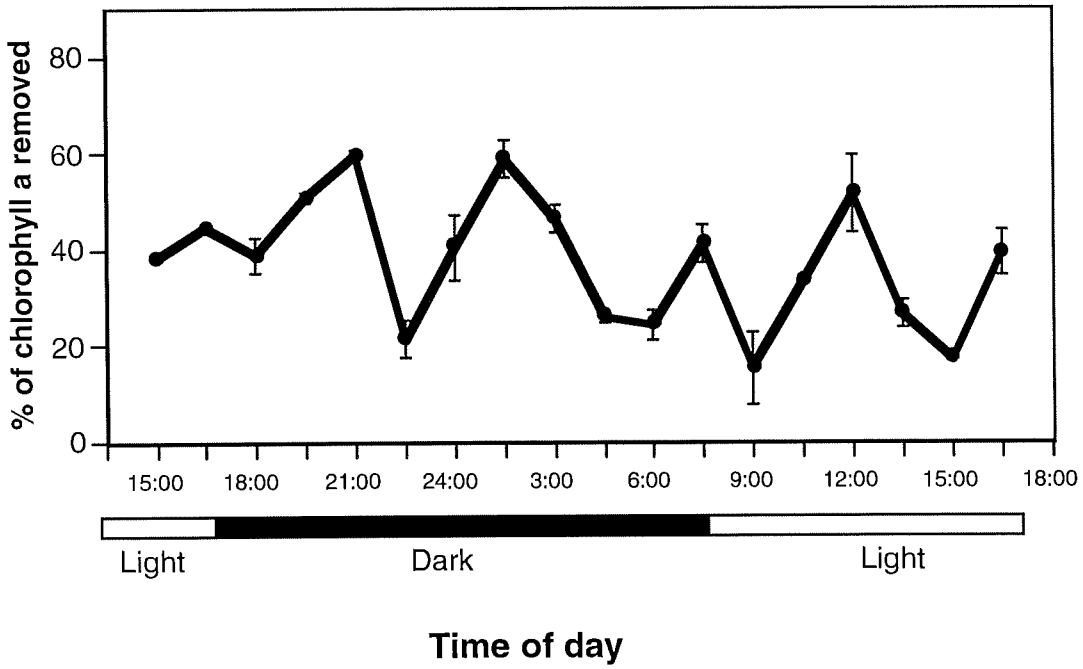


Fig. 6. Removal of chlorophyll *a* from water passing through upwellers by juvenile oysters over a day-night cycle. Values are average ± range/2.

6.2.2. Methods for assessment of growth rate of oysters and survival

For the first five feeding trials, oyster growth was measured by the changes in dry weight (DW) and organic weight (OW) of sub-samples from upwellers. Data for oyster DW were used to statistically compare the growth rates in these trials. However, variances within treatment groups were high, which made it difficult to show that differences were statistically significant. Errors due to sub-sampling the oyster population were a major contributor to the variance. We tested the volumetric measurement of the oyster populations as an alternate measure of oyster biomass, and for calculating growth rate. Growth rate calculated using volume of oysters was linearly related to growth rate calculated using DW ($R^2 = 0.62$) and OW ($R^2 = 0.74$). The relationships were defined by the regression equations:

$k_{DW} = 1.004\ k_{VOL} - 0.005$
$k_{OW} = 1.104\ k_{VOL} - 0.001$

where k_{VOL}, k_{DW}, and k_{OW} are growth rates from measurements of oyster volume, DW and OW, respectively. Because the within-group variance for k_{VOL} was significantly less, and closely correlated to k_{DW} and k_{OW}, it was used for statistically comparing growth rate data for feeding trials subsequent to trial 4. Also, *A posteriori* power analysis showed the minimum difference in growth rate (Δk) that an experiment could have detected as being significant ($P < 0.05$) with a power of at least 0.8 was $\Delta k = 0.015$ for k_{DW} (based on Trials 1-5) and 0.010 for k_{VOL} (based on Trial 6).

At the commencement of trials, mortality in batches of oysters was <5%. The exception was Trial 14 (15%). There were no significant changes in oyster survival in any of the experiments.

6.2.3. Concentration of supplementary diet

Trial 1: Concentration of supplementary-feed (discontinuous feeding)

Pavlova pinguis CS-375, a microalgae isolated from Pipe Clay Lagoon, was used in the initial phase of the project as the reference diet. In trial 1 we aimed to assess the effectiveness of *P. pinguis* (grown under 12:12 h L:D) as supplementary feed at different concentrations. Three treatments received the same flow-through of water as the control upwellers for 22h d^{-1}, but received supplementary food at different concentrations for the remaining 2h d^{-1}. The mean concentrations added to each treatment were 53, 106 and 212 mg d^{-1} (dry weight). The feed was delivered as a single ration, between midday to 2 p.m. weekdays, over an 18 d trial. The rations were held constant throughout the trial.

The growth of oysters increased with food concentration (ANOVA; $P < 0.001$) (Fig. 7)[1]. The oysters fed 106 and 212 mg had approximately 32% faster growth rates than control oysters ($P < 0.001$), whereas those fed 53 mg day^{-1} grew no faster than the control oysters. As the growth rates differed very little between oysters fed the 106 and 212 mg rations, subsequent experiments during year 1 of the project used a ration of approximately 100 mg d^{-1}.

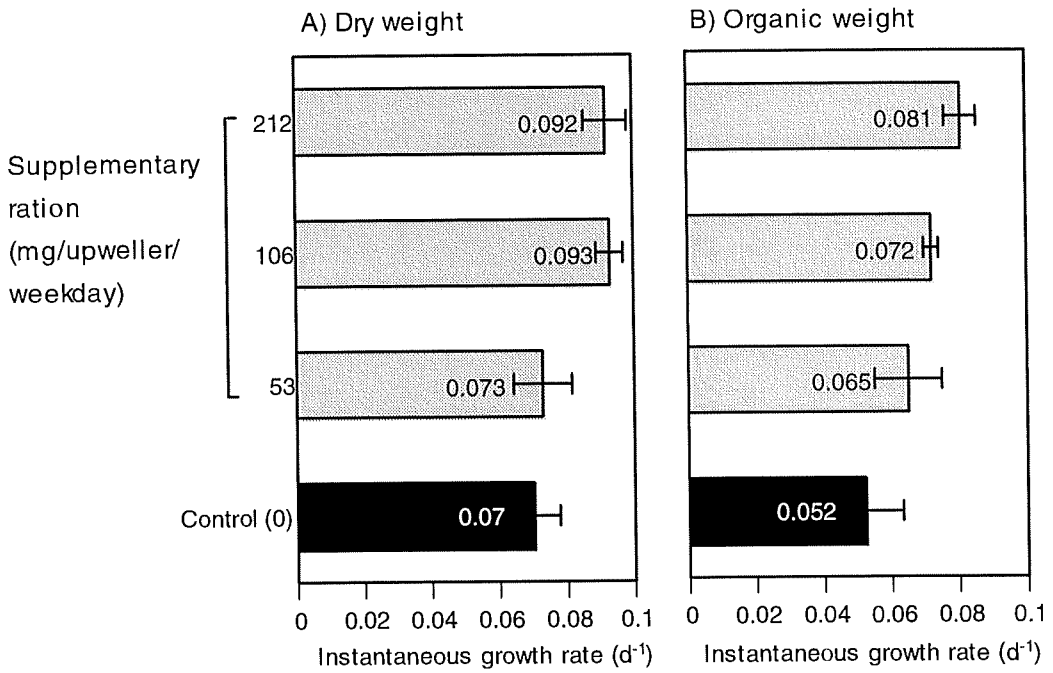


Fig. 7. Dry weight and organic weight growth rates of 700 µm juvenile oysters fed differing concentrations of supplementary food (*P. pinguis*) for 18 d in comparison to control oysters. Error bars are standard deviation.

[1] Instantaneous growth rate values (k) may be readily converted to doubling time by the fomula:
doubling time = 0.693/k.
For example, in the above graphs this gives a doubling in dry weight of control oysters (k = 0.07) of 10 days.

Trial 13: Concentration of supplementary feed (continuous feeding)

Supplementary feeding was re-evaluated at different concentrations of algae, delivered continuously (c.f Trial 1 page 21; single 2 h feed weekday^{-1}) over a 17 d period. This re-evaluation was necessary because we observed that at high feed concentrations (200 mg upweller^{-1} d^{-1}) oysters reached satiation after feeding on 1/2 to 3/4 of the ration over 1-1.5 h with the discontinuous feeding methods (as determined by chlorophyll *a* concentration entering and exiting the upweller). Delivering the food over 24 h might therefore allow the oysters to ingest and assimilate the microalgae more efficiently at the higher rations – even though at lower rations (98 mg upweller^{-1} weekday^{-1}) we found no difference in growth between oysters fed continuously and discontinuously (Trial 3; page 24).

Oysters were fed either 58, 116 or 174 mg (initial ration) *Isochrysis* sp. (T.ISO) (grown under 24:0 h L:D) each day. Oysters were fed continuously (except during the daily cleaning) for 7 d week^{-1}. Rations were increased weekly in proportion to the growth increase seen in oysters receiving the initial 58 mg d^{-1}, and remained at the ratio of 1:2:3. The growth of the supplementary-fed oysters was compared to a flow-through control.

There were significant growth-rate differences between treatments (ANOVA; $P < 0.01$), with a trend of increased oyster growth with increased supplementary food (Fig. 8).

Supplementary-fed oysters had significantly higher growth than the control oysters ($P < 0.05$). Oysters initially fed 116 mg and 174 mg had faster growth than oysters initially fed 58 mg ($P < 0.05$), yet there was no significant difference between the 116 and 174 mg d^{-1} treatments. The growth rate of oysters fed the highest ration ($k = 0.11$) was greater than rates achieved with supplementary feeding in the supplementary trials 1-12 ($k \leq 0.093$). Also, since there was no apparent levelling out of growth response with increasing supplementary food in this trial, further growth increases are possible at even higher rations.

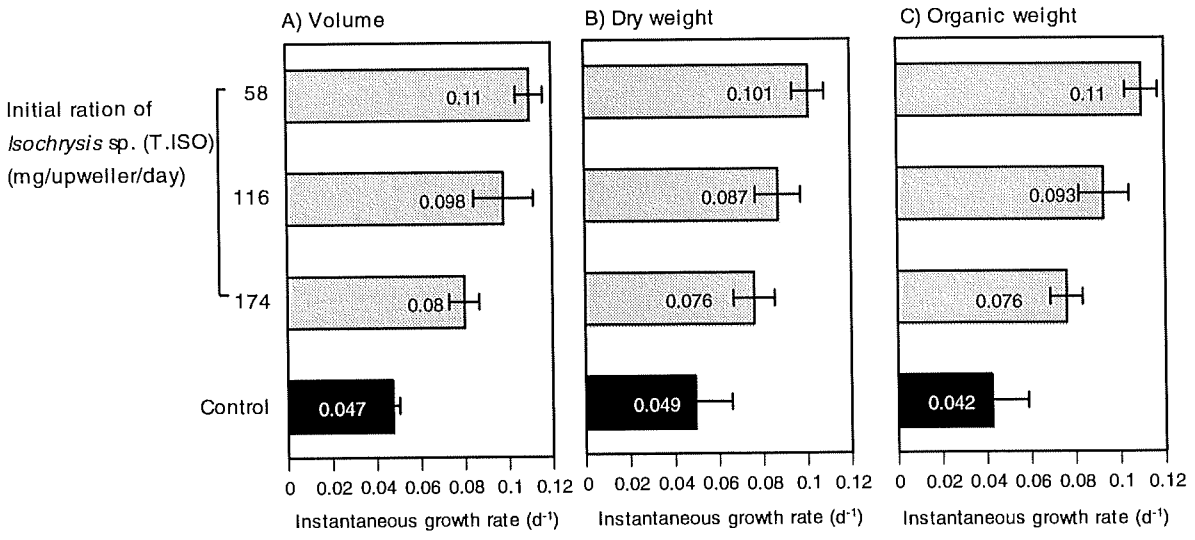


Fig. 8. Volumetric, dry weight and organic weight growth rates of juvenile oysters (≈1100-1500 µm) fed different concentrations of supplementary *Isochrysis* sp. (T.ISO) over 17 d. Rates are compared to control oysters. Error bars are standard deviation.

Discussion: Concentration of supplementary food

Trials 1 and 13 demonstrated that growth rates of oysters increased with increasing concentrations of supplementary diet. In trial 1 where oysters were fed over a 2 h period, the growth response did not improve above the 106 mg upweller^{-1} weekday^{-1} (≡ 3.8 mg mL.oyster^{-1} d^{-1}) ration. However, in trial 13 where oysters were fed continuously, there was no apparent levelling off of growth response (highest ration ≡ 14.6 mg mL.oyster^{-1} d^{-1}).

Another trial (Trial 3; page 24) demonstrated that at the lower food ration of 3.5 mg mL.oyster^{-1} d^{-1} that there was no significant difference in growth of oysters fed daily in 2 h rations, versus continuously. However, comparison of data from trials 1 and 13 suggests that at higher rations, the feed cannot be efficiently assimilated during a 2 h period. We verified this in a subsequent short-term study. We fed oysters stocked at 20 mL upweller^{-1}, three different rations of *P.pinguis*, i.e. 3.8, 7.6 and 15.2 mg mL.oyster^{-1} over a 2 h period, and estimated filtration efficiency by measuring the chlorophyll *a* removed (as a measure of phytoplankton) from water passing through the upwellers. Efficiency was highest at the 3.8 mg ration (average 40% chlorophyll a removed) with significant reductions in efficiency at the higher concentrations (25% and 10% at the 7.6 and 15.2 mg rations, respectively). In particular, oysters fed the 15.2 mg ration were not removing any chlorophyll *a* after 1.5 h of feeding. These results concur with others, showing that beyond an optimum feed concentration, ingestion capacity is saturated and filtration rates decrease with increasing food (Navarro and Winter, 1982; Bricelj and Malouf, 1984; Beiras et a., 1993). The net effect is a regulation of food uptake whereby the amount of particles filtered per unit time is relatively constant. For clam *Venerupis pullastra* seed (Beiras et al., 1993) and oyster *Ostrea edulis* seed (Beiras et al., 1994) in open-flow systems, this optimum feed concentration was 30 cells µl^{-1} of *Isochrysis* sp. (T.ISO. In comparison, we calculated that by feeding *P. pinguis* in upweller at a ration of 3.8 mg mL.oyster^{-1} (as per trial 1) would give cell concentration of up to 50 cell µl^{-1} within the upweller, i.e. near or perhaps beyond optimum food concentration during the feeding period.

Maximum growth rates were not established in trial 13, though possibly only modest growth increases could be given at higher rations. For example, we calculated that at the highest ration, the average cell concentration of *Isochrysis* sp. (T.ISO) flowing through upwellers ranged from 10 cells µl^{-1} (week 1) to 30 cells µl^{-1} (week 3) (c.f. optimum feed concentrations of 30 cells µl^{-1} for other mollusc seed; Beiras et al., 1993, 1994). Also, the instantaneous growth rate at the highest ration (k = 0.11 d^{-1}) is comparable to maximum growth rates of bivalve seed seen in other studies, e.g. k = 0.13 d^{-1} for *C. virginica* (Urban et al., 1983), k = 0.14d-1 for *O. edulis* (Beiras et al., 1994), k = 0.12 for *Saccostrea commercialis* (O'Connor et al., 1992) and 0.070 for C. gigas (Enright et al., 1986a).

6.2.4 Presentation of supplementary food

Trial 3: Duration of feeding

The effects of duration of supplementary feeding was tested by distributing a fixed ration (98 mg upweller^{-1}) of supplementary *P. pinguis* (12:12 h L:D) on weekdays over an 18 d trial, as follows:

a) over 2 h (standard method)
b) 2 x 1 h - Supplied daily with two supplementary half-rations, each over 1 h, at midday and midnight (discontinuous method).
c) continuously

Oysters fed using the discontinuous and standard feeding methods (i.e. a) and b)) had 31-51% faster growth rates than control oysters ($P < 0.05$) (Fig. 9). Oysters fed continuously had growth rates intermediate between the control oysters and those fed using the 2 x 1 h regimen (not statistically significant). Because the discontinuous method appeared the most effective at increasing the animal's growth rate at this feeding ration, this regimen was adopted for subsequent feeding trials (trials 4-10 and 12).

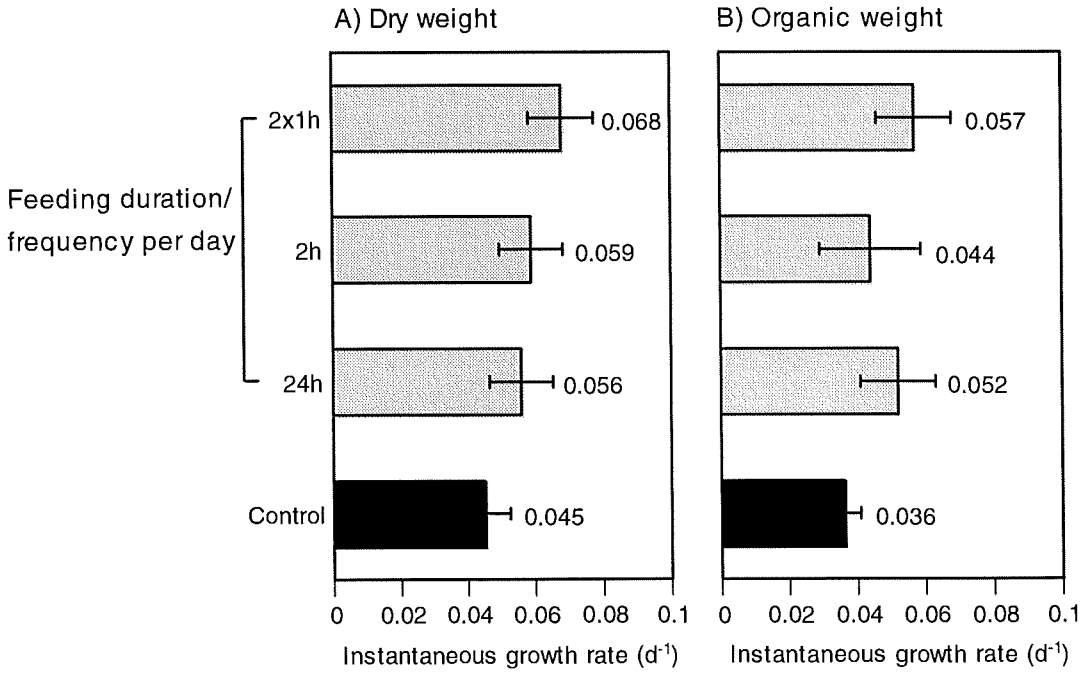


Fig. 9. Dry weight and organic weight growth rates of 700 µm juvenile oysters fed a supplement of 98 mg of *P. pinguis* upweller^{-1} d^{-1} using continuous and discontinuous feeding methods over a 18 d compared to control oysters. Error bars are standard deviation.

Trial 5: Duration of supplementary feeding - repeated

This experiment comparison of 1 versus 2 feeds per day again (c.f. trial 3, page 24) but using volumetric analyses to compare the growth rate of the different treatments. In addition, a parallel trial was undertaken under similar conditions (apart from scale) in the commercial nursery. This is discussed in the section on nursery-scale trials.

Experimental treatments were fed as described in trial 3, excluding a continuous feeding treatment. Supplementary-fed oysters received 87 mg *P. pinguis* (12:12 h L:D) upweller^{-1} d^{-1}, fed during weekdays over an 18 d trial.

Growth rate assessment showed no difference between supplementary-fed and control oysters (Fig. 10).

Control oysters grew significantly slower in this trial than subsequent trials, despite background chlorophyll *a* levels being 2 to 4 times greater than in previous trials (i.e. 2.9 µg L^{-1}, compared to 0.69 to 1.47 µg L^{-1}; Table 1, page 5). Lower water temperatures in this trial (average daily range 9 to 12°C; Table 1) compared to previous trials (average daily range ≥ 12 to 15°C) most likely contributed to the lower growth in both control and supplementary-fed oysters.

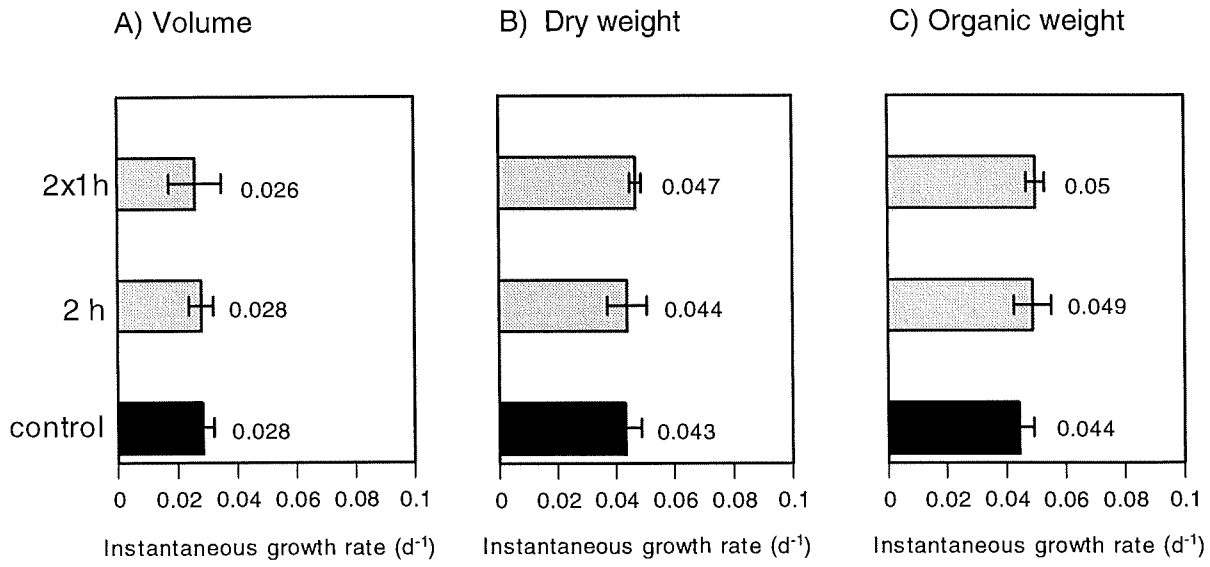


Fig. 10. Volumetric, dry weight and organic weight growth rates of 1300 µm juvenile oysters fed an average supplement of 97 mg *P. pinguis*/upweller^{-1}/weekday. Error bars are standard deviation.

Trial 2: Recirculation versus single passage of feed

The trial aimed to compare different methods for delivering the diet of *P. pinguis* (12:12 h L:D) to oysters over an 18 d trial:

a) Drip 1 - Supplementary food was delivered in a single ration over 2 h in addition to normal flow-through.

b) Drip 2 - The flow-through water was stopped whilst supplementary feeding took place. Supplementary food was added over 1 h, followed by the addition of seawater for a further hour at the same rate. Normal flow-through was then resumed.

c) Recirculation - Flow-through water was turned off and one-third of the total supplementary ration was added to the upweller. The water within the upweller was recirculated for 30 min with an aquarium pump. The system was then flushed with 10 min of flow-through water at the normal flow rate. This was repeated twice with the remaining 2 rations, over a total period of 2 h. The ration (103 mg upweller^{-1} weekday^{-1}) was held constant throughout the trial, with the feeding occurring only on weekdays.

Growth rates of oysters fed using drip treatments were 8% higher, but not statistically different ($P > 0.05$) to those fed by the recirculating method (Fig. 11). Oysters in the "Drip 1" and "Drip 2" treatments showed similar growth rates, with mean values 25% higher than the control oysters. However, this could not be statistically substantiated due to the large variance in the control group (Fig. 11). As all feeding methods appeared equally effective, we chose the "Drip 1" method for subsequent trials, because it was the least labour-intensive

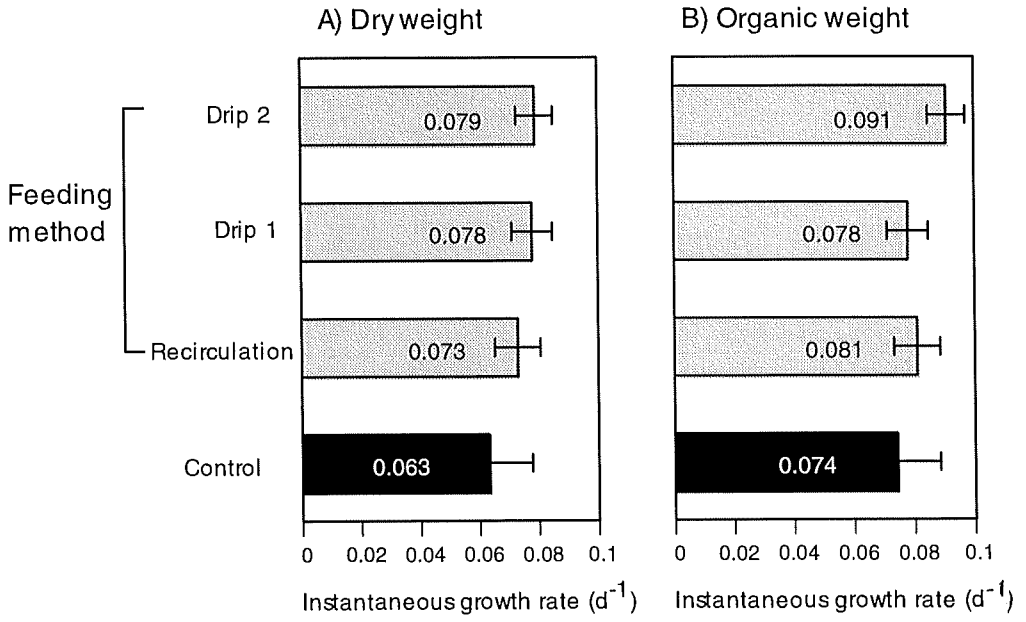


Fig. 11. Dry weight and organic weight growth rates of 700 µm juvenile oysters fed an average supplement of 103 mg *P. pinguis* upweller^{-1} weekday^{-1} using drip and recirculation methods over 18 d. Error bars are standard deviation.

Trial 15: Recirculation versus single passage of food - repeated

This trial aimed to re-examine different methods for delivering supplementary food. We initially investigated this aspect during one of our early trials, and found that a "single-pass" of supplementary food was equally if not more effective as "recirculating" the feed (Trial 2). This question was re-examined as our later trials used higher feed concentrations, and delivered the food over a 24 h period (not 2h, as in Trial 2).

The diet was *Isochrysis* sp. (T.ISO) (24:0 h L:D) fed at a ration of 196 mg upweller^{-1} d^{-1}. Food was delivered continuously. Three different systems were assessed for delivering the feed:

1. normal flow: i.e. food delivered with a total flow rate of 700 mL min^{-1}
2. reduced flow: food delivered with a total flow rate of 230 mL min^{-1}, and
3. water recirculated within the upweller, with feed constantly dripping in at a rate to provide 8 water changes/day

The rationale for testing the latter 2 methods was that they effectively provided longer "passage" times of algal cells through the oyster bed and therefore they might provide a more efficient filtration.

Control oyster growth rates were substantially lower that rates typically seen in year 1 and 2 of the project (i.e. Trials 1 to 11)), as were average chlorophyll *a* levels (0.36 µg L^{-1}; 0.48 ± 0.18 in year 3; 1.1 ± 0.6 µg L^{-1} in years 1 and 2). The normal-flow method (i.e. 700 mL min^{-1} upweller^{-1}) produced significantly greater growth in supplementary-fed oysters than the alternate methods (Fig. 12).

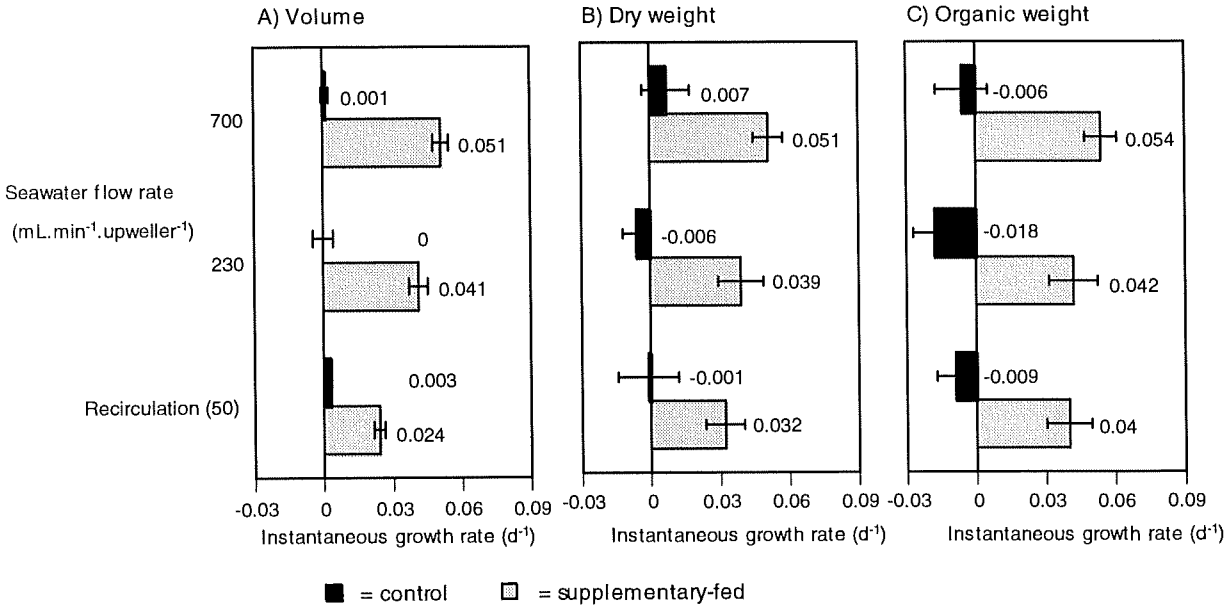


Fig. 12. Volumetric, dry weight and organic weight growth rates of oysters, with or without supplementary feeding with *Isochrysis* sp. (T.ISO) using different methods. Error bars are standard deviation.

Discussion: Presentation of supplementary food

At feed concentrations used during the first year of the project (eg. Trials 3 and 5; ≤ 3.5 mg d^{-1} mL^{-1} of oysters) there were no significant differences in growth rates between oysters fed 2 x 1 h d^{-1}, 1 x 2 h d^{-1} and continuously. From trial 3, the 2 x 1 h feeding was apparently most effective, though the large treatment variances meant differences were not significant. As discussed in the previous discussion on concentration of supplementary feed (p.23), at higher feed concentrations (eg. as used in Trials 6 and onwards; $\geq$ 6 mg d^{-1} mL^{-1} of oysters) we established that filtration of microalgae by oysters was less efficient, and for that reason feed was either delivered continuously or in 2 x 2 h rations.

In another study, Langton and McKay (1974) demonstrated better growth of Pacific oyster spat fed discontinously (6 h on, 6 h off regime) compared to continuously, though this finding has not been substantiated by subsequent reports. In natural environments, bivalves do exhibit periodicity in feeding behaviour (see reviews of Higgins, 1980 and Hawkins *et al.,* 1983). Higgins (1980) found that oysters (*C. virginica*) fed continuously showed no 24 h periodicity of feeding activity, and the initiation of periods of activity and quiescence was apparently random. In contrast, Higgins (1980) found oysters subjected to discontinuous feeding often exhibited 24 h periodicity in activity, which appeared to be influenced by the availability of food.

Trials 2 and 15 examined different flow rates and recirculation of water. Though no significant differences in growth were observed in Trial 2, in trial 15 recirculation of water significantly impacted on oyster growth. Reasons for this were not investigated, though a likely explanation is that this method produced a greater build-up of waste products (e.g. ammonia, faecal material) in the system which negatively impacted on growth. This may also explain why oysters fed at a seawater flow rate of 230 mL min^{-1} $upweller^{-1}$ grew less than oysters fed at a flow rate of 700 mL min^{-1} $upweller^{-1}$. Also, the latter flow rate ($\equiv$ 28 mL min^{-1} g^{-1} of oysters) is within the range of 20 to 50 mL min^{-1} g^{-1} of oysters suggested to be optimal for the feeding of juvenile *C. gigas* in upwelling systems (Spencer *et al.,* 1986). At these flow rates, approximately 20% of suspended material is filtered by *Crassostrea gigas* (Spencer *et al.,* 1986). This and other studies found that lower flow rates do produce higher levels of filtration and therefore a better use of food (Rodhouse and O'Kelly, 1981), but they also give reduced growth. For example, Manzi *et al.* (1986) found that growth of mussel *Mercenaria mercenaria* was reduced when more than 20% of ambient chlorophyll a was removed from water passing through upwellers. Kirby-Smith (1972) found scallop growth was reduced when more than 40% of chlorophyll *a* was removed from inflowing water.

6.2.5 Different diets of cultured microalgae as feed

Trial 6: Pavlova species

The aim of this study was to compare the value of Australian *Pavlova* species, grown under 12:12 h L:D. We also re-examined the effects of increasing concentrations of supplementary food when it was split into two rations day^{-1} (cf. Trial 1 when food was given as a single daily ration). Supplementary-fed oysters received the same flow-through of water as the control upwellers for 20h/day, but received one of three different diets for the remaining 4 h (2 x 2 h) during weekdays over an 18 d trial:

a) *P. pinguis* (grown under 12:12 h L:D) at a ration of 90 mg day^{-1}.
b) *P. pinguis* (12:12 h L:D) at a ration of 180 mg day^{-1}.
c) *Pavlova* sp. (CS-63) (12:12 h L:D) at a ration of 180 mg day^{-1}.

Growth rates (volumetric assessment) of oysters increased with supplementary food concentration ($P < 0.0001$) (Fig. 13). Oysters fed 90 mg *P. pinguis* had 46% faster growth than control oysters ($P < 0.001$). Doubling the supplementary concentration enhanced the rates; oysters fed 180 mg *P. pinguis* had 14% faster growth rates than those fed 90 mg ($P < 0.05$), and 66% faster growth than control oysters ($P < 0.0001$). *P. pinguis* was a more effective supplementary feed than the *Pavlova* sp. (CS-63) at the 180 mg ration ($P < 0.02$), producing a 9% greater growth rate.

In trial 1 (page 21) we examined the effects of supplementary feed concentration on oyster growth. In that study, the feed was delivered as a single daily ration (over 2h). Oyster growth rates increased with feed concentration up to a 106 mg/day ration. One purpose of this trial was to assess whether growth rates improved at the higher rations (i.e. at ≈ 200 mg) by splitting the feeding into day and night-time rations. We demonstrated this was possible. This trial included our first comparison of different microalgae as supplementary food, showing that *Pavlova pinguis* was superior to *Pavlova* sp. CS-63.

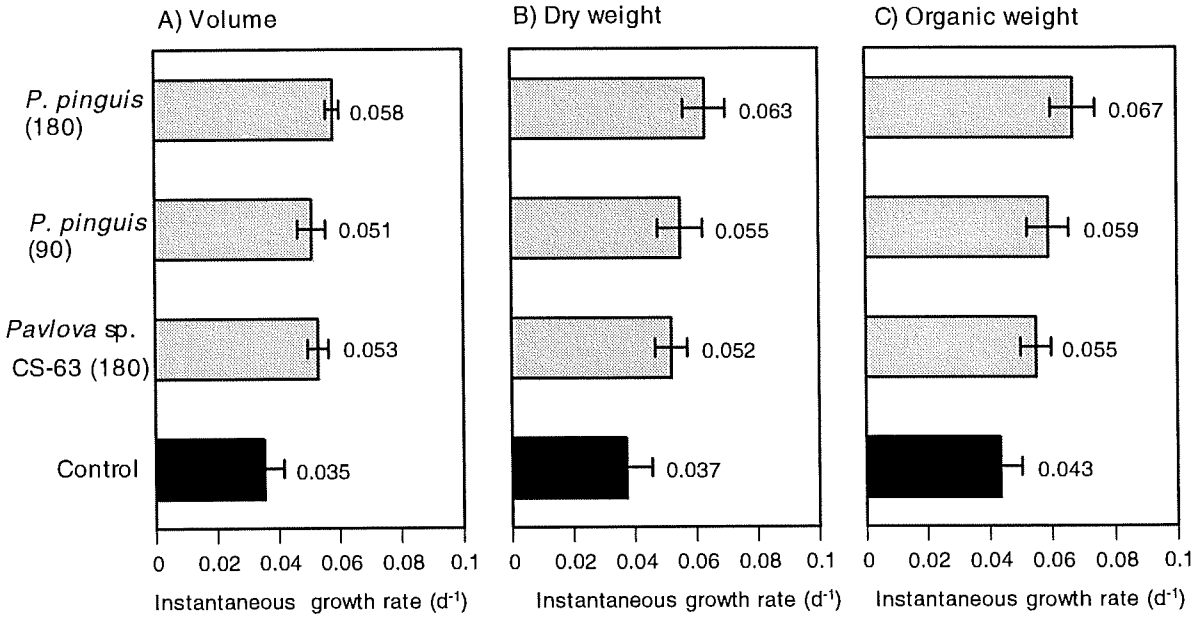


Fig. 13. Volumetric, dry weight and organic weight growth rates of 700 µm juvenile oysters fed different concentrations of as supplementary food, and fed different supplementary diets. The rations (mg upweller^{-1} weekday^{-1}) are shown in parentheses. Error bars are standard deviation.

Trial 7: P. pinguis grown under different light conditions

This experiment assessed the effects of two different light regimes (i.e. 12:12 and 24:0 L:D regime) on the nutritional value of *P. pinguis* used as supplementary food for juvenile oysters.

Methods were identical to trial 6, except that the average daily ration of supplementary food was less (140 mg). Two different supplementary diets were compared to each other and control oysters over an 18 d trial. These diets were:

a) *P. pinguis* cultured under 12:12 h L:D regime, and
b) *P. pinguis* cultured under 24:0 h L:D regime

Oysters fed the supplementary diets had faster growth rates (35 to 44% more) than the control oysters (Fig. 14) ($P < 0.0001$). Oysters fed on *P. pinguis* cultured under 24:0 h L:D grew 8% faster than oysters fed the algae cultured under 12:12 h L:D ($P < 0.0005$).

The light photoperiods chosen in this study represent the two most commonly used by hatcheries for culturing microalgae. 12:12 h L:D is used by hatcheries with outdoor algal cultures taking advantage of the natural day:night photoperiod. Hatcheries that grow microalgae indoors tend to use from between 16:8 h L:D to 24:0 (i.e. continuous illumination), because most microalgae grow faster and their production cost is less using longer light photoperiod.

The difference in nutritional quality of microalgae grown under different light conditions may be related to their biochemical composition. We found that *P. pinguis* contained more lipid when grown under 24:0 h L:D than when grown under 12:12 h L:D (Table 3). Typically, microalgae grown under 24:0 h L:D are more energy-rich (particularly in carbohydrate and triglyceride) than cells grown under 12:12 h L:D (Vårum et al., 1986, Brown et al., 1996).

On the basis of the results, and an improved production rate of the microalgae, subsequent trials used microalgae cultured under 24:0 h L:D.

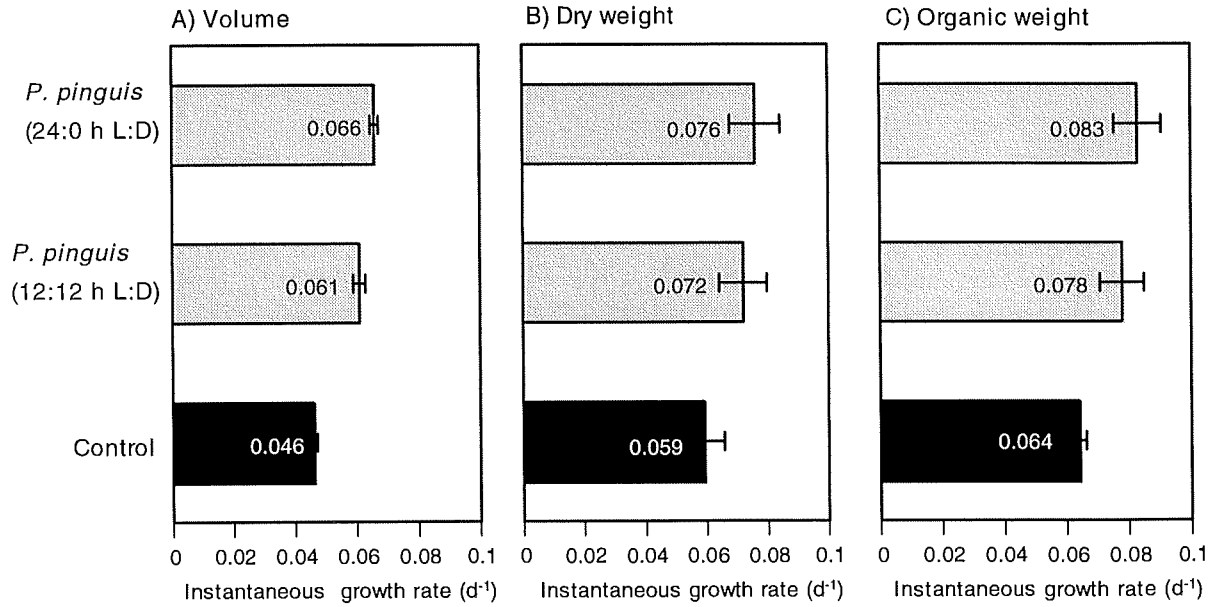


Fig. 14. Volumetric, dry weight and organic weight growth rates of 700 µm juvenile oysters fed *Pavlova pinguis* grown under 12:12 h L:D or 24:0 h L:D. Error bars are standard deviation.

Trial 8: Australian microalgae and mixed diets

This experiment compared Australian microalgae and a mixed-diet of the two as supplementary feed for juvenile oysters. Supplementary-fed oysters received the same flow-through of water as the control upwellers for 20h d^{-1}, but received one of three different diets for the remaining 4 h (2 x 2h), at a fixed ration of 190 mg $upweller^{-1}$ $weekday^{-1}$ over an 18 d trial.

a) *P. pinguis*
b) *R. salina* CS-24
c) A mixed diet of the above 2 species

Supplementary-feeding produced an increase in oyster growth rate of between 53 to 72% (Fig. 15). *R. salina* was more effective than *P. pinguis* ($P < 0.05$). Growth of oysters fed the mixed diet was not significantly different from either of the uni-algal diets. Mixed diets often produce better growth rates than uni-algal diets in "closed-system" trials because they provide a better balance of nutrients. However, in our flow-through system, oysters are already receiving a complex mixture of algae and other food particles from the background water, hence there was no additional benefit of having a mixed diet as supplementary feed.

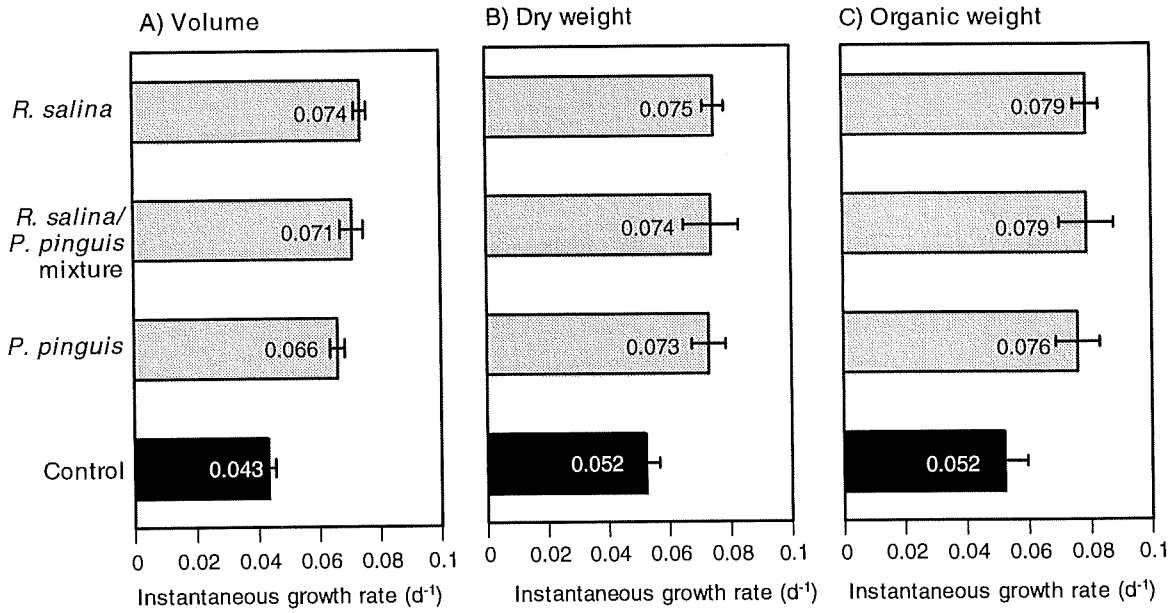


Fig. 15. Volumetric, dry weight and organic weight growth rates of 700 µm juvenile oysters fed 190 mg $upweller^{-1}$ $weekday^{-1}$ of *R. salina, P. pinguis* or a mixture of the two, compared to control oysters. Error bars are standard deviation.

Trial 10: Microalgae with different PUFAs

This trial compared microalgae containing different compositions of polyunsaturated fatty acids (PUFAs) as supplementary food for juvenile oysters. *P. pinguis* contained both 20:5(n-3) and 22:6(n-3), *Isochrysis* sp. (T.ISO) lacked 20:5(n-3) but contained 22:6(n-3), and *D. tertiolecta* lacked both PUFAs (Appendix i).

Supplementary-fed oysters received the same flow-through of water as the control oysters for 20h d^{-1}, but received one of three diets for the remaining 4 h (2 x 2h), at a fixed ration of 190 mg upweller^{-1} weekday^{-1} over a 17 d trial:

a) *P. pinguis*
b) *Isochrysis* sp. (T.ISO)
c) *D. tertiolecta*

Supplementary-feeding produced an increase in oyster growth rates of between 44 and 74% (Fig. 16). *Isochrysis* sp. (T.ISO) and *D. tertiolecta* were equally effective as supplementary feeds, and both diets performed significantly better than *P. pinguis* ($P < 0.05$).

The effectiveness of *D. tertiolecta*– an algae deficient in 20:5(n-3) and 22:6(n-3) – indicated that the oysters were being sustained in these essential fatty acids from natural phytoplankton in the inflowing water. The fatty acid compositions of oysters were assessed at the end of the trial and compared to their respective diets. This data are presented and discussed in full in the appended manuscript (Appendix i).

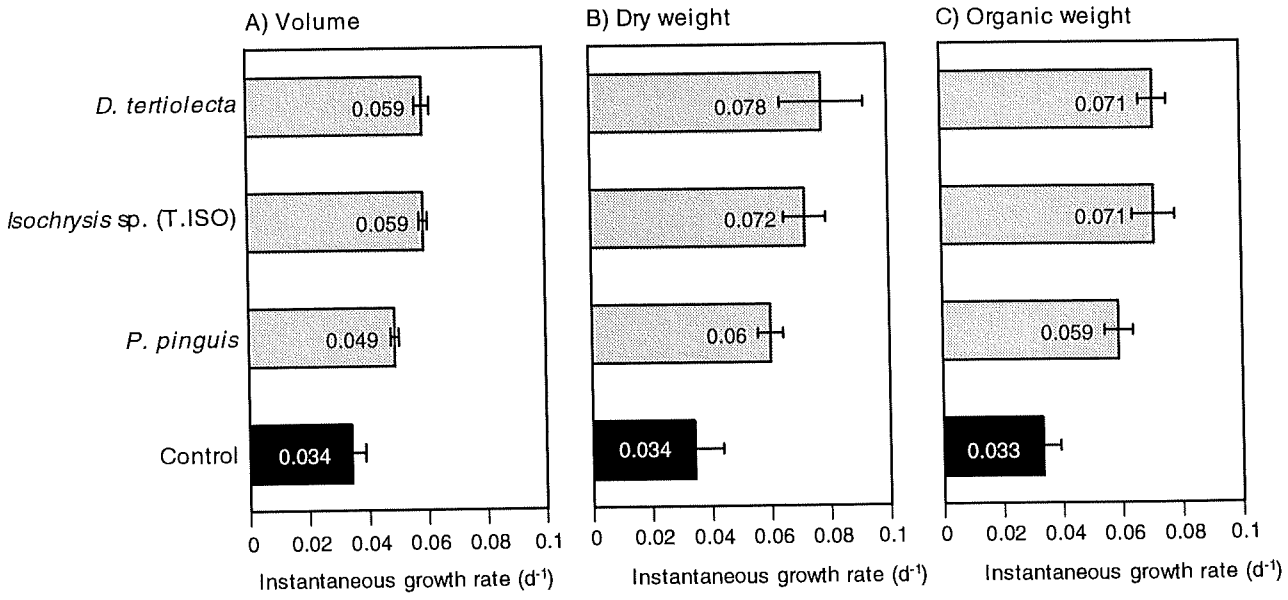


Fig. 16. Volumetric, dry weight and organic weight growth rates of 700 µm juvenile oysters fed 190 mg upweller^{-1} weekday^{-1} of supplementary diets with different PUFA composition, compared to control oysters. Error bars are standard deviation.

Discussion: Different diets of cultured microalgae as food

The supplementary feeding experiments 6, 7 8 and 10 compared different live diets as supplementary feeds. A live diet of *C. calcitrans* was also compared to *P. pinguis* in trial 9 (page 35). All the species examined had previously been found to be of high nutritional value in recirculation systems, either as a uni-algal diet or as part of a mixed species diet (Enright et al. 1986a; Laing and Millican, 1986; O'Connor et al., 1992; Brown et al., 1998). Studies here also confirmed the value of these microalgae as dietary components.

The gross biochemical composition of individual species was assessed (Table 3). Analysing data across all feeding trials we found no correlation between the gross composition of supplementary feed, and diet performance. Several factors would account for this: 1) the composition of (available) protein, carbohydrate and lipid of background seston flowing into upweller must also be considered. This was not determined in the trials because of the analytical difficulty in accurately measuring these fractions in complex mixtures, 2) differences in other nutrient factors, or digestibility of microalgae also affecting dietary performance and 3) differences in the culture environment between trials also affecting the interaction between supplementary food composition and growth performance. Some of these factors are eliminated or reduced by making within-trial comparisons– in particular trial 7 where the *P. pinguis* of different composition (by virtue of its different culture environments) was tested. *P. pinguis* (24:0 h L:D) performed better than *P. pinguis* 12:12 h L:D, and this may have been related to it containing higher concentrations of energy-rich lipid.

For reasons mentioned in 3) above, it is also difficult to make inter-trial comparison of the relative effectiveness of the different diets. Nevertheless, the trials demonstrated that most of the diets were very effective. The % apparent growth efficiency (AGE) provided a measure of how efficiently the supplementary-fed microalgae were converted to oyster (organic) biomass. Values ranges from 21% (*P. pinguis* in trial 10) to 67% (*R. salina/P. pinguis* mix in trial 8) and were directly correlated to the oyster growth rates ($r^2 = 0.83$). In general though, *P. pinguis* was less effective than other diets, except for *Pavlova* sp. CS-63 (trial 7)

Differences in diet performance may be unlikely to be related to any deficiencies of micronutrients (eg. vitamins, PUFAs) because a) ambient seawater may have been providing adequate concentrations, and b) the relatively short experimental period. This was evident in the excellent performance of the PUFA-deficient *D. tertiolecta*. Nevertheless, composition analysis of oyster spat at the completion of the experiment showed *D. tertiolecta*-fed oysters contained approximately 50% less of the PUFA 22:6(n-3) than *Isochrysis* sp. (T.ISO)-fed oysters (Appendix i). Therefore, prolonged feeding with this alga, or feeding during periods when ambient seawater concentrations of PUFAs were lower may reduce it's effectiveness. For the above reasons, differences in the effectiveness of diets was most likely related to subtle differences in their amount of available protein and energy for oysters – a combined function of their ingestion rate, digestibility and their proportions of protein, carbohydrate and lipid.

Table 3. Gross biochemical composition (% of dry weight) of microalgal diets tested in the supplementary feeding trials of juvenile Pacific oysters *Crassostrea gigas*.* Values are mean ± 1 s.d. n.d. = not determined. Means in columns sharing a common superscript are not significantly different (P > 0.05).

Feeding trial and species	Protein	Carbohydrate	Lipid	Ash
Trial 7				
P. pinguis (12:12 h L:D)	18 ± 6 ^{c}	30 ± 9 ^{a}	10 ± 1 ^{c}	n.d.
P. pinguis	22 ± 2 ^{c}	34 ± 12 ^{a}	18 ± 3 ^{a}	n.d.
Trial 8				
P. pinguis	36 ± 7 ^{b}	28 ± 5 ^{a}	18 ± 4 ^{a}	14 ± 4 ^{c,d}
R. salina	59 ± 20 ^{a}	19 ± 5 ^{b}	19 ± 3 ^{a}	10 ± 3 ^{d}
1:1 mixture of above	48 ± 18 ^{a,b}	24 ± 7 ^{a,b}	19 ± 5 ^{a}	12 ± 5 ^{c,d}
Trial 9				
P. pinguis	39 ± 11 ^{b}	31 ± 8 ^{a}	18 ± 4 ^{a}	15 ± 5 ^{b,c,d}
C. calcitrans	47 ± 8 ^{a,b}	23 ± 3 ^{a,b}	19 ± 4 ^{a}	23 ± 7 ^{a}
C. calcitrans paste	29 ± 3 ^{b}	17 ± 4 ^{b}	18 ± 3 ^{a}	22 ± 2 ^{a,b}
S. costatum paste	31 ± 9 ^{b}	19 ± 2 ^{b}	19 ± 4 ^{a}	19 ± 2 ^{a,b,c}
Trial 10				
P. pinguis	35 ± 15 ^{b}	29 ± 6 ^{a}	13 ± 1 ^{a}	18 ± 10 ^{a,b,c}
D. tertiolecta	39 ± 12 ^{b}	28 ± 10 ^{a}	23 ± 1 ^{a}	12 ± 4 ^{c,d}
Isochrysis sp. (T.ISO)	34 ± 7 ^{b}	30 ± 3 ^{a}	24 ± 5 ^{a}	13 ± 6 ^{c,d}

* Samples were also analysed from Trial 6. However, because insufficient material was taken for analysis, this led to an over-estimation of ash in these samples and an underestimation of other gross components. However, the relative proportion of gross components in the two diets were similar: *Pavlova* CS-63; 18 ± 5% protein, 16 ± 5% carbohydrate, 14 ± 2% lipid; *P. pinguis*; 20 ± 6% protein, 15 ± 5% carbohydrate, 15 ± 0.1 % lipid.

6.2.6. Alternate supplementary diets

Trial 9: Algal pastes

We compared algal pastes with live diets as supplementary food for juvenile oysters. Supplementary-fed oysters received the same flow-through of water as the control upwellers for 20 h d^{-1}, but received one of four different diets for the remaining 4 h (2 x 2h feeds), at a fixed ration of 180 mg $weekday^{-1}$ over an 18 d trial:

a) *P. pinguis* (grown under 24:0 h L:D)
b) *C. calcitrans* (18:6 h L:D)
c) *C. calcitrans* paste (18:6 h L:D)
d) *S. costatum* paste (24:0 h L:D)

Algal pastes were prepared at NSW Fisheries using a continuous super-centrifuge, and transported immediately in sealed containers by air-freight to CSIRO, Hobart. Upon arrival they were stored at 4°C in the dark. Additional pastes were supplied mid-way through the trial, so that pastes used on any given day were between 3 days and 2 weeks old,

Supplementary-feeding produced an increase in oyster growth rates of between 62 to 88% (Fig. 17). The *S. costatum* paste and live *C. calcitrans* culture were equally effective, and produced the highest oyster growth rates. The live *C. calcitrans* culture was (apparently) slightly superior to the *Chaetoceros calcitrans* paste (not statistically different). These diets all were significantly better than *P. pinguis* ($P < 0.02$). The highlight of this study was the success of both of the algal pastes.

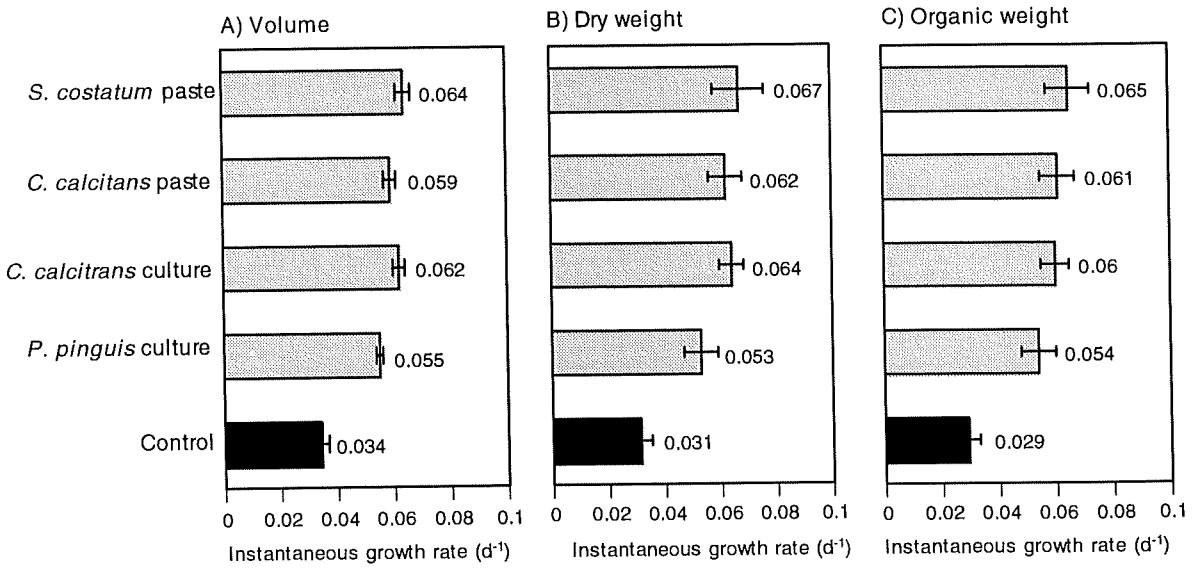


Fig. 17. Volumetric, dry weight and organic weight growth rates of 700 µm juvenile oysters receiving 180 mg $upweller^{-1}$ day^{-1} of supplementary live algal cultures and algal pastes, compared to control oysters. Error bars are standard deviation.

Trial 12: Dried diets

We compared commercial "off-the-shelf" diets with cultured microalgae as supplementary feeds. Graded 500-700µm oysters were supplementary fed one of three diets (weekdays, 2 x 2 h d^{-1}) in addition to normal flow-through water of Pipe Clay Lagoon water for 26 d. The supplementary ration was initially 130 mg d^{-1}, and was increased weekly, in proportion to the growth of the oysters. The diets were tested against a control receiving only flow-through water. The diets were:

a) Cultured *Isochrysis* sp. (strain T ISO)
b) Microfeast® MB-30; yeast and lipid emulsion
c) Bio-Marine Algamac-2000; freeze-dried algae (*Schizochytrium* sp.)

Supplementary-feeding produced an increase in oyster growth rates of between 167 to 500% (Fig. 18). This large effect was a function of the control growth rate seen in this trial (0.009) which was significantly lower than control growth rates in all previous trials (≥ 0.028). The low control growth rates might have been due, in part, to below average chlorophyll *a* values in this trial (cf. 0.55 mg L-1, compared to 1.1 ± 0.6 for previous trials). The *Isochrysis* sp. (T ISO) diet out-performed the commercial feeds ($P < 0.01$). Of the commercial diets, the yeast-based Microfeast® MB-30, gave a greater increase in oyster growth than Algamac-2000 ($P < 0.05$)*.

This study demonstrated that two commercial "off-the-shelf" products could be used effectively as supplementary diets to enhance oyster growth. Though they were less effective in growth enhancement than microalgae, their lower cost indicates they may be a viable alternative to microalgae for supplementary feeding. Further studies are required to fully evaluate the relative cost-effectiveness of the different diets.

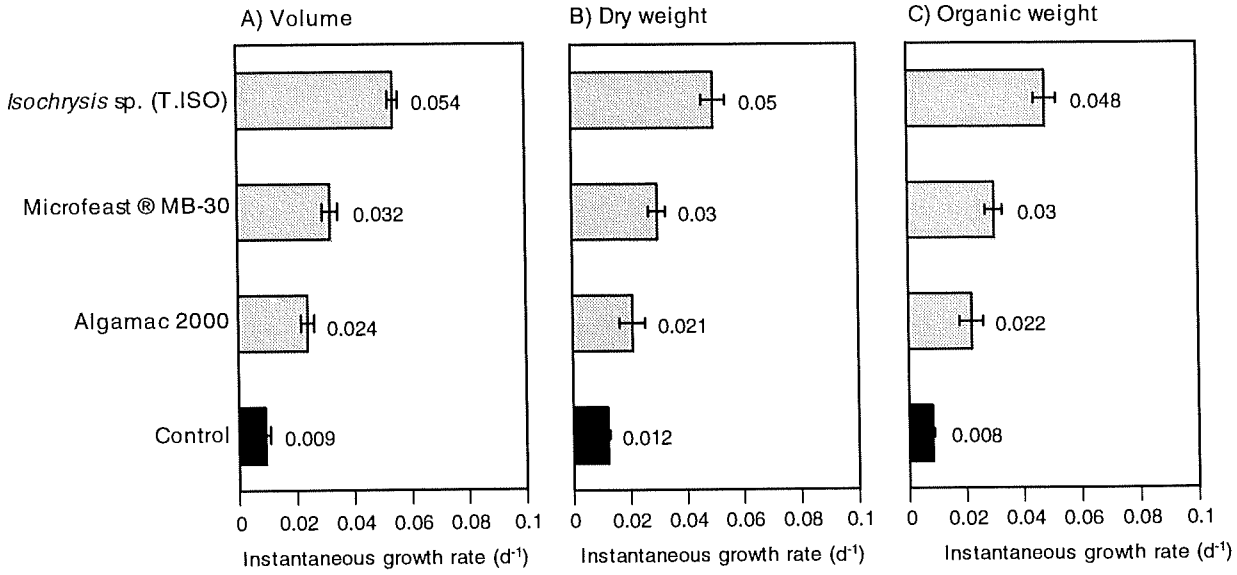


Fig. 18. Volumetric, dry weight and organic weight growth rates of 500-700 µm juvenile oysters fed commercial dried diets, and microalgae as supplementary feed, at an initial ration of 130 mg upweller^{-1} day^{-1}. Rates are compared to control oysters. Error bars are standard deviation.

* The results give a comparison of the two commercial products under the specific conditions of this one experiment are not meant to reflect generally the superiority of one product over the other. The relative effectiveness of these products (to each other, and to microalgae) may change under different concentrations and composition of ambient inflowing phytoplankton, different feeding rations, methods of feed presentation and other culture variables.

Discussion – Alternative supplementary diets

Over the last few decades, much effort has been directed at developing and assessing alternatives to live microalgae for the hatchery and nursery rearing of bivalve molluscs. Reasons for their assessment include their potential cost-effectiveness and ease of use. Continuous microalgal production can otherwise constitute a major component of a hatchery's operating cost. Products assessed include artificial microcapsule diets (Langdon and Waldock, 1981; Knauer and Southgate, 1997), bacteria (Robert and Trintignac, 1997), yeast (Nell et al., 1996), dried algae (Curatolo et al., 1993; Boeing, 1997) and pasted algae (Nell and O'Connor, 1991). A general concensus is that though some of these products are significantly cheaper than live microalgae, their nutritional value is significantly inferior to make them useful as complete diets. Nevertheless, some may be cost-effective as partial replacement of live microalgae. Their use has recently been reviewed by Robert and Trintignac (1997). These authors concluded that concentrated and dried microalgae currently appear to be the best alternative for juvenile bivalves, while microcapsules are a useful tool for studying nutritional requirements.

The potential of algal pastes as supplementary feeds were confirmed in trial 9. *Skeletonema* sp. and *C. calcitrans* pastes (average age = 8 d) were of a similar nutritional value as live *C. calcitrans*. Diatoms have previously been shown to withstand the centrifugation process better than flagellates used in aquaculture, and consequently have a better keeping quality (Nell and O'Connor, 1991, Heaseman pers. comm., Knuckey pers. comm). On the basis of results produced here, more research is warranted on pastes prepared from other species, and to assess whether their nutritional quality can be sustained over longer periods.

The two dried products, Microfeast ® MB-30 (yeast-based) and AlgaMac 2000 (algal-based) were successful as supplemental diets, though their growth performance were not as good as live algae. However, their significantly lower cost cost (≈ $AUS 80 to 100 kg^{-1} compared to $375 kg^{-1} for microalgae) means that their cost-effectiveness warrants further evaluation. Also, even cheaper alternatives are available from the producers of these products, eg. RotiMac (≈$AUS 40) and Microfeast 100 (≈$AUS 30), which may prove equally or more cost-effective.

6.2.7 Size class and stocking density of oysters

Trial 4: Size class

The experiment assessed the effects of supplementary feeding oysters of different size classes (900 µm and 1300 µm). Oysters were stocked initially at an equivalent organic weight biomass. The supplementary diet (106 mg upweller^{-1}) of *P. pinguis* (grown under 12:12 h L:D) was delivered during weekdays in two fixed rations (at noon and midnight; 1 h feeding duration) over an 18 d trial.

Supplementary-fed oysters grew 24% faster than control oysters in the 900 µm size class ($P < 0.01$) and 20% faster in the 1300 µm ($P < 0.05$) size class (Fig. 19). Supplementary feeding therefore had similar effects in promoting growth in both the 900 µm and 1300 µm oysters.

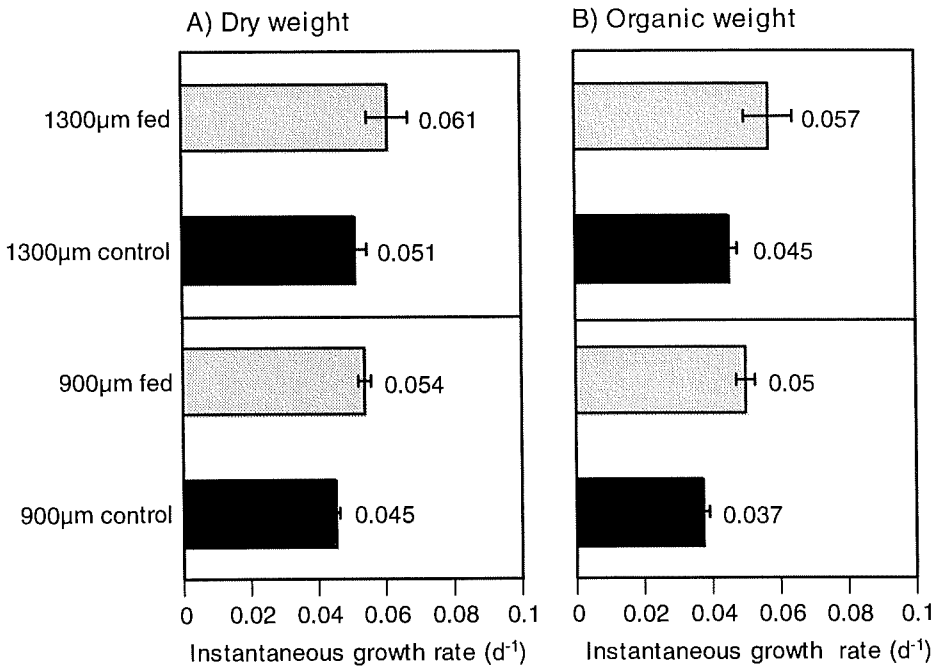


Fig. 19. Dry weight and organic weight growth rates of 900 µm and 1300 µm oysters fed a supplement of 106 mg *P. pinguis* upweller^{-1} weekday^{-1} compared to control oysters.

Trial 11: Stocking volume – no supplementary feeding

The trial investigated the relationship between initial oyster stocking volume and the growth rate. Oysters (1100 µm) were placed in upwellers at 5 different stocking volumes, i.e. 10, 15, 20, 30 and 40 mL. Oysters were maintained in the usual manner, but did not receive supplementary feeding. Growth was assessed at the end of the 18 day trial.

There were significant differences in growth rate between oyster stocked at different volumes with a trend toward faster growth rates at lower volumes (Fig. 20). Regression analysis showed that growth rate was correlated to stocking volume according the following equation:

$k_{VOL} = 0.0985 - 0.0220*\ln(\text{volume})$ ($r^2 = 0.998$; $P < 10^{-5}$)

where k_{VOL} = the growth rate calculated using oyster upweller volumes, and volume = volume (mL) of oyster population in upweller.

The initial stocking volume of 20 mL is equivalent to the normal commercial stocking volume (≡ 400 ml in commercial upwellers), and similar to that used in the experimental scale trials. The reduced growth rates at higher volumes was most likely related to reduced food availability (per oyster), though other factors, such as an increased concentration of waste products within the oyster bed could also reduce growth.

Of note, when the volume was halved (i.e. to 10 mL), the increase in growth was approximately equivalent to the increase seen in Trial 10 (page 32) from supplementary feeding with *P. pinguis*. This shows that a reduction of stocking volume can also enhance production, and could be considered as an alternative to supplementary feeding when additional upweller units are available in the commercial nursery.

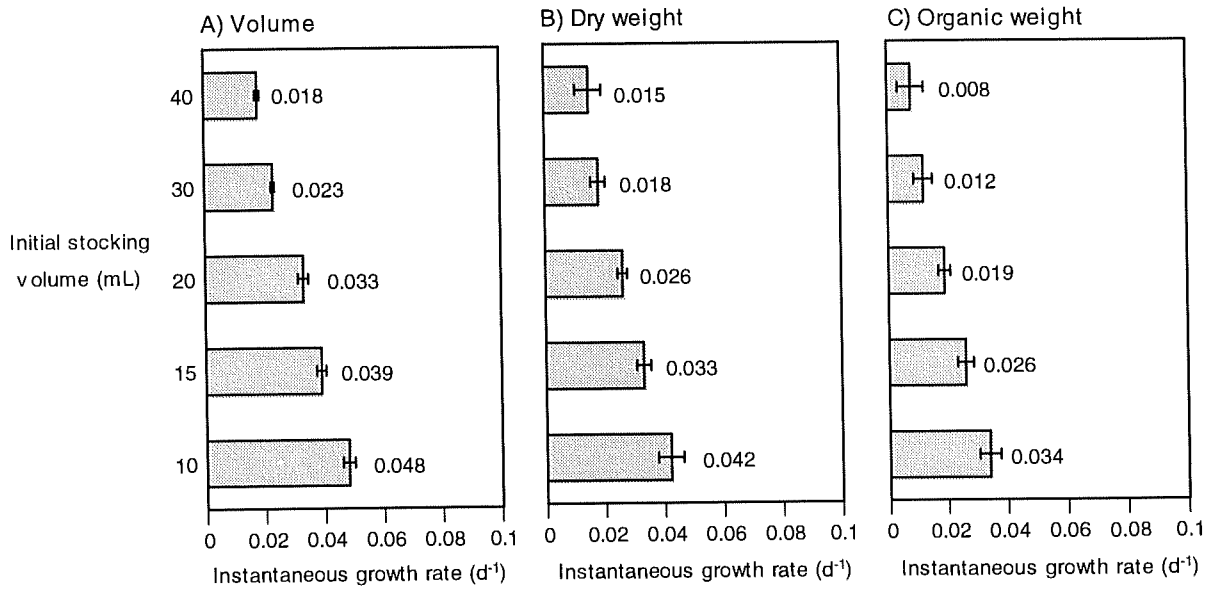


Fig. 20. Volumetric, dry weight and organic weight growth rates of 1100 µm oysters without supplementary feeding, stocked at different initial volumes.

Trial 14: Stocking volume and supplementary feeding

The experiment aimed to assess growth rates and the utilisation of supplementary microalgae at different oyster stocking volumes.

Oysters (500 µm) were stocked in 10 l upweller systems at initial volumes of 10, 20 and 30 mL. At each volume, oysters either received no supplementary feed (control) or were supplementary fed *Isochrysis* sp. (T.ISO) at an initial daily ration of 200 mg upweller^{-1} delivered over 24 h. The supplementary feed ration was adjusted weekly, in proportion to the growth rate of the fastest growing experimental group. The trial was conducted for 16 d. The utilisation of the microalgae at the different oyster densities was also calculated as the % AGE.

Control oyster growth rates in this trial were again significantly lower that rates typically seen in year 1 and 2 of the project (i.e. Trials 1 to 11), as were average chlorophyll *a* levels (0.3 µg L^{-1}; compared to 1.1 ± 0.6 µg L^{-1}). Supplementary-fed oysters stocked at 10 mL had significantly greater growth than oysters stocked at 20 and 30 mL, though their utilisation of the microalgae was less efficient (Fig. 21). As the production of microalgae would constitute the major cost in supplementary feeding, stocking volumes should be optimised to maximise the utilisation of the microalgae yet maintain significant increases in growth rate. On this basis, the stocking density of 20 mL appears the most suitable for supplementary feeding – though the utilisation of supplementary-fed microalgae may well change under conditions of higher background level of phytoplankton in inflowing water.

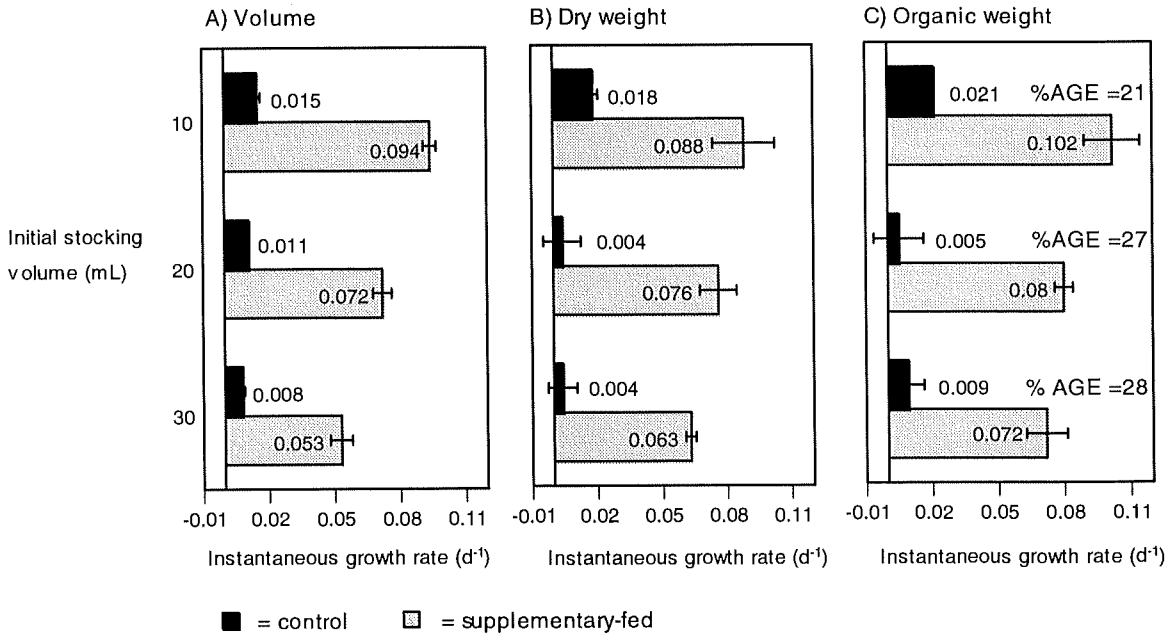


Fig. 21. Volumetric, dry weight and organic weight growth rates of 500 µm oysters at different stocking volumes, with or without supplementary feeding with *Isochrysis* sp. (T.ISO) at an initial ration of 200 mg upweller^{-1} day^{-1}.

Discussion: size class and stocking density of oysters

Trials 9 found a similar growth response to supplementary feeding of 900 µm and 1300 µm oysters. This is consistent with other studies, eg. Urban *et al.* (1993) found the instantaneous growth rate (k) was constant in *Crassostrea virginica* juveniles over a 3 week trial, during which oysters increased from 225 mg to 3 g live weight. In another study on feeding of Pacific oysters from juvenile (5 mg) to adult (800 mg) stages, Gerdes (1983) found no relationship between body size and the assimilation efficiency. However Gerdes (1983) did find that the weight-specific, daily ingestion rates did gradually decrease with increasing oyster mass, from 0.5 mg to 0.4 mg algae per mg (dry weight) of oyster, as oysters increased from 5 to 50 mg dry weight.

A reduced food availability (per unit of oyster biomass) was most likely the principal reason for the reduction in growth rate in both supplementary fed and control oysters with increasing stocking volume. Similarly, Holliday et al. (1993) found an inverse relationship between stocking density and growth of juvenile Sydney rock oysters *Saccostrea commercialis* grown in mesh frames, within an intertidal lease. At higher densities, a buildup in the concentration of oyster excretory products (faeces, ammonia) could also have negative impact on growth.

A reduction in the stocking volume of commercial upwellers is an alternative to supplementary feeding to improve growth, and could be used when sufficient upweller space is available. However, benefits with this approach could be offset against the extra labour cost to maintain additional upweller units. While supplementary feeding with a lower stocking density of 10 mL in trial 14 gave the best growth, conversion of supplementary algal biomass (which represents the major cost of supplementary feeding) was most efficient between 20 and 30 mL, as represent by higher %AGE values. Therefore, densities within this range (i.e. ≡ 400 to 600 mL in commercial upwellers) are recommended for the best utilisation of feed, at the feeding rations used in this trial.

6.2.8. Combined results from Trials 12 and 13 – longer term supplementary feeding

Trial 13 (page 22) was run immediately after Trial 12 (page 36). The T.ISO-fed oysters from Trials 12 were used as the treatment (T-ISO-fed) and control within Trial 13. In parallel to Trial 13, we also restocked control oysters from Trials 12 at the same organic-weight biomass as Trial 13 oysters. This served as a second control, and allowed us to assess supplementary feeding over the longer term.

Supplementary feeding of oysters over 43 days effected a dramatic increase in growth compared to control oysters (Figs. 22 and 23). From an initial stocking volume of 9 mL, the volume of supplementary-fed oysters increased to 240 mL, whereas control oysters had only increased to 22 mL.

There were significant differences between oyster sizes (Fig. 24). At the completion of the experiment, control oysters were mostly less than 1500 μm. In comparison T.ISO-fed oysters were all greater than 1500 μm, with the most being in the 3100-3500 μm range. In fact, 84% of the supplementary-fed oysters would no longer require nursery culture at the end of the 43 days as they were large enough for tray culture (i.e. >2300μm). In comparison, the control oysters would take at least a further 55 days (98 days in total) to reach a comparable biomass.

The low growth rate of the control oysters (especially in Trial 12) possibly magnified the effect of supplementary feeding over the 43 days. Nevertheless when control growth rates are as low as seen here, supplementary feeding in the nursery becomes necessary to maintain continuity of seed supply. This study demonstrated that supplementary feeding can substantially reduce the amount of time that oysters spend in the nursery stage at Pipe Clay Lagoon at times when production would otherwise be very low. Because maximum growth rates were not identified (i.e. a "plateauing" of growth with increasing food concentrations), improved growth rates may still be possible.

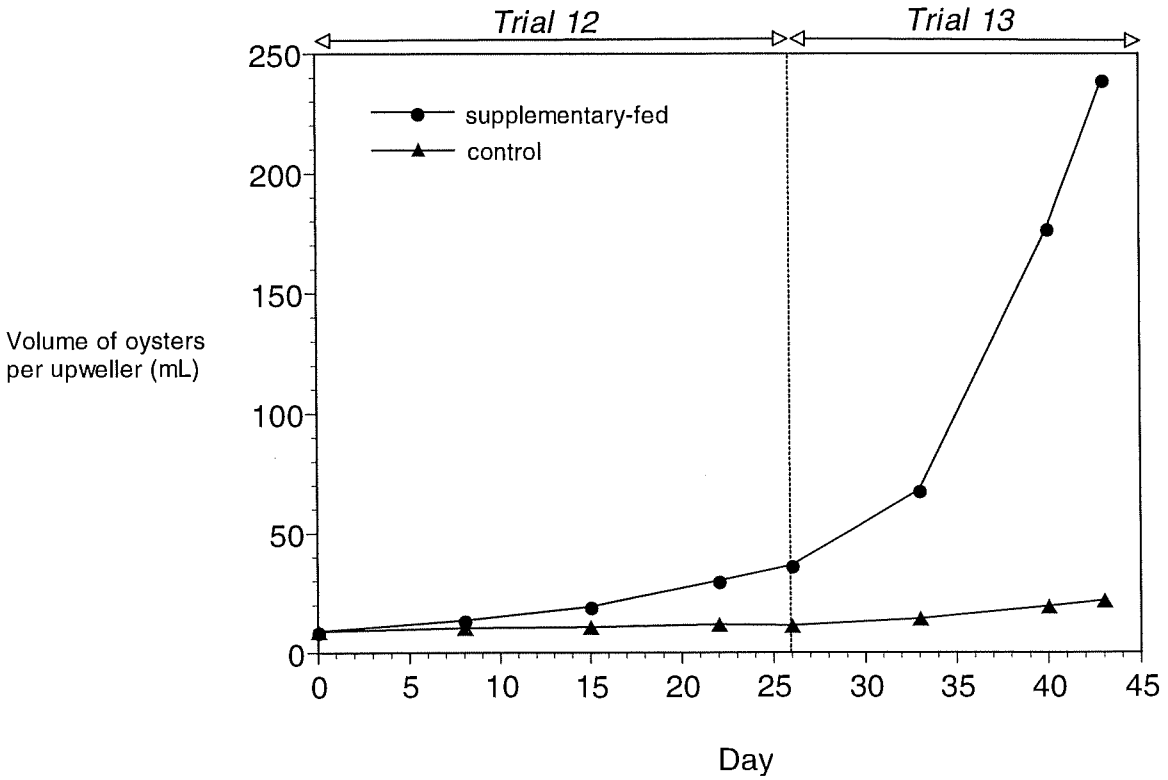
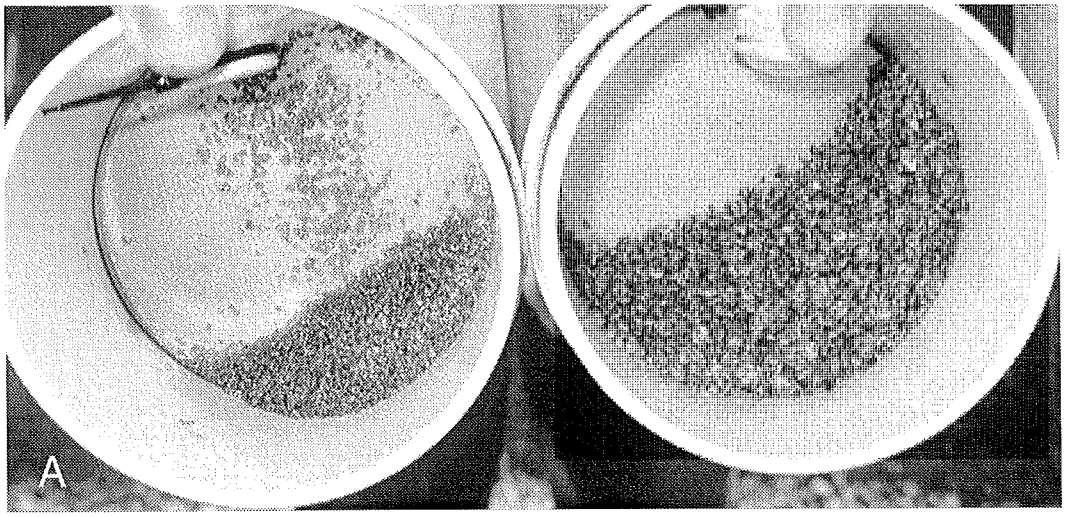


Fig. 22. The volumetric growth rate of supplementary-fed and control oysters over 43 d (combined results from Trials 12 and 13). Control oysters and T.ISO-fed oysters were restocked at equivalent (organic weight) biomass at the completion of Trial 12, to form control and T.ISO-fed groups within Trial 13. Oyster volumes displayed after day 26 were mathematically derived from the observed growth rates.



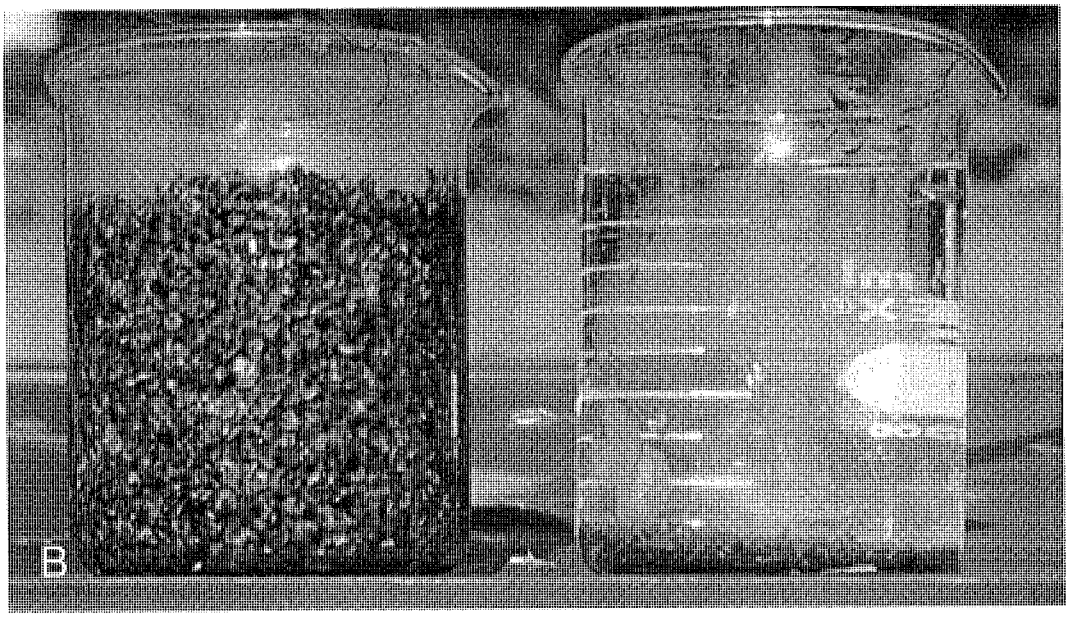


Fig. 23.

A. Comparison of the size of oysters (initial size 500-700 μm) after 43 days without supplementary feeding (left chamber) and with supplementary feeding (right chamber).
B. Comparison of the increase in biomass of oysters (initial volume 9 ml; size 500-700 μm) after 43 days without supplementary feeding (right beaker) and with supplementary feeding (left beaker). Beaker size = 250 mL.

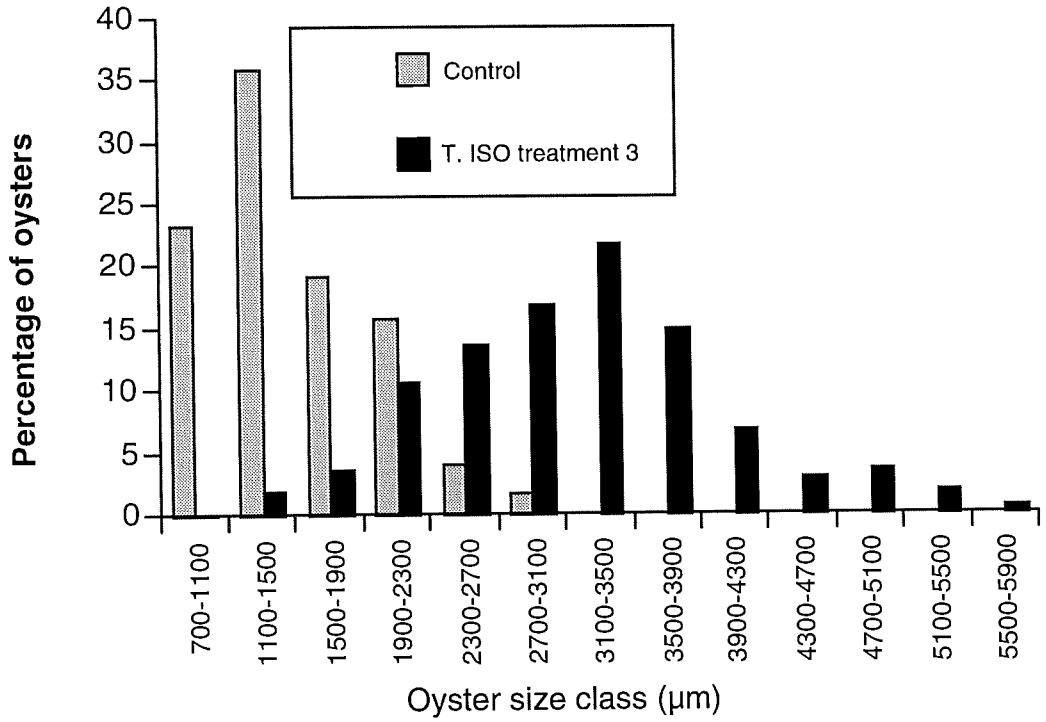


Fig. 24. Comparison of size ranges for control and supplementary-fed oysters at the end of 43 days. The majority of supplementary fed oysters (treatment 3) were of a size suitable for growing in trays. The initial size of the oysters was 500-700 µm.

6.2.9. Commercial-scale nursery trials

Trial A: Supplementary feeding at the Pipeclay Lagoon

The experiment aimed to assess whether supplementary feeding was effective at the commercial-scale. This trial was undertaken in parallel to the experimental-scale Trial 5 (see page 25), under (apart from scale) otherwise identical conditions.

Upwellers were stocked with 520 mL of 1300 µm oysters. Oysters received either no supplementary food (i.e. control) or 1.67 g *P. pinguis* (12:12 h L:D) upweller^{-1} day^{-1}. Food was delivered during weekdays in two fixed rations (2 h duration each) over an 11 d trial.

Growth rate assessed by volumetric analysis showed no difference between supplementary-fed and control oysters (Fig. 25). When assessed by dry weight and organic weight, there was a trend ($P > 0.05$) for improved growth rate with the supplementary feeding. The growth rates and trends in the commercial-scale, were also evident in experimental-scale trial 5.

Control oysters grew significantly slower in this trial than previous experimental-scale trials, despite background chlorophyll *a* levels being 2-4 times higher. Lower water temperatures in this trial (average daily range 9 to 12°C; Table 1) compared to other previous trials (average daily range ≈ 12 to 15°C in trials 1-4) most likely contributed to the poor growth in both control and supplementary-fed oysters.

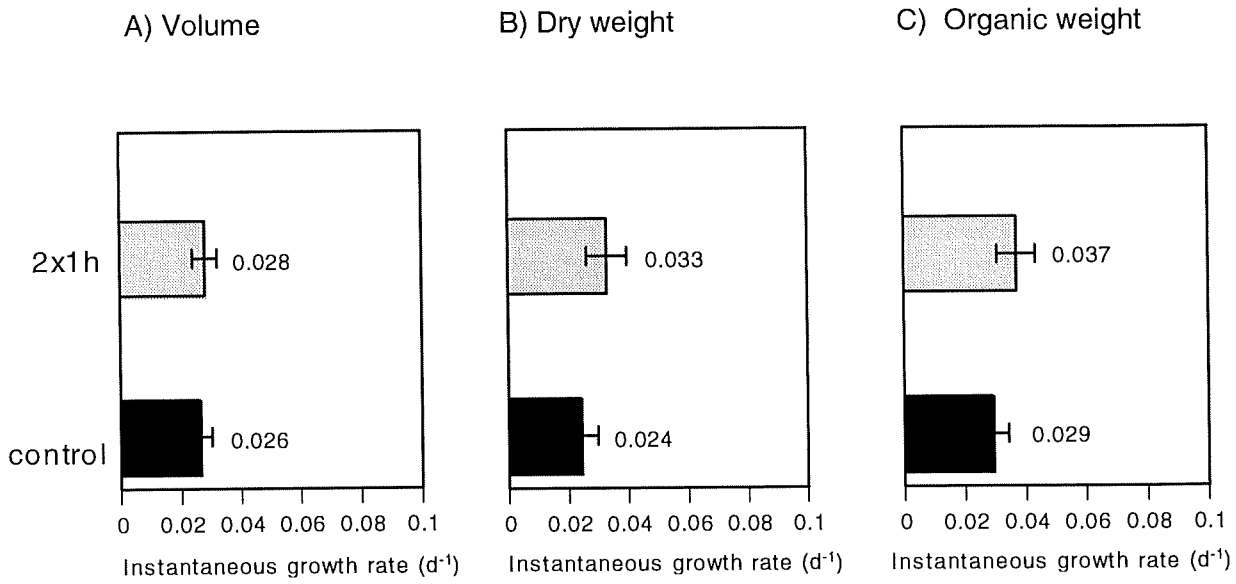


Fig. 25. Volumetric, dry weight and organic weight growth rates of 1300 µm oysters fed an average supplement of 1.67 g *P. pinguis* upweller^{-1} weekday^{-1}. Error bars are standard deviation.

Trial B: Supplementary feeding at Pipeclay Lagoon

Our second commercial-scale feeding trial was conducted in conjunction with Trial 7 (page 30). It aimed to evaluate *P. pinguis* cultured under 12:12 h L:D as a supplementary feed.

Commercial upwellers were stocked with 250 mL of 700 µm oysters. Oysters received either no supplementary-food (control) or 1.3 g *P. pinguis* day^{-1}. Food was delivered during weekdays in two fixed rations (2 h duration each) over an 18 d feeding trial.

There was a 10% improvement in growth as a result of supplementary feeding (Fig. 26). Though this modest growth increase was encouraging, we had initially planned to feed these oysters three times the actual rate fed - but we had problems maintaining the required algal production rate because of logistical constraint at the Pipe Clay research facility. Based on our other experiments, we predicted that the result can be significantly improved by a) tripling the food ration and b) utilising *P. pinguis* grown under 24:0 h L:D regime (c.f. Trial 7).

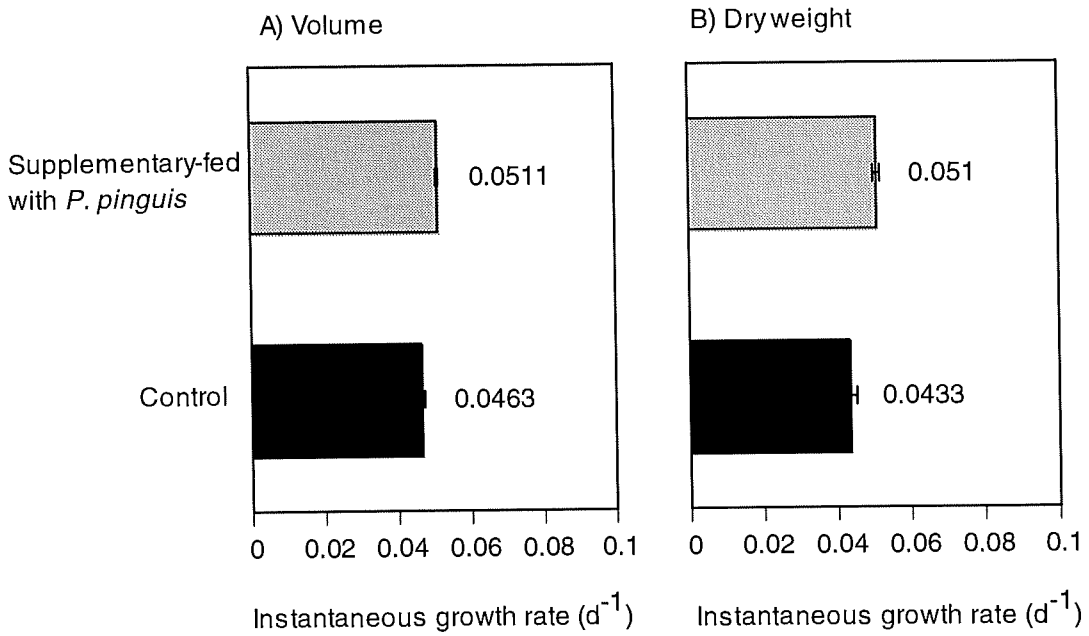


Fig. 26. Volumetric and dry weight growth rates of 700 µm juvenile oysters fed 1300 mg weekdays^{-1} of *P. pinguis* (12:12 h L:D) compared to control oysters, grown on a commercial scale. Error bars are standard deviation.

Discussion: commercial scale trials

Though trial A did not establish an improvement in growth through supplementary feeding, the parallel experimental-scale trial conducted at the same time (under similar conditions, apart from scale) also failed to detect a response. Most likely, a contributing factor was the lower water temperatures during the trial (compared to other trials) and hence a reduced "scope-for-growth" (Buxton et al., 1981).

Trial B established that supplementary feeding improved growth on the commercial scale. While improvements were modest, later experimental-scale experiments showed much higher responses were possible at 2 to 3 times the feed ration. For logistical reasons, it was not feasible to produce food in such quantities at the Pipe Clay Lagoon site, to test higher feed rations for commercial scale feeding.

TRIALS C AND D: Comparing control growth rates at Pipe Clay Lagoon and Little Swanport

The project had aimed to compare the effects of supplementary feeding at Pipe Clay Lagoon and Little Swanport. We chose to first compare growth rates of control (i.e. non-supplementary fed) oysters at these sites. On the basis of the results, we would then assess whether supplementary feeding at Little Swanport should be investigated.

Four upwellers were stocked with 300 mL of 900 µm oysters at the Pipe Clay Lagoon nursery. Two of the upwellers were transported to the Little Swanport nursery, and allowed to acclimate for 2 d to the local water conditions. Volumes of the oysters in all upwellers were measured (=initial volume) and then subjected to normal daily maintenance by industry. The growth comparison was undertaken at the sites twice, i.e. from 14 to 24 Feb 97 (10 d) and from 22 April to 5 May (13 d). At the end of the trial, the volumes of the oysters were recorded and their growth rates assessed. During each trial water quality (chlorophyll *a*, particulate matter, T) parameters were measured.

The growth rate of control oysters was significantly greater at Little Swanport than at Pipe Clay Lagoon for each of the testing occasions (Fig. 27). During these trials, levels of chlorophyll *a* were ≈ 3-8 times greater at Little Swanport than at Pipe Clay Lagoon. The growth rate at Little Swanport in the Feb '97 trial far exceeded any rate seen for supplementary-fed oysters at Pipe Clay Lagoon during the 3 yr study period (Fig. 27). The growth rate of control oysters at Little Swanport were lower during the April '97 trial, though this rate exceeded average rate of control oysters at Pipe Clay Lagoon from '95-97, and was only slightly less than the average growth rate of supplementary-fed oysters at Pipe Clay Lagoon. The poor growth rates of control oysters at Pipe Clay Lagoon in the two trials were typical of those seen throughout the 96/97 production season and significantly lower than the previous two years.

In view of the results, supplementary feeding at Little Swanport may provide only a minor (if any) increase in oyster growth, and may not be cost-effective. Alternatively, it may only prove useful at specific times of the year, when the biomass of natural phytoplankton is reduced.

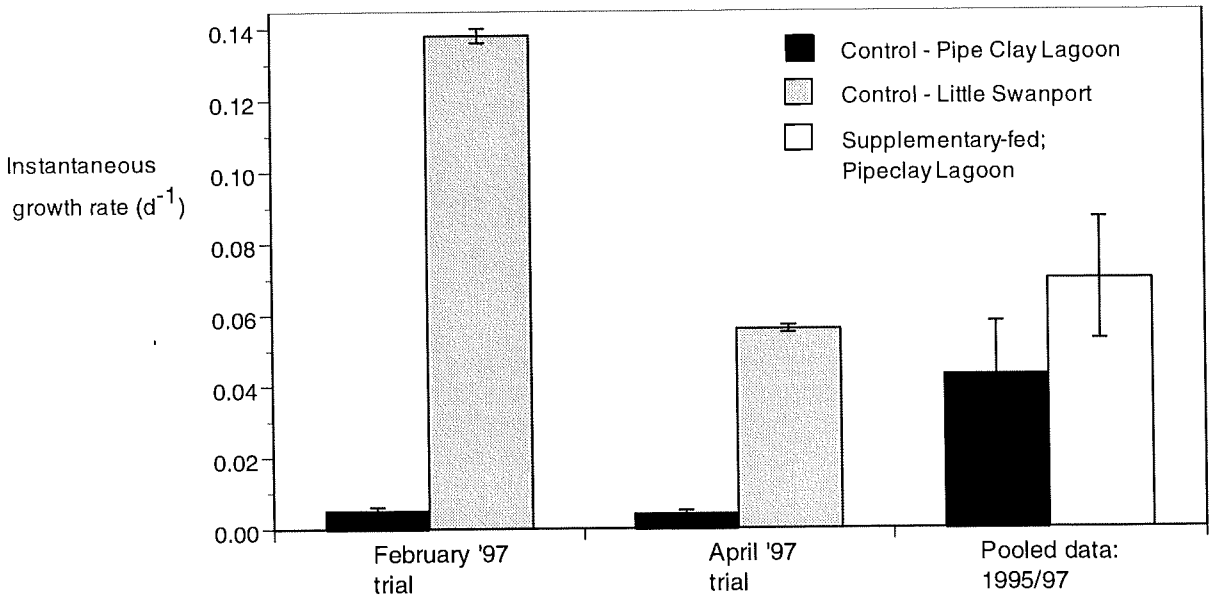
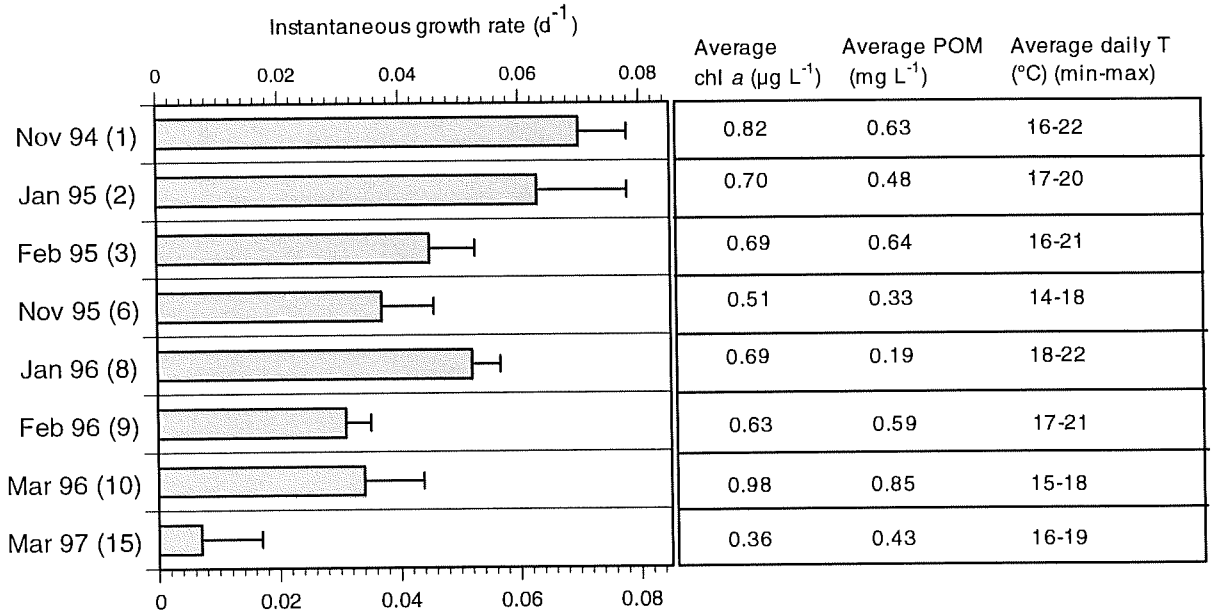


Fig. 27. The volumetric growth rates of control oysters (non-supplementary fed) at Little Swanport and Pipe Clay Lagoon. Rates are also compared to the growth rates (poled data) of control and supplementary fed oysters at Pipe Clay Lagoon.

6.2.10. Effects of season (across trials)

In a number of the trials, the initial experimental parameters (size and stocking volume of oysters, diet type and ration) were similar. This enabled us to make some assessment of season on the growth rates of control and supplementary fed oysters. Because volumetric analysis of growth rate was not initiated until trial 5, the between-trial growth rates were compared on the basis of total dry weights (Figs. 28, 29).

The growth rate of control oysters showed significant differences between trials (ANOVA; $P < 0.0001$), ranging from $k = 0.007$ to $0.070\ d^{-1}$ (Fig. 28). Regression analysis did not establish a statistical relationship between k and nutritional factors (concentrations of chlorophyll *a*, particulate organic matter) or temperature. Nevertheless, poorest growth occurred in Trial 15, when concentrations of chlorophyll *a* were significantly lower than in all other trials.

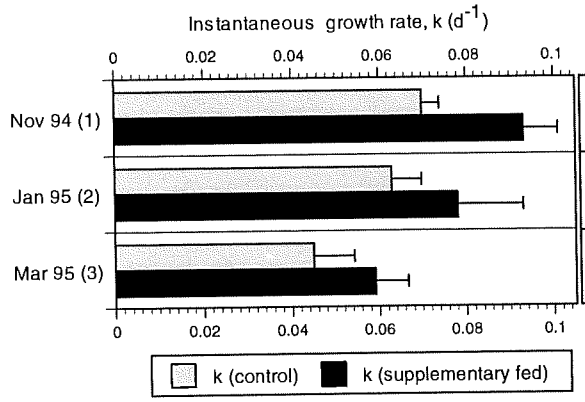


Average chl *a* (µg L^{-1})	Average POM (mg L^{-1})	Average daily T (°C) (min-max)
0.82	0.63	16-22
0.70	0.48	17-20
0.69	0.64	16-21
0.51	0.33	14-18
0.69	0.19	18-22
0.63	0.59	17-21
0.98	0.85	15-18
0.36	0.43	16-19

Fig. 28 Comparison of the growth rate of control (non-supplementary fed) oysters between trials. Growth rate was calculated on the basis of total oyster dry weight. Initially, oysters were 700 µm and stocked at 19 to 20 mL. The trial identity is shown in parentheses. POM = particulate organic matter.

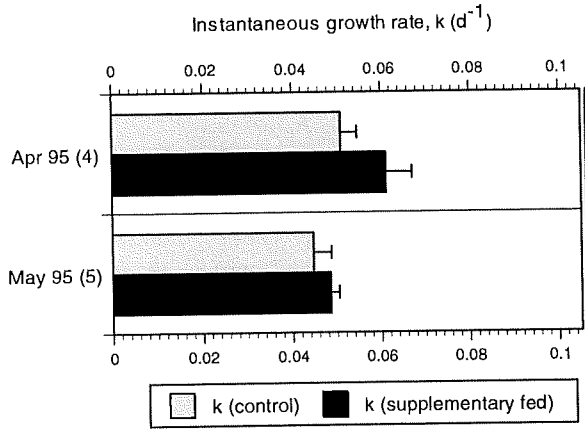
In supplementary feeding trials, the increase in growth rate attributed to supplementary feeding was calculated as the difference in the rate of supplementary fed oysters and control oysters (i.e. $k_{sup} - k_{cont}$ in Fig. 29). This value was significantly greater in trial 1 (0.023) than in trials 2 and 3 (0.014 to 0.015) ($P < 0.05$; Fig. 29A). There was no correlation between $k_{sup} - k_{cont}$ in trials 1-3 and nutritional parameters or temperature. In trial 4, $k_{sup} - k_{cont}$ (0.011) was significantly greater than in trial 5, where supplementary feeding had little impact ($k_{sup} - k_{cont} = 0.004$) ($P < 0.05$; Fig. 29B). Most likely, this difference was due to the lower water temperature in trial 5 (9-12°C) compared to trial 4 (12-15°C). In trials 8-10, $k_{sup} - k_{cont}$ was not significantly different (0.022 to 0.026) (Fig 29C).

A)

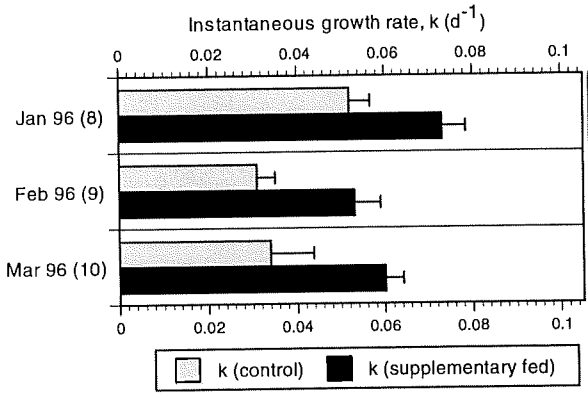


	$k_{sup.} - k_{cont}$	Avge chl a (µg L^{-1})	Avge POM (mg L^{-1})	Avge daily T (°C) (min-max)
Nov 94 (1)	.023 ± .003	0.82	0.63	16-22
Jan 95 (2)	.015 ±.004	0.70	0.48	17-20
Mar 95 (3)	.014 ± .003	0.69	0.64	16-21

B)



	$k_{sup.} - k_{cont}$	Avge chl a (µg L^{-1})	Avge POM (mg L^{-1})	Avge daily T (°C) (min-max)
Apr 95 (4)	.011 ± .001	1.47	1.01	12-15
May 95 (5)	.004 ± .0004	2.90	0.77	9-12



	$k_{sup.} - k_{cont}$	Avge chl a (µg L^{-1})	Avge POM (mg L^{-1})	Avge daily T (°C) (min-max)
Jan 96 (8)	.023 ± .002	0.69	0.19	18-22
Feb 96 (9)	.022 ± .003	0.63	0.59	17-21
Mar 96 (10)	.026 ± .003	0.98	0.85	15-18

C)

Fig. 29. Supplementary feeding between trials. Growth rate is given on the basis of oyster dry weight. In the different trials, oysters were initially:

a) 700 µm, stocked at 20 mL, fed 98 to 106 mg *of P. pinguis* (12:12 h L:D) upweller^{-1} weekday^{-1}

b) 1300 µm, stocked at 25-27 mL, fed 97 to 106 mg *of P. pinguis* 12:12 h L:D) upweller^{-1} weekday^{-1}

c) 700 µm, stocked at 19 mL, fed 180 to 190 mg *of P. pinguis* (24:0 h L:D) upweller^{-1} weekday^{-1}.

The trial identity is shown in parentheses. POM = particulate organic matter.

6.3. MONITORING PROGRAM AT OYSTER NURSERIES

One of the objectives of the project was to document changes in the water quality and growth rates of juvenile Pacific oysters (*C. gigas*) from commercial nurseries at Pipe Clay Lagoon and Little Swanport. Data from the monitoring program are presented in Figures 30 to 38, to show the seasonal trends and a comparison between the nursery sites. This data are also summarised by yearly averages in Table 4. The complete data set is given in Appendices ii to iii.

6.3.1 Inorganic nutrients

Silicate concentrations were relatively stable at Pipe Clay Lagoon (average 4.8 µM), except between October 1994 to May 1995, where values typically exceeded 10 µm (Fig. 30A). Concentrations at Little Swanport were significantly higher (average 23 µm), and occasionally exceeded 300 µM (between January and March 1996; Fig. 30A).

Nitrate concentrations showed seasonal variation at Pipe Clay Lagoon with maximum values occurring from July to September (average 0.41 µM). Seasonal trends were not apparent at Little Swanport (average 0.36 µM) (Fig. 30B).

Phosphate concentrations showed seasonal variation at Pipe Clay Lagoon with maximum values occurring from June to August (average 0.32 µM). Seasonal trends were not apparent at Little Swanport (average 0.24 µM) (Fig. 30C).

6.3.2. Particulate matter and dissolved organic carbon

Total particulate matter (TPM), particulate organic matter (POM) and dissolved organic carbon (DOC) concentrations were all greater at Little Swanport (average values of 4.2, 1.2 and 2.5 mg L^{-1}, respectively) than at Pipe Clay Lagoon (average values of 3.8, 0.79 and 1.5 mg L^{-1}, respectively) (Fig. 31). These parameters showed significant intra-annual variation and no seasonal trends.

6.3.3. Chlorophyll *a*

Approximately 75% of the total chlorophyll *a* at both sites was contained in the < 20 µm fraction. Seasonal trends were apparent at Pipe Clay Lagoon (maximum levels July to September), but not at Little Swanport. Average chlorophyll *a* concentrations at Little Swanport (2.7 µg L^{-1} total; 2.0µg L^{-1} in < 20 µm fraction) were approximately double those of Pipe Clay Lagoon (1.3 µg L^{-1} total; 1.0 µg L^{-1} in < 20 µm fraction). (Fig. 32).

At Pipe Clay Lagoon, chlorophyll *a* concentrations were correlated to nitrate and phosphate concentrations:

Chl *a* = 1.13 + 0.834*nitrate (adjusted r^2 = 0.35; $P < 0.005$ for nitrate and intercept)
Chl *a* = 0.355 + 2.74*phosphate (adjusted r^2 = 0.13; $P < 0.03$ for phosphate and intercept)

6.3.4. Salinity, rainfall and temperature

Salinity was stable at Pipe Clay Lagoon, even during periods of high rainfall (average 34.1 ± 0.6 ppt). Salinity at the more estuarine site of Little Swanport (average 31.5 ± 7.5 ppt) was prone to rapid fluctuation – particularly during periods of high rainfall when values dropped below 5 ppt (Fig. 33). The sites received similar rainfall over the project period (Pipe Clay Lagoon 11.2 ± 15.1 mm $week^{-1}$; Little Swanport 10.2 ± 20.0 mm $week^{-1}$). Temperature profiles at both sites showed typical seasonal trends (maximum in summer, minimum in winter). Temperatures were generally1-2°C warmer at Little Swanport (average 14.3 ± 3.8°C) than at Pipe Clay Lagoon (12.7 ±3.2°C).

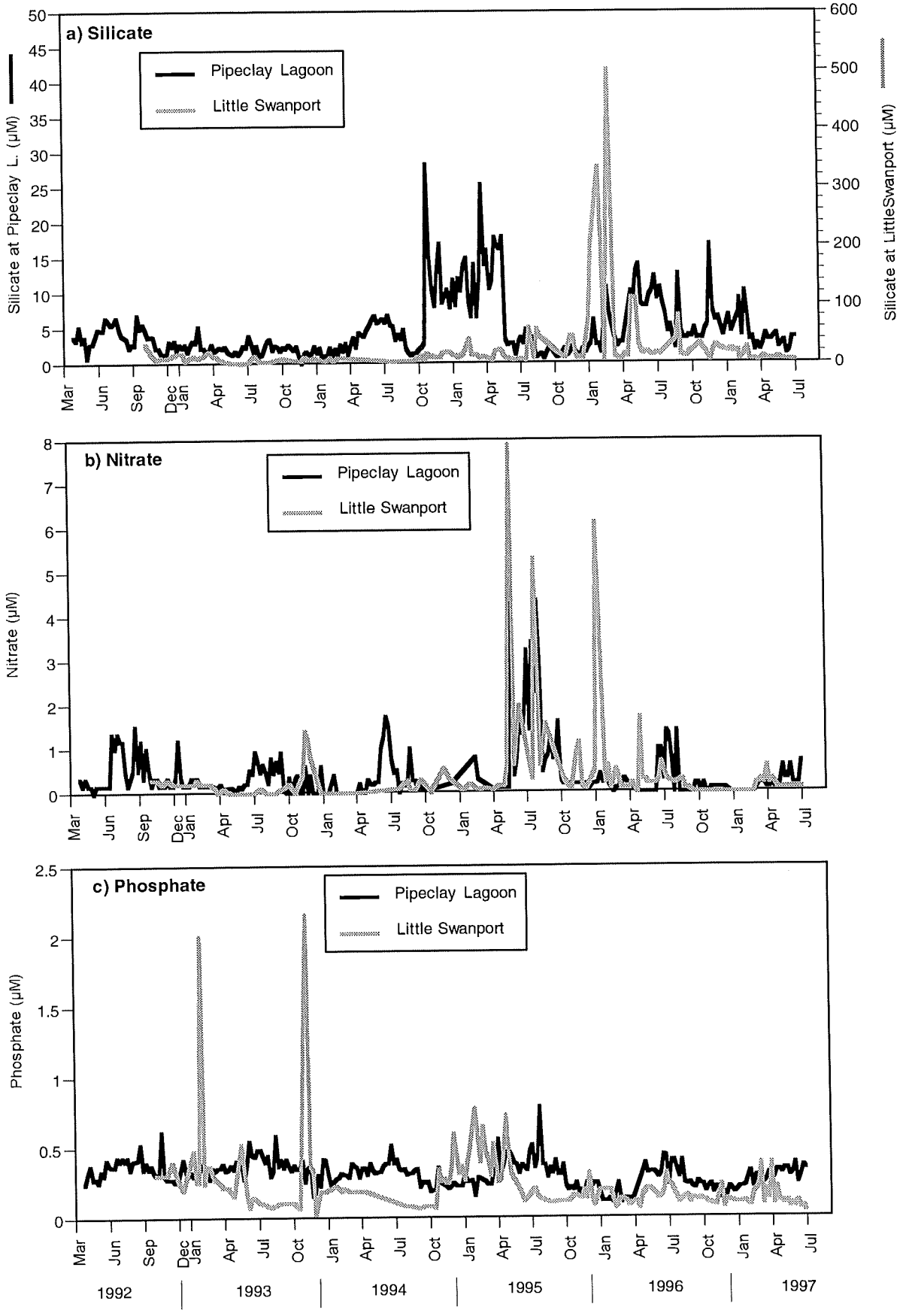


Fig. 30. Nutrient concentrations at Pipe Clay Lagoon and Little Swanport from 1992 to 1997.

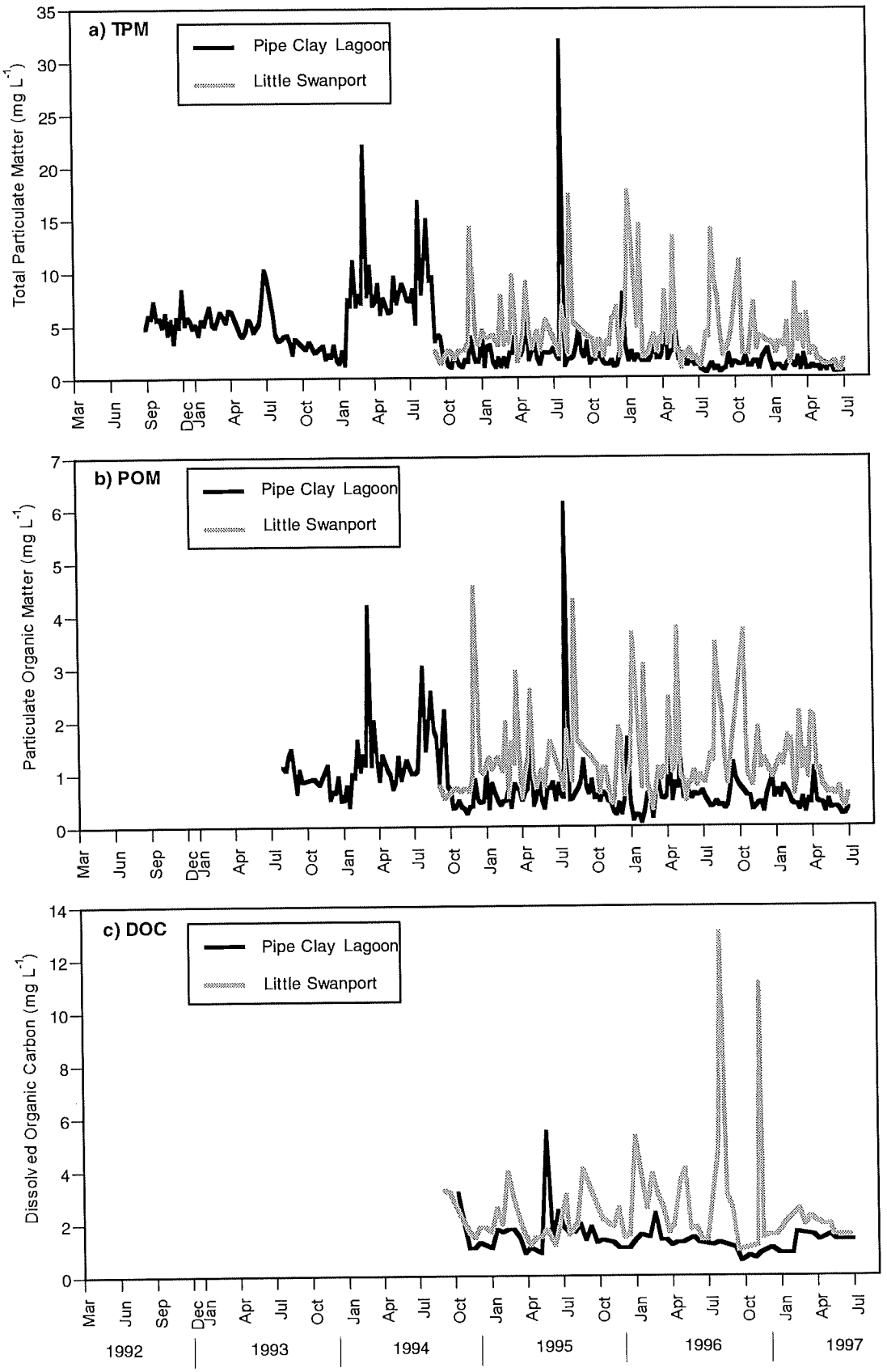


Fig. 31. Concentrations of total particulate matter, particulate organic matter and dissolved organic carbon at Pipe Clay Lagoon and Little Swanport from 1992 to 1997.

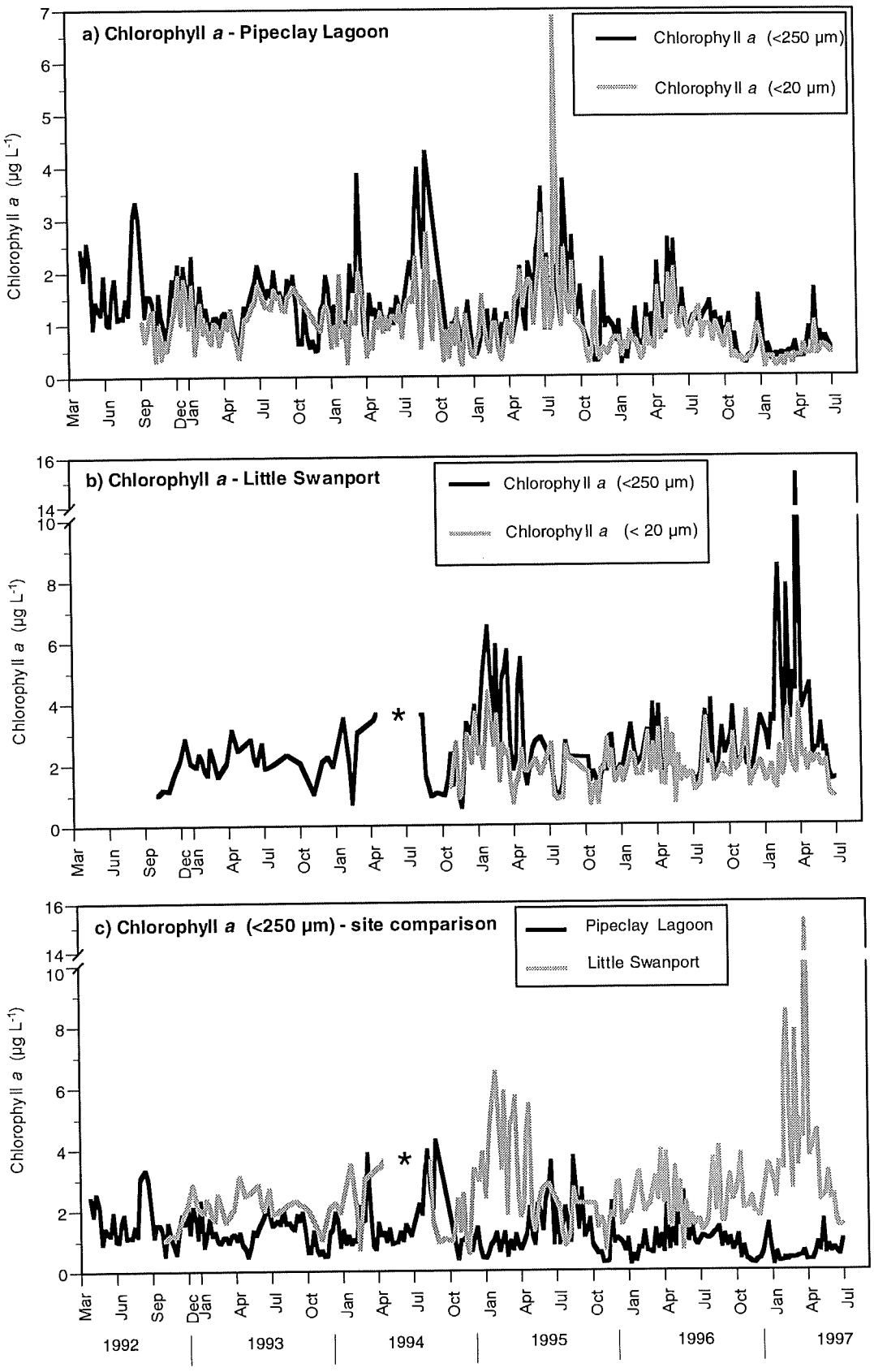


Fig. 32. Concentrations of chlorophyll *a* at Pipe Clay Lagoon and Little Swanport from 1992 to 1997.
* =sample not available from Little Swanport during this period

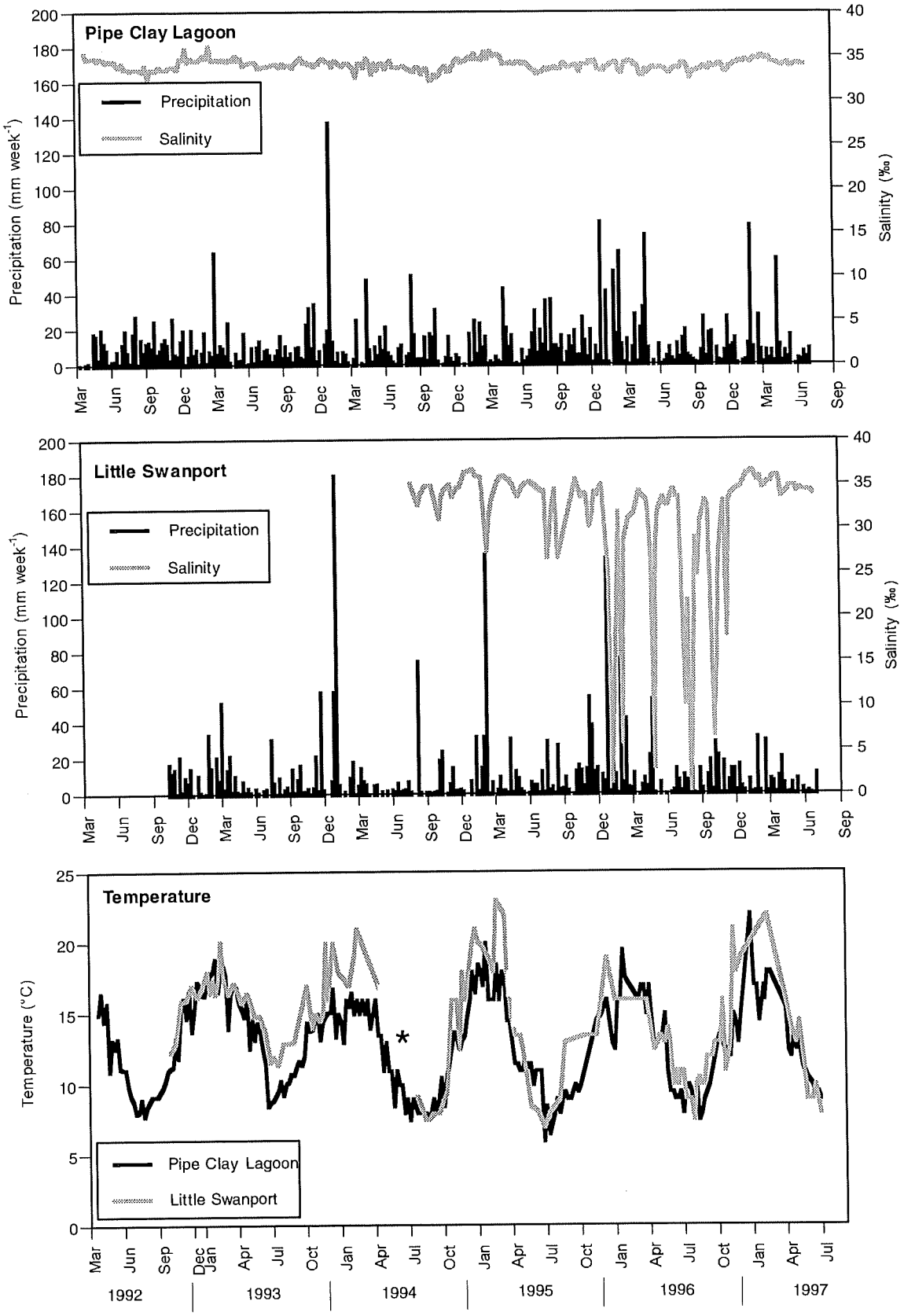


Fig. 33. Salinity, rainfall and temperature profiles from Pipe Clay Lagoon and Little Swanport from 1992 to 1997. * = samples not available from Little Swanport during this period.

Table 4. Water quality data from Pipeclay Lagoon and Little Swanport – Annual (from July to June) average (± s.d.) data. k = instantaneous growth rate.

Site/Year	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg L^{-1})	chl. a (<20µm) (µg L^{-1})	TPM (<20µm) (mg L^{-1})	POM (< 20 µm) (mg L^{-1})	DOC (mg L^{-1})	Oyster k (day^{-1})
Pipeclay Lagoon											
1992/93	13.6 (± 3.4)	34.2 (± 0.7)	3.0 (± 1.5)	0.37 (± 0.37)	0.37 (± 0.07)	1.4 (± 0.6)	1.0 (± 0.4)	5.6 (± 1.2)			0.019 (± 0.010)
1993/94	12.8 (± 2.6)	34.1 (± 0.4)	2.7 (± 1.6)	0.33 (± 0.42)	0.35 (± 0.08)	1.3 (± 0.6)	1.2 (± 0.4)	6.0 (± 3.6)	1.1 (± 0.6)		0.011 (± 0.018)
1994/95	12.4 (± 3.7)	34.1 (± 0.8)	8.9 (± 6.4)	0.43 (± 1.06)	0.31 (± 0.09)	1.5 (± 1.0)	1.2 (± 0.7)	3.6 (± 3.4)	0.85 (± 0.60)	1.7 (± 1.0)	0.022 (± 0.016)
1995/96	12.2 (± 3.4)	33.7 (± 0.5)	4.3 (± 3.8)	0.57 (± 0.91)	0.27 (± 0.12)	1.2 (± 0.7)	1.1 (± 1.0)	2.8 (± 4.3)	0.72 (± 0.83)	1.5 (± 0.3)	0.013 (± 0.013)
1996/97	12.5 (± 3.3)	34.1 (± 0.5)	5.0 (± 3.0)	0.22 (± 0.35)	0.27 (± 0.07)	0.7 (± 0.4)	0.6 (± 0.3)	1.2 (± 0.5)	0.52 (± 0.21)	1.2 (± 0.3)	0.009 (± 0.024)
Average (± s.d.)	**12.7 (± 3.2)**	**34.1 (± 0.6)**	**4.8 (± 4.2)**	**0.38 (± 0.68)**	**0.32 (± 0.09)**	**1.26 (± 0.71)**	**1.01 (± 0.65)**	**3.79 (± 3.49)**	**0.79 (± 0.63)**	**1.45 (± 0.65)**	**0.015 (± 0.017)**
Little Swanport											
1992/93	15.9 (± 1.9)		10.9 (± 7.8)	0.15 (± 0.11)	0.35 (± 0.37)	2.12 (± 0.54)					
1993/94	16.3 (± 3.0)		6.9 (± 3.4)	0.26 (± 0.45)	0.29 (± 0.49)	2.28 (± 0.83)					
1994/95	14.5 (± 5.2)	34.6 (± 1.8)	12.4 (± 7.4)	0.40 (± 1.33)	0.33 (± 0.19)	2.93 (± 1.61)	2.15 (± 0.84)	4.19 (± 2.64)	1.22 (± 0.81)	2.10 (± 0.77)	
1995/96	12.9 (± 2.7)	29.8 (± 8.4)	57.7 (± 101.8)	0.68 (± 1.28)	0.16 (± 0.06)	2.08 (± 0.77)	1.85 (± 0.69)	4.73 (± 4.11)	1.29 (± 0.92)	2.55 (± 1.08)	
1996/97	12.6 (± 3.8)	30.6 (± 8.7)	15.4 (± 14.4)	0.10 (± 0.13)	0.14 (± 0.07)	3.41 (± 2.43)	2.00 (± 0.70)	3.76 (± 2.87)	1.33 (± 0.75)	3.00 (± 3.00)	
average (±s.d.)	**14.3 (± 3.8)**	**31.5 (± 7.5)**	**23.3 (± 54.2)**	**0.35 (± 0.93)**	**0.24 (± 0.26)**	**2.66 (± 1.65)**	**1.98 (± 0.74)**	**4.21 (± 3.30)**	**1.29 (± 0.82)**	**2.56 (± 1.98)**	

6.3.5. Fatty acid concentrations and composition

The concentrations of the long-chain PUFAs at Little Swanport were approximately double that at Pipe Clay Lagoon (Figure 34). Between November 1994 and October 1995, average values of 22:6(n-3) were 735 ± 501 ng L^{-1} at Pipe Clay Lagoon compared to 1380 ± 1100 ng L^{-1} at Little Swanport. Average values of 20:5(n-3) were 787 ± 432 ng L^{-1} at Pipe Clay Lagoon compared to 1660 ± 1300 ng L^{-1} at Little Swanport.

Whilst the relative concentrations of the PUFAs at the sites tended to reflect the total chlorophyll *a* concentrations at the sites (i.e. Little Swanport approximately double that of Pipe Clay Lagoon) the percentage composition data presented additional information on the algal communities. The percentage composition of total PUFAs was significantly greater at Little Swanport (38.3 ± 5.8%) than at Pipe Clay Lagoon (32.3 ± 7.3) ($P < 0.05$; Appendix iv, v). The percentage of 20:5(n-3) at Little Swanport (9.7 ± 1.6%) was significantly greater than at Pipe Clay Lagoon (7.3 ± 2.1%) ($P < 0.05$; Appendices iv, v).

6.3.6. Pigment concentrations

Concentrations of chlorophyll and accessory pigments (as determined by HPLC) from water samples are shown in Figs 35 to 37, and Appendix vi.
For most pigments, concentrations at Little Swanport were usually greater than at Pipe Clay Lagoon. This was not surprising, as the levels of chlorophyll *a* (from spectrophotometric assays) were typically double at Little Swanport compared to Pipe Clay Lagoon – indicating a twofold algal biomass. However, the relative proportions of diadinoxanthin (common in many microalgae but lacking in chlorophytes and cryptophytes; Jeffrey *et al.*, 1997) were greater at Pipe Clay Lagoon (Fig. 36B). Alloxanthin (present in cryptophytes; Jeffrey *et al.*, 1997) concentrations at Little Swanport were 4-times those at Pipe Clay Lagoon (Fig. 36C). These two observations suggest the concentrations of cryptophytes were proportionately greater at Little Swanport. Chlorophylls c1 + c2 (one or other in diatoms, dinoflagellates, chrysophytes and prymnesiophytes; Jeffrey *et al.*, 1997) were in higher concentrations at Pipe Clay Lagoon) (Fig. 34A). 19'-Hexanoyloxyfucoxanthin (characteristic of prymnesiophytes) was periodically detected at Pipe Clay Lagoon, but never at Little Swanport (Appendix vi).

6.3.7. Nutrient parameters and oyster growth rates at Pipe Clay Lagoon

Growth rates of oysters showed significant seasonal and interannual variation (Fig. 38). Multiple regression analysis of the complete data set showed no direct correlation between oyster growth and nutrient parameters and temperature. However, the low growth rates observed from January 1996 to June 1997 (average $k = 0.003 \pm 0.023$) was associated with significantly lower chlorophyll *a* concentrations over that period (0.6 µg L^{-1}).

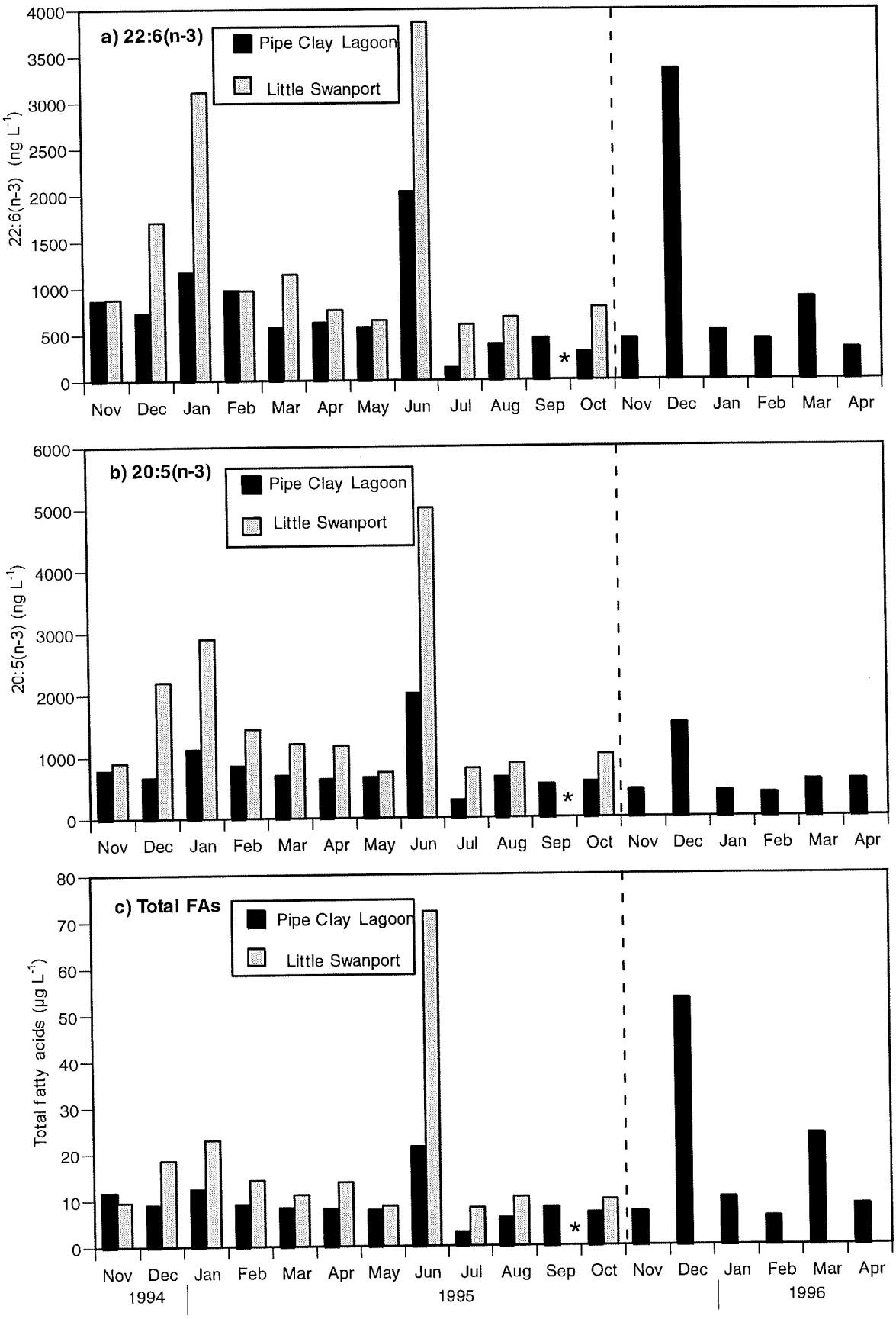


Fig. 34. PUFA concentrations in the particulate matter from Pipe Clay Lagoon and Little Swanport. * = samples not analysed (Little Swanport only).

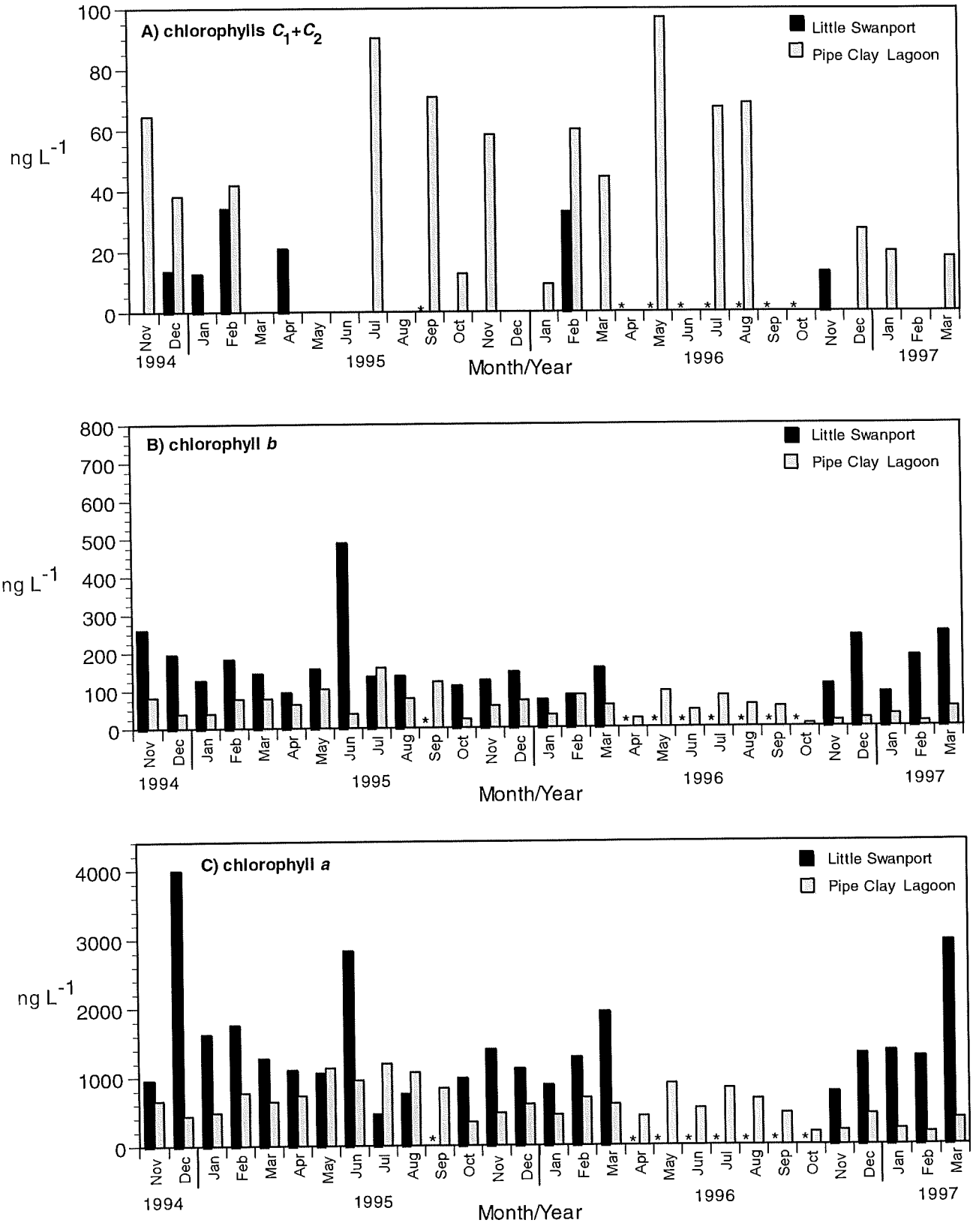


Fig. 35. Concentrations of chlorophyll pigments (measured by HPLC) in monthly-integrated samples (4-5 samples/month taken at weekly intervals) in seawater at Pipe Clay Lagoon and Little Swanport.
* = samples not analysed (Little Swanport only).
chl. c_1 + c_2 – present in cryptophytes, diatoms, dinoflagellates, prymnesiophytes, chrysophytes and raphidophytes; chl. b – chlorophytes, prasinophytes and euglenophytes; chl a – all microalgae (Jeffrey et al, 1997).

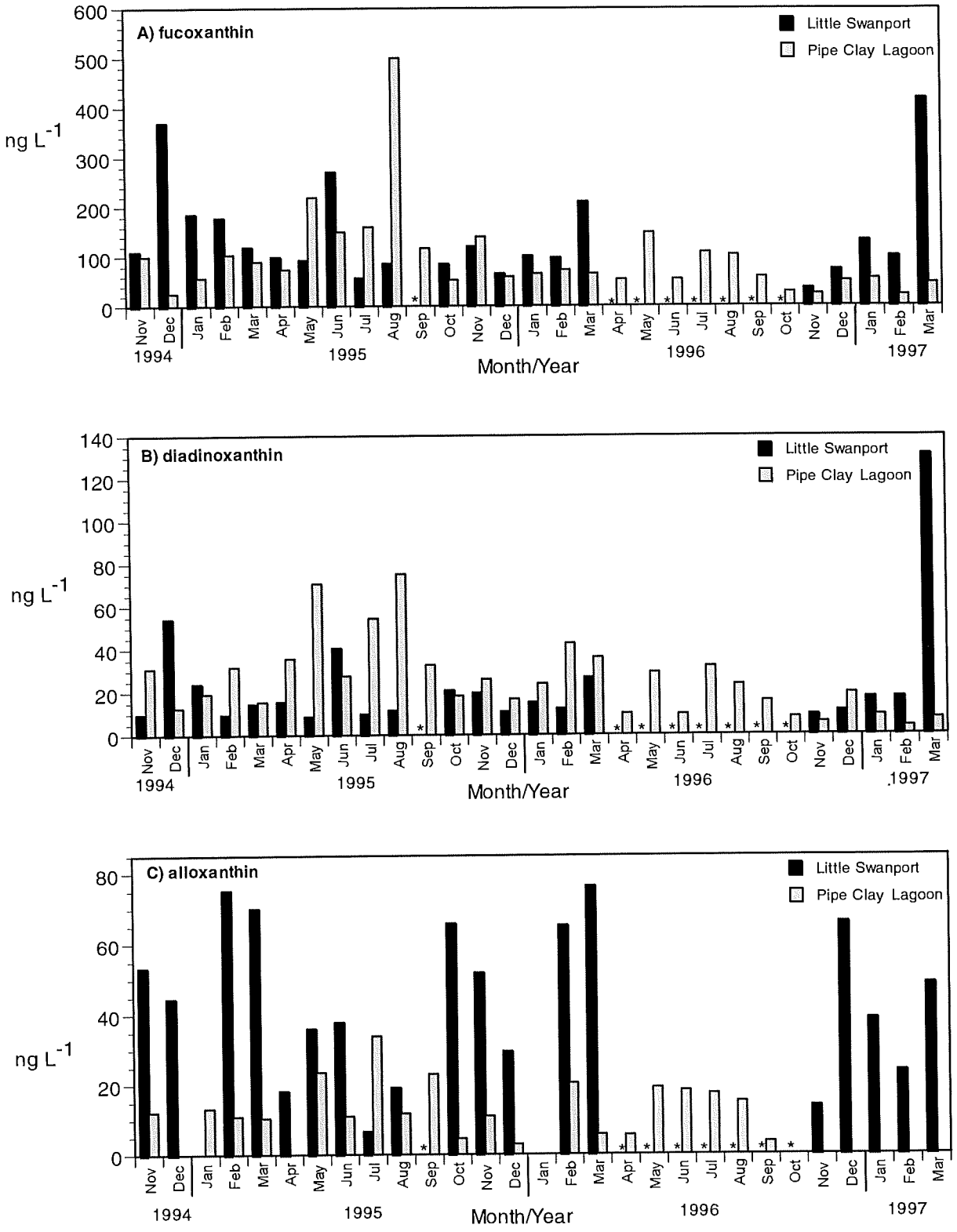


Fig. 36. Concentrations of fucoxanthin, diadinoxanthin and alloxanthin in monthly-integrated samples (4-5 samples/month taken at weekly intervals) in seawater at Pipe Clay Lagoon and Little Swanport.

* = samples not analysed (Little Swanport only)

fucoxanthin – present in diatoms, chrysophytes, raphidophytes and some prymnesiophytes;
diadinoxanthin – in diatoms, chrysophytes, raphidophytes, prymnesiophytes, dinoflagellates and euglenophytes;
alloxanthin - in cryptophytes (Jeffrey et al., 1997).

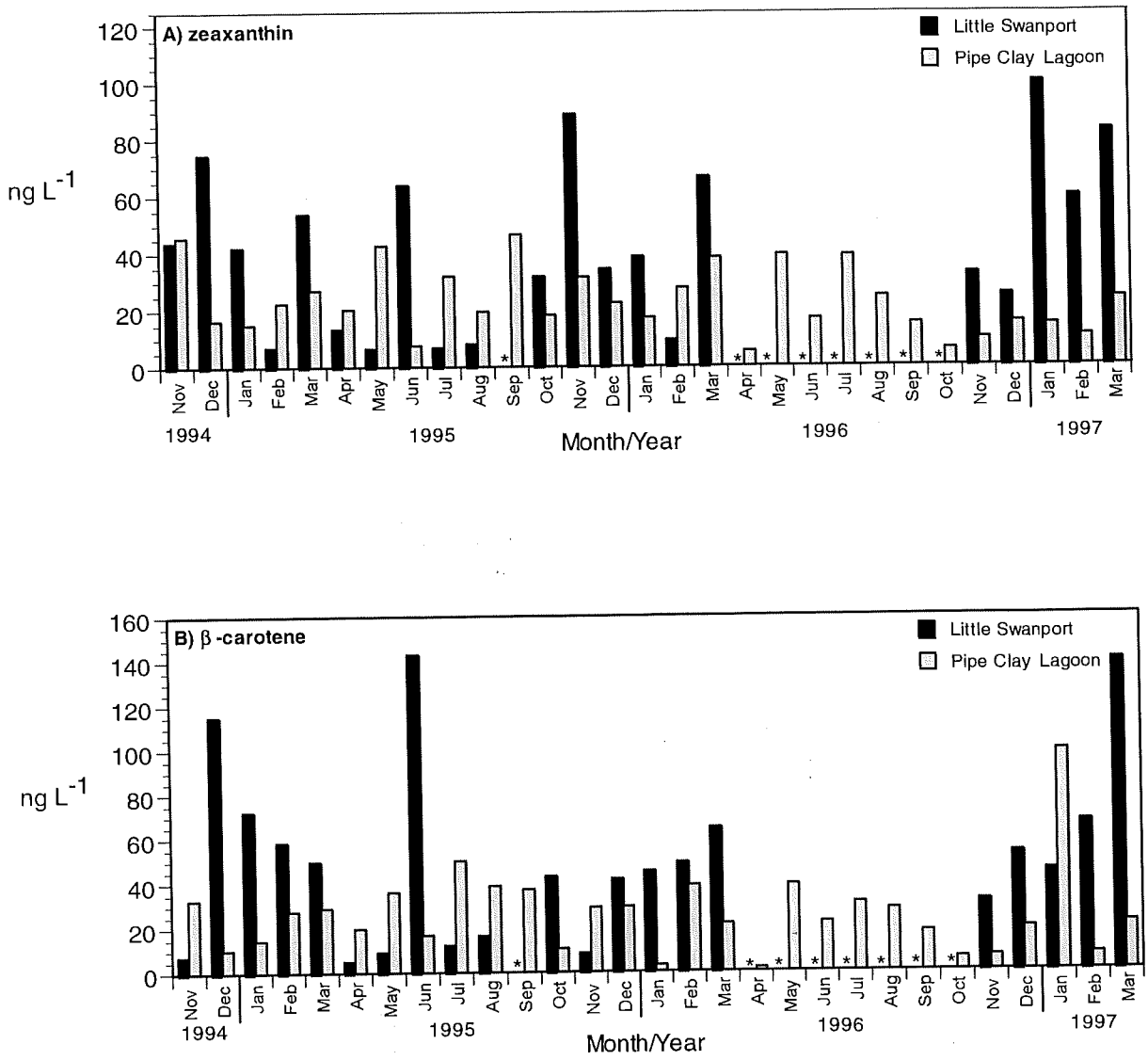


Fig. 37. Concentrations of zeaxanthin and β-carotene in monthly-integrated samples (4-5 samples/month taken at weekly intervals) in seawater at Pipe Clay Lagoon and Little Swanport.

* = samples not analysed (Little Swanport only).

zeaxanthin – present in cyanobacteria, prochlorophytes, rhodophytes, chlorophtes and (trace amount) eustigmatophytes;
β-carotene – present (usually) as a minor pigment in most microalgae, except rhodophytes and cryptophytes (Jeffrey et al., 1997).

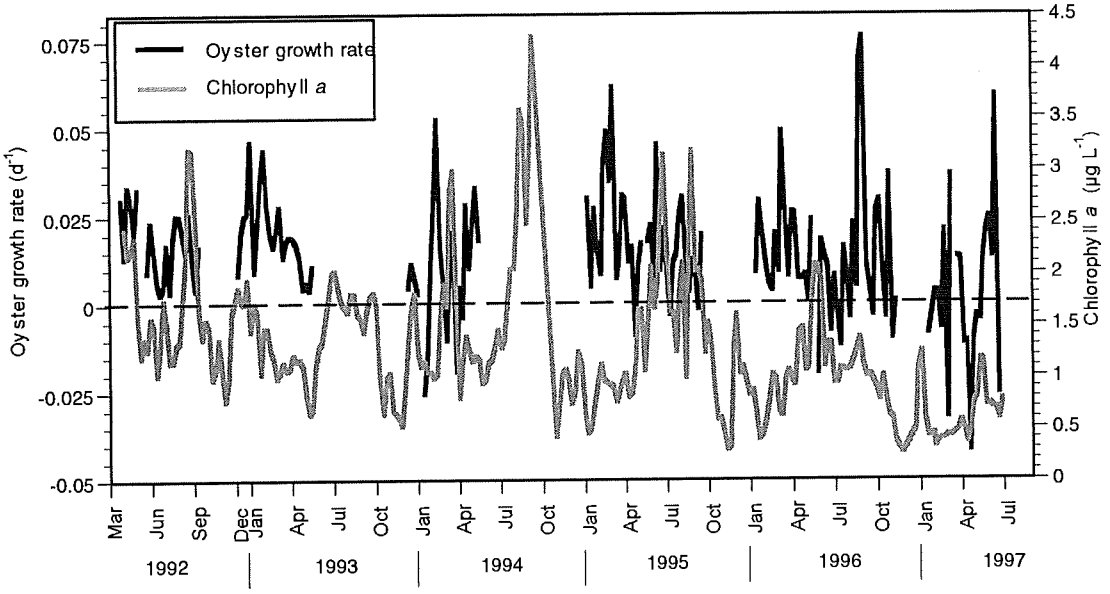


Fig. 38. The growth rate of oysters compared to concentrations of chlorophyll *a* at the Pipe Clay Lagoon site, from 1992 to 1997.

6.4. DISSEMINATION OF RESULTS

Results were transferred to our industry partner through 6 monthly FRDC progress reports, and additional research reports.

Results were also presented at scientific and industry conferences (AMSA, Tas Aquaculture Research Exchange, Australasian Society for Phycology and Aquatic Botany; see Appendix vii). A feature article on the research was published in the Australian newspaper (Appendix viii) and a radio interview was given to the ABC. An article was also published in Austasia Aquaculture (Appendix ix) and the Tasmanian Mercury. One manuscript has been submitted to the journal Aquaculture (Appendix i) and we have prepared a draft of another (Appendix x).

7. DISCUSSION

General introductory comments

Juvenile oysters are usually grown in land-based nurseries using upweller (or downweller) systems, where local seawater is pumped through to provide food particles (Rodhouse et al., 1981). This method is effective, but a major disadvantage is its reliance on the productivity of the associated waterway for oyster growth. In the present study, this limitation was evident during the 1996/97 production season when oyster growth rates at Pipe Clay Lagoon were significantly lower than in previous seasons, and apparently associated with lower concentrations of phytoplankton passing into the nursery.

Approaches for enhancing the growth of juvenile bivalves in upweller systems have included feeding them entirely on cultured microalgae (Walsh et al., 1987), or supplementing their natural diet either a) with cultured microalgae (e.g. current study) or b) by fertilisation of seawater to stimulate microalgal bloom formation (Spencer, 1988). The economics of the first approach (i.e. complete algal feeding) are equivocal and depend on the scale and operating parameters of the nursery (including methods used for algal mass culture), environmental factors and the phytoplankton ecology of the associated waterway (Persoone and Claus, 1980; Walsh et al., 1987; Myers and Boisvert, 1990). Fertilisation of seawater to promote natural blooms can be more cost effective and is an approach used by some Australian nurseries. However, results are unpredictable as it is difficult to control the growth of selected (eg. high nutritional value) microalgal species within a bloom where environmental conditions are not controlled (Spencer et al., 1986; Spencer, 1988). Moreover, fertilisation of seawater can occasionally lead to a reduction in oyster growth, compared to unfertilised seawater (Spencer et al., 1986).

The major project aim was to assess supplementary diets for enhancing production at the Pipe Clay Lagoon site. The diets included microalgae having a high nutritional value in recirculation culture systems, either as a uni-algal diet or as part of a mixed species diet (Enright et al. 1986a; Laing and Millican, 1986; O'Connor et al., 1992; Brown et al., 1998), algal pastes and off-the-shelf commercial diets. Short-term (16 to 26 d) experiments were conducted comparing the nutritional value of the different diets using culture systems (apart from scale) identical to industry – and thus applicable to industry. We established that supplementary feeding was effective in enhancing growth rates of juvenile oysters at the site. It's effectiveness varied according to diet type and presentation, concentration and seasonal factors (prevailing water quality parameters), though on average the feeding increased oysters' growth rates by 60%.

Nutritional value of microalgal diets and biochemical composition

It was not a project objective to define the absolute nutrient (eg. protein, carbohydrate, etc.) requirements of juvenile Pacific oysters. Other studies have addressed this using recirculating systems (see reviews by Webb and Chu, 1983, Brown et al, 1997), which are more suited to nutritional studies than flow-through systems because with the latter, results are confounded by a constant (changing) input of feed from ambient seawater. Another limitation of flow-through systems is that it is difficult to accurately measure nutrients (protein, carbohydrate, lipid) in the seawater, and even if it were possible, values might not necessarily reflect availability or intake by oysters. Even with recirculation systems though, correlation has proven difficult because differences in the size, digestibility and concentrations of other nutrient fractions (apart from those being assessed) between different microalgae have confounded the interpretation of trials. Algal diets rich in carbohydrate are reported to produce the best growth for juvenile oysters (*Ostrea edulis*, Enright et al., 1986b) and larval scallops (*Patinopecten yessoensis*, Whyte et al., 1989), while high dietary protein provides best growth for juvenile mussels (*Mytilus trossulus*, Kreeger and Langdon, 1993). The gross biochemical composition was assessed for different supplementary diets used in the trials, but diet performance was not correlated with composition. However, in trial 7, *P. pinguis* grown under 24:0 h L:D was more effective than the same alga grown under 12:12 h L:D. This might have been attributed to higher concentrations of energy-rich lipid in the 24:0 h L:D cultures.

Although it was not practicable to correlate diet performance with gross composition, it seems likely that differences in the available protein and energy within diets has a major contribution. Assessment of the digestibility of the different supplementary diets was also beyond the scope of the project. However, identical or similar strains (i.e. *D. tertiolecta, I. galbana, Pavlova lutheri* and *R. salina*) are efficiently ingested (≥ 81%) and assimilated (≥78%) by the Bay scallop, *Argopecten irradians concentricus* (Say). Of the other species, *Chaetoceros calcitrans* and *Skeletonema* sp., have a high nutritional value for Sydney rock oyster spat (O'Connor et al., 1992; Heasman pers. comm.), indicating they are efficiently digested. We did however, assess the apparent growth efficiency (%AGE) of each diet, as a measure of the efficiency by which the food was used. % AGE values for algal diets (including pastes) were ≥ 21% indicating efficient assimilation of algal biomass by oysters. In costrast %AGE values for Microfeast MB-30 (11%) and AlgaMac 2000 (7%) were significantly lower.

Differences in diet performance may be unrelated to any micronutrient deficiencies (eg. vitamins, PUFAs) because background seawater may have been supplying adequate concentrations, and also the relatively short experimental period. For example, the PUFA-deficient *D. tertiolecta* proved to be an excellent diet in Trial 10, though at the completion of the experiment oysters fed the alga contained approximately half the PUFA 22:6(n-3) than *Isochrysis* sp. (T.ISO)-fed oysters. Prolonged feeding with *D. tertiolecta* would not be recommended, especially during periods when background PUFA concentrations in seawater were lower (as would have occurred from December 1996 to June 1997 when chlorophyll *a* were lower).

One should be careful in comparing diets between trials, because of the differences in biotic (phytoplankton and other food particles concentrations and species composition) and abiotic (temperature, nutrients, dissolved organics) parameters of background seawater, and their interaction with growth and diet performance. Not only did this contribute to the differences in control (i.e. non-supplementary fed) oyster growth rates, but it also contributed to differences in the effectiveness of supplementary feeding (see section 6.2.10; Effects of seasonality). To assist in making some diet evaluation within and between trials though, we included *Pavlova pinguis* as a reference diet during the first two years of the project (i.e. trials 1 to 10). *P. pinguis* was generally the least effective diet, though is was more effective than *Pavlova* sp. CS-63. Within-trial comparison showed that *Isochrysis* sp. (T. ISO), *D. tertiolecta, R. salina, C. calcitrans* (live culture and paste) and *S. costatum* (paste) were the most effective microalgal diets, though for the reason mentioned, we did not discriminate between their effectiveness.

Trial 8 assessed a mixed-species diet as a supplementary feed. In recirculating culture systems, mixed-species diets generally give better growth of oysters than monospecific diets because they contain a more balanced mix of essential nutrients (Webb and Chu, 1983). However, there was no benefit in providing a mixed supplementary diet of *R. salina* plus *P. pinguis*. Again, the reason is probably that the background natural phytoplankton fulfils most of the oysters' need for micronutrients, and supplementary feeding is simply providing extra protein and energy for additional growth.

Three Australian microalgal strains were assessed as supplementary feeds because they may have been more suited to local environmental conditions (eg. water quality, ambient temperatures if cultures were to be grown in outdoor ponds). Additionally, future quarantine laws and council regulations might restrict the use of overseas strains requiring reliance on local strains. *Pavlova* sp. CS-63 and *P. pinguis* were less effective diets than the other local strain, *R. salina.* Nevertheless, the cell division rate of *R. salina* would need to be improved to make it as cost-effective as overseas strains. In another study, two local diatom strains, *Attheya septentrionalis* and *Entomoneis* sp., had rapid cell division rates, and a nutritional value for oyster spat that was almost equivalent to *T. pseudonana* (Knuckey and Brown, unpublished) – an alga recognised as having excellent nutritional value (Brown and Jeffrey, 1992). These two species warrant further examination as local alternatives for supplementary feeding.

Algal pastes and dried diets

As well as live algal diets, we tested four "off-the-shelf" diets – two algal pastes prepared by centrifugation, Microfeast® MB-30 (a yeast-based dry diet) and Alga-Mac 2000 (a dried preparation of *Schizochytrium* sp., an algal-like heterotrophic microorganism). Products similar to the above (and others including bacteria and yeast) have been assessed as alternatives to live algae for molluscs, because they may be more cost-effective and can be used at remote nursery sites that lack algal mass-culture facilities. To date, most have had too low a nutritional value to make them useful as a complete replacement diet (Jones et al., 1993; Nell et al., 1994; Robert and Trintignac, 1997).

S. costatum and *Ch. calcitrans* pastes performed as well as live microalgal supplementary-diets. This indicated that a) little nutritional loss occurred during the pasting, transportation and storage period and/or b) the natural phytoplankton compensated for any nutritional inadequacies of the pastes. Pastes of these species have also proven effective for *S. commercialis* larvae, though they were not as effective as the live microalgae (Nell and O'Connor, 1991). The pastes used in the latter study, and ours, were stored for less than 14 d prior to use. The storage life of these diets may be limited to this short period, and further studies are needed to establish their effectiveness after longer-term storage (eg up to and beyond 1 month).

Of the dried diets tested as supplementary feed in trial 12, Microfeast® MB-30 was more effective than Alga-Mac 2000; both were less effective than live *Isochrysis* sp. (T.ISO)*. The dried products cost less (available commercially at ≈ AUS $ 80-100 kg^{-1}) than live microalgae produced in hatcheries (range of ≈A$80 to $500 kg^{-1} dry weight, depending on scale and technology; Coutteau and Sorgeloos, 1992). The trial showed that both products are promising alternatives to live microalgae for supplementary feeding, and their cost-effectiveness needs to be evaluated further. Both the control growth rates evident during this trial (k = 0.009 d^{-1}) and the average levels of chlorophyll *a* (0.55 µg L^{-1}) in inflowing water, were significantly lower that in the previous 11 trials (k = 0.028 to 0.070 d^{-1}; chl *a* = 0.63 to 2.9 µg L^{-1}). Therefore, the diets were acting more as "complete" diets, than "supplementary" diets and they may be even more effective under normal ambient (i.e. higher) concentrations of chlorophyll *a*. Also, other dried products are also available from these companies ($30 to $40 kg^{-1}), and these may also warrant evaluation.

Cost effectiveness of feeding and extension to the commercial nursery

Though some of the live diets differed in their performance, their cell division rates (i.e. production cost) may be a more important factor in determining their overall cost-effectiveness. *R. salina*, *Ch. calcitrans*, *Isochrysis* sp. (T.ISO) and *D. tertiolecta* all were excellent diets and outperformed *P. pinguis*. However, the cell division rates of *Isochrysis* sp. (T.ISO) and *D. tertiolecta* were 20-30% faster than *R. salina*, and would therefore be more cost-effective. *Ch. calcitrans* had equivalent growth rates when grown in carboys to *Isochrysis* sp. (T.ISO) and *D. tertiolecta* grown in bags – but *Ch. calcitrans* is not suited to growth in bags, which is the most common microalgal technology currently used within local hatcheries.

Preliminary cost/benefit assessment of supplementary feeding was undertaken. In general terms, this will depend on the balance between costs of producing microalgae, or for other alternate diets, and savings in labour costs from a reduction in the nursery phase. The growth rate of oysters fed the natural diet (i.e. control) is a major influence, and at Pipe Clay Lagoon it varied seasonally and affected supplementary feeding. For example, the production season of 1996/97 (see trials 12-15 and data from the monitoring program) was a sustained period of low growth rates for control oysters. We ran two trials (12 and 13) in succession during this time to determine benefits of longer-term supplementary feeding. Based on a microalgal production costs of A$ 375 (eg. "average" commercial hatchery; Coutteau and Sorgeloos, 1992) the direct additional feed costs would approximate $0.35 per

* The relative effectiveness of these commercial products (to each other, and to microalgae) may change under different concentrations and composition of ambient inflowing phytoplankton, different feeding rations, methods of feed presentation and other culture variables.

thousand oysters for growth from 0.5 to 3.0 mm. This compares to an "average" production cost of ≈ $15 per thousand oysters of 5 mm size (M. John, Shellfish Culture, pers. comm.) – though during this period of low growth the "effective" production costs for control oysters would have been significantly greater. Almost certainly, supplementary feeding was cost-effective in increasing production during this time. During periods when control growth rates and/or background phytoplankton concentrations are already high, the cost-effectiveness of supplementary feeding may be equivocal. Nevertheless, supplementary feeding may be an important strategy even during these periods, to increase production to meet seasonal demands by farmers (i.e. continuity of supply).

Based on the findings of this research, together with their own commercial-scale R and D and economic assessment, Shellfish Culture are undergoing a major development of infrastructure at the Pipe Clay site. This has involved the building of a new nursery, with double the upweller capacity. To support the oyster production, Shellfish Culture will now be adopting supplementary feeding as routine. As an extension of the current project, we propose to continue collaboration with Shellfish Culture to assess the most cost-effective method to supply mass-cultured microalgae to their nursery at a commercial level. We estimate that up to 20,000 L of microalgae will be required per day to meet future production targets of the nursery. To meet this need for microalgae, potential culture systems include the "Bayes" system of continuous culture (see Fish Farming Int., Feb 1996, pp. 44-45), batch or semi-continuous systems of open tanks (Donaldson, 1991), or variations of the two. The Bayes system is based on units of 40 x 500 L bags and suitable for production of 4,000 L per day – suitable for hatcheries/nurseries of moderate size, but below production required at the Pipe Clay site. We plan to investigate a series of indoor and outdoor tanks (1,000 to 20,000 L) and outdoor ponds (up to 200,000 L).

In general terms, cheaper production of microalgal biomass would make supplementary (or complete) feeding of spat more attractive. Artificial illumination is a major cost in growing microalgae using the standard hatchery methods of batch and semi-continuous cultures within tanks and bags. Moreover, as cell concentrations increase in tanks and bags, light penetration becomes rapidly attenuated due to self-shading and cultures can quickly become light-limited. This reduces the production rate, and effectively increases the production cost. Over the last decade or so, new technologies have been developed that are capable of significantly reducing algal production costs. Photobioreactors are algal culture systems comprising of coils or tubing, with a corresponding high surface area to volume ratio. In these systems, light limitation is less of a problem, and productivity is significantly greater than conventional tank and bag cultures. Therefore, production costs are significantly lower; eg. < A$ 20 depending on species (Borowitzka, 1992). Some microalgae are capable of heterotrophic growth (i.e. by utilising an added carbon source in the absence of light) using fermentation technology; costs are less than $30 kg^{-1}. With improvements in growth conditions, costs could drop below $2 kg^{-1}. (Gladue, 1991). Both procedures require a significant capital investment, which may make their adoption uneconomical to many hatcheries. Alternatively, these products could be produced from a central location and sold as wet pastes or dried products to hatcheries or nurseries.

Other aspects influencing juvenile oyster growth rates at Pipe Clay Lagoon

Increases in oyster growth were achieved by reducing their stocking volume. Trial 11 demonstrated that by halving the "standard" stocking volume (20 mL for experimental upwellers), growth rates were increased by 45%. This approach could be useful, but has limitations. A reduction in stocking volume would correspondingly increase the labour associated with the daily cleaning or "servicing" of the additional upwellers. During peak season, the Pipe Clay Lagoon nursery is usually operating at full capacity and there is little scope for increasing the number of upwellers. However, a reduction in stocking volume might be a cost-effective method for increasing growth during the low season (late autumn to spring) when upweller space is available, growth rates are generally lower, and staff have more time available for servicing the upwellers.

We did not establish a general relationship between water temperature and the growth of control oysters (or efficacy of supplementary feeding). Other studies have examined the relationship between temperature and oyster growth. In juvenile *C. gigas* maintained in flow-through seawater, Malouf and

Breese (1977) found no growth advantage to temperature greater than 15°C. During periods of low food availability, they found an inverse relationship between growth and temperature, from 10°C to 23°C. In more controlled laboratory experiments, Buxton et al (1981) found the "scope-for-growth" (and assimilation efficiency) of *Ostrea edulis* was greatest at 15°C to 20°C, but rapidly diminished below 10°C as food ingestion also reduced. Bougrier et al. (1995) assessed clearance rates of *C. gigas* from 5°C to 32°C, and found maximum rates at 19°C.

In trial 5 when average temperatures ranges from 9°C to 12°C, we found supplementary feeding was ineffective. Two approaches could be used to enhance growth rates at Pipe Clay Lagoon during late autumn to spring when ambient water temperatures are below this level: a) incorporate engineering systems (e.g. heat exchangers) to heat the water flowing into the nursery or b) recirculate sea-water, enriched in supplementary feed, through the upwellers. The former method may be impractical because of the costs associated with heating the large volumes of flowing seawater – up to 1.5 kL of seawater min^{-1} is pumped through the Pipe Clay Lagoon nursery. The latter approach – which is effectively more "complete" feeding rather than supplementary feeding – is feasible; oysters can be grown to market size (Epifanio and Mootz, 1976), though it's cost-effectiveness would need to be established at the Pipe Clay Lagoon site.

We investigated various methods of "presenting" the supplementary feed. We found that a single "passage" of feed through the oyster bed at standard flow rates was better than recirculation of feed (with some water exchange) or a reduced flow rate. Most likely, better growth rates were achieved with the former method because it provided a more efficient removal of waste products within the oyster bed and environment – which otherwise may have reduced growth. In early trials, we established that discontinuous feeding (1 or 2 ration d^{-1}; each ration over 1-2 h period) was as effective as continuous feeding. However at double the food concentration, we found that oysters reached satiation after delivering 1/2 to 3/4 of the ration with the discontinuous feeding methods (as determined by chlorophyll *a* concentrations entering and exiting upwellers; data not shown). Thereafter, continuous feeding was chosen to deliver supplementary feed in feeding trials (where feasible, given experimental constraints).

Effect of site on juvenile oyster growth rates, and extension of results to other sites

The growth rates of control oysters at Little Swanport and Pipe Clay Lagoon were compared in February and April 1997. The growth at Little Swanport during February 1997 was significantly greater than that ever achieved at Pipe Clay Lagoon – even with supplementary feeding. During these trials, levels of phytoplankton (based on chlorophyll *a*) were 3–8 times higher at Little Swanport. Higher levels of dissolved organic carbon (DOC) may also have contributed to higher growth rates at Little Swanport; oysters and other filter feeders are able to utilise small organic molecules such as amino acids and organic acids present within this fraction (Manahan and Stephens, 1983). Though we only compared the growth rates of control oysters at the two sites twice, anecdotal information from industry suggests that (in general) growth is significantly greater at the Little Swanport site. Again, higher food concentrations and water temperatures, as shown from the monitoring program data, undoubtedly account for this. Because of the higher control oyster growth rates, supplementary feeding was not investigated at Little Swanport. Supplementary feeding at this site may only be useful, and cost-effective, if sustained periods of poor growth are identified and associated with a decline in food concentrations in the water. Despite the higher productivity of Little Swanport, salinities can drop dramatically during periods of high rainfall making it unsuitable for oyster cultivation for several weeks of a year (Martin John, pers. comm.). During periods of low salinity, oyster may cease feeding (even if ambient food concentration are high) or growth may be depressed because of the metabolic cost associated with maintaining osmotic balance (Brown, 1988).

Though our results suggested supplementary feeding may not be useful at Little Swanport, the technology could be adopted in other regions with similar background phytoplankton concentrations to Pipe Clay Lagoon. Crawford and Mitchell (1998) examined site and seasonal variation of environmental factors in 5 oyster grow-out regions of Tasmania – i.e. Pipe Clay Lagoon, Little Swanport, Pittwater, Georges Bay and Simpsons Bay – from 1991 to 1994. Average chlorophyll *a*

concentrations during their survey were only slightly lower at Pipe Clay Lagoon (2.4 μg L^{-1}) than the other sites (2.8 to 2.9 μg L^{-1}). This suggests that supplementary feeding could be effective at other sites within Tasmania, particularly when their ambient phytoplankton concentrations are reduced.

Data from the monitoring program of water quality

Multiple regression of data from the monitoring program failed to demonstrate a relationship between growth rates of oysters and nutrient or physical factors. Below average oyster growth rates from January to June 1997 were associated however with lower chlorophyll *a* concentrations, which was corroborated by data from the supplementary feeding trials.

In a broader study assessing 10 sites over 14 months, Brown (1988) correlated environmental factors with seasonal and site-related growth variation in Pacific oysters. He found from multiple regression analysis that food supply (particulate organic matter, phytoplankton), temperature, and to a lesser degree, salinity were the major factors affecting monthly growth rate. Also, chlorophyll *b* concentrations were more strongly correlated to oyster growth than chlorophyll *a*, which was attributed to the high concentrations of microalgae belonging to the Chlorophyceae and Euglenophyceae classes.

At the Pipe Clay Lagoon and Little Swanport sites, concentrations of chlorophyll *a* in the <20 μm were approximately 75% of that within the <250 μm fraction. We found a similar value for the fractionation of POM (data not shown). Algae from 2 to 30-40 μm may be assimilated by juvenile *Crassostrea* (De Pauw, 1981), though particles >10 μm may be cleared less efficiently that 5-10 μm-sized particles (Jørgensen, 1996). Based on the above considerations, we assumed that the bulk of the available food for small spat would be contained within the <20 μm seawater fraction – hence most of the seawater analysis (TPM, POM, chlorophyll a, fatty acids and pigments) were undertaken from this fraction.

Concentration of chlorophyll *a* at Pipe Clay Lagoon were similar to those found during a 4-yr survey from 1985-89 in nearby Storm Bay (Harris et al., 1991). In a later study, concentrations of chlorophyll *a* reported by Crawford and Mitchell (1998) at Little Swanport (2.9 μg L^{-1} for <500 μm fraction) between 1991-93 were similar to those observed during our monitoring from 1992 to 1997 (2.7 μg L^{-1} in the <250 μm fraction); though we found average concentrations at Pipe Clay to be approximately half those observed by these authors. Some of the difference between these two data sets from Pipe Clay Lagoon could be attributed to within-site variation of concentrations between sampling stations, which Crawford and Mitchell (1998) found to be significant, or differences in methodology. Despite the lower phytoplankton concentrations at the Pipe Clay Lagoon site, it was the most productive for on-growing oysters, which was attributed (in part) to the site's rapid flushing rate (Crawford and Mitchell, 1998). In another related study, nutrient and chlorophyll *a* showed high spatial and seasonal variability at six stations associated with mussel farming in Pelorus Sound, New Zealand (Gibbs et al., 1992).

Similarly, site and temporal variation, and methodology could account for differences between our POM concentrations (averages of 0.8 mg L^{-1} at Pipeclay Lagoon and 1.5 mg L^{-1} at Little Swanport, in the <20 μm fraction) and those found by Mitchell (pers. comm.) during 1997-98 (3.4 mg L^{-1} and 2.8 mg L^{-1} respectively, at the aforementioned sites, in <500 μm fraction). POM values reported by us are not unusually low however, and are comparable (or greater) than values reported by Gibbs et al. (1992) in Pelorus Sound.

Data from the monitoring program characterised the water quality parameters of the two sites and their seasonal and interannual changes. In broad terms, Little Swanport was characterised by greater food availability (higher concentrations of chlorophyll *a*, POM and DOC). There were also differences in the food quality (phytoplankton species composition) between the sites, as evidenced by differences in pigment profiles of water samples. Chlorophyll *a* concentrations at Pipe Clay Lagoon were correlated with concentrations of nitrate and phosphate, and were highest from winter to early spring. Gibbs et al. (1992) also noted seasonal variation in chlorophyll *a* in Pelorus Sound; highest concentrations

occurred during summer for the inner stations, and winter for the outer stations. A reduced grazing pressure of grow-out oysters in the Pipeclay Lagoon (1995 production of 8 million oysters; Crawford and Mitchell, 1998) during winter) may have in part contributed to the winter peak in phytoplankton concentrations at that site.

Summary

In conclusion, the project demonstrated significant increases in growth rates of juvenile oysters at the Pipe Clay Lagoon site through supplementary feeding. On average, growth rates increased by 60% supplementary feeding, though in several trials there were at least 6-fold increases. Though some of the diets differed in their effectiveness, important findings were that algal pastes and low-PUFA diets were as effective as PUFA-rich microalgal diets. Two dried diets were also effective; though not as effective as live diets. Supplementary feeding appears warranted at Pipe Clay Lagoon to maintain continuity of seed supply, as there were periods throughout the project where growth rates where otherwise poor because of a lack of food. A more detailed examination of the cost-effectiveness of supplementary feeding is needed under commercial conditions. Nevertheless, the technology was successfully transferred to industry, as Shellfish Culture plan to routinely use supplementary feeding at the site. Reduction in the production costs of microalgae, through the adoption of new culture technology, could make supplementary feeding even more attractive.

8. BENEFITS

The research developed methods for increasing the production of juvenile Pacific oysters at the Pipe Clay Lagoon site, when growth rates of oysters were otherwise low because of an inadequate food supply. Shellfish Culture Ltd, the major seed producer in Tasmania, will therefore be the primary beneficiary of the research. As a result of this study - and Shellfish Culture's own commercial-scale R and D and economic assessment - they now plan to use supplementary feeding at their Pipe Clay Lagoon nursery. A fuller assessment of the economic benefits will only be evident after adoption of supplementary feeding for one or several production seasons. Nevertheless, supplementary feeding will allow them to reduce their production cost (e.g. a 10-20% reduction could give a saving of up to $100 K p.a.) and/or increase their overall seed production.

Methods developed and used within this project could also have a similar benefit to other shellfish nursery sites – particular those characterised by low or fluctuating food availability and spat growth.

Benefits of the research will flow on to the Pacific oyster on-growers (farmers). The industry is valued at ≈$20m p.a, and, with the recent allocation of new lease sites in Tasmania by the state government (more than doubling the existing area), is set to rapidly expand in the next decade. At present nearly all product is sold on the domestic market. With an expansion of oyster production, the industry is well positioned to develop a high-value export market (particularly to SE Asia), by taking advantage of its product quality and the "clean-green" image that the industry has achieved. Any future expansion depends on a corresponding increased juvenile oyster or "seed" production (valued at $2-3 M p.a.). Extension of our research to Shellfish Culture and other seed producers will contribute to this.

9. INTELLECTUAL PROPERTY

Intellectual property generated from the research includes:

- The nutritional value of microalgae, algal pastes and dried diets as supplementary food for oysters
- Methods used for delivering supplementary diets most effectively to oysters
- Data from the weekly monitoring of water samples from Pipe Clay Lagoon and Little Swanport

This information is fully contained in this report and will be therefore be available to producers of mollusc seed throughout Australia. Results of the research will also be published as scientific manuscripts (eg. Appendices i and x).

10. FURTHER DEVELOPMENT

On the basis of our results with experimental-scale supplementary feeding, Shellfish Culture and CSIRO plan further collaboration to extend the results to commercial-scale feeding of juvenile oysters and scallops. A major factor for this extension will be the development of cost-effective mass algal culture systems capable of 20,000 L per day production at Shellfish Culture's newly developed Pipe Clay Lagoon site. We plan to submit a proposal to the FRDC to undertake this extension.

In general, adoption of newer technologies for mass-culturing microalgae (i.e. photobioreactors, heterotrophic culture) by either:

a) commercial nurseries (eg. Shellfish Culture) and/or
b) a central, specialised facility, supplying microalgal biomass (as paste or other product) to nurseries at a cost,

would make supplementary feeding more attractive economically. CSIRO is currently undertaking research at the laboratory scale to isolate and identify microalgae that are capable of low cost and rapid growth using these technologies. Once we have identified suitable strains, we will investigate their mass culture (collaboration with other CSIRO Divisions or industry partners) and then their use as feed for aquaculture – including supplementary food for juvenile oysters.

11. STAFF ON PROJECT

From CSIRO Division of Marine Research:

Malcolm Brown, 40%

Malcolm McCausland, 100%

Graeme Dunstan 2%

Shirley Jeffrey 2%

The assistance of work-experience students (each providing ≈ 1-2 weeks) is also acknowledged: David Lecossois, Kris Kowalski, Greg Smith, and Dawn Ring.

From Shellfish Culture Ltd:

Martin John, Greg Hollingsworth, 5% total

Technical officers, 5% total

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Evaluation of live and pasted microalgae as supplementary food for juvenile Pacific oysters (Crassostrea gigas)

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Abstract

Supplementary feeding was an effective method of enhancing growth of juvenile Pacific oysters (Crassostrea gigas) at a nursery site with low to moderate levels of natural phytoplankton (chlorophyll a, 0.68 ± 0.29 μg l^{-1}). Over five experiments, oysters (500 to 700 μm) in upwellers were fed supplementary rations of live or pasted microalgal diets. Supplementary diets were added to naturally occurring seston at a ration of 7 mg $d^{-1}ml^{-1}$oysters (initial oyster bed volume). These levels increased the total phytoplankton concentration by 50 to 207 %. Variation in the environmental conditions between experiments influenced oyster growth-rates. Increases in oyster growth-rates were high in animals supplemented with Chaetoceros calcitrans (instantaneous growth-rate, k = 0.062 d^{-1}), Dunaliella tertiolecta (k = 0.059 d^{-1}), Isochrysis sp. (T. ISO) (k = 0.059 d^{-1}), an Australian isolate of Rhodomonas salina (k = 0.074 d^{-1}) and pasted Skeletonema costatum (k = 0.064 d^{-1}) and Chaetoceros calcitrans (k = 0.059 d^{-1}). These rates were significantly greater than in the oysters fed a reference supplementary diet, Pavlova pinguis (range for experiments; k = 0.049 to 0.066 d^{-1}) and in the control (i.e. non-supplementary fed) oysters (range for experiments, k = 0.034 to 0.043 d^{-1}). Pavlova pinguis was a more effective diet when it was grown under a 24:0 h L:D (k = 0.066 d^{-1}) than when grown under a 12:12 h L:D regime (k =0.061 d^{-1}). Pasted (4 to 14 d old) C. calcitrans (k = 0.059 d^{-1}) gave similar growth to live C. calcitrans (k = 0.062 d^{-1}), which indicated the promise of pastes as an off-the-shelf alternative to live diets. The effectiveness of D. tertiolecta – a diet lacking the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) – indicated that the oysters received sufficient quantities in background phytoplankton to sustain a high growth-rate. The essential nature of these fatty acids was demonstrated when all oysters retained higher percentages of 20:5(n-3) and 22:6(n-3) than the total fatty acids. Initial cost estimates for supplementary feeding show that it will add 2 to 3% to juvenile oyster production costs during the nursery phase when oysters are 0.5 to ~3 mm in size.

Keywords: Bivalve; Nutrition; Pavlova pinguis; Phytoplankton; Polyunsaturated fatty acid; Rhodomonas salina; Algal pastes

1. Introduction

Pacific oyster (Crassostrea gigas) production in many countries relies on hatcheries and nurseries to rear larvae and juveniles (Chew, 1990; Dix, 1991; Donaldson, 1991). Production systems are similar throughout the industry. Larvae and newly settled juveniles are grown in land-based hatcheries on a nutritious diet of cultured microalgae, most commonly Isochrysis sp. (strain T. ISO), Chaetoceros calcitrans, Pavlova lutheri and Thalassiosira pseudonana (Coutteau and Sorgeloos, 1992). Juveniles are typically on-grown from ~500 μm to 2-5 mm in tanks through which seawater is pumped continuously from an estuary or bay (Rodhouse et al., 1981). The oysters feed on the naturally occurring seston in the water. This method has low costs associated with the pumping of water (M. John, pers. comm.), but oyster growth at the nurseries varies with the changing concentrations and quality of food (principally phytoplankton) (Wilson, 1987; Brown, 1988). The changes in species composition and productivity are caused by variations in environmental factors and by competitive grazing from filter feeders in the waterway (Spencer and Gough, 1978; Alpine and Cloern, 1992; Gibbs et al., 1992).

Two approaches have been used to increase phytoplankton concentrations in nurseries to improve oyster growth. In the first, large ponds or tanks of seawater are fertilised to stimulate natural phytoplankton blooms for use in recirculating or semi-recirculating nursery systems. This has had varying degrees of success. Juvenile Ostrea edulis growth-rates either showed no response or increased up to 130%,compared to a uni-algal control diet, depending on the composition of the induced bloom (Rodhouse et al., 1983). Across five experiments, juvenile C. gigas and O. edulis receiving fertilised seawater were an average 60 % heavier

after six weeks than oysters receiving unfertilised sewater (Spencer et al., 1986). This would have been higher, however one experiment showed inferior growth and low survival in oysters fed in the fertilised seawater (Spencer et al., 1986).

In the second approach, the naturally occurring seston is supplemented at the nursery with algal monocultures. The cost of food production is higher than the first approach; however, it provides greater control over the oysters' diet (and consequently their growth-rates and production) and as a result may be more cost-effective.

The nutritional qualities of the microalgal species have been measured when used in enclosed, single- and multi-species feeding experiments (Flaak and Epifanio, 1978; Enright et al., 1986a, b; O'Connor et al., 1992). However, when used as a supplementary food source the nutritional qualities of the total diet will differ. Hence, the purpose of this study was to assess algal species as supplementary diets for juvenile Pacific oysters at a major nursery site in Australia. Diets were chosen from microalgae in different taxonomic classes (i.e. diatoms, prymnesiophytes, a cryptophyte and a chlorophyte) with different biochemical compositions, including polyunsaturated fatty acids (PUFAs). The chosen algae were; the common aquaculture strains Dunaliella tertiolecta, Chaetoceros calcitrans, Isochrysis sp. (strain T. ISO); new Australian strains Pavlova pinguis, Pavlova sp. and Rhodomonas salina; and algal pastes of C. calcitrans and Skeletonema costatum (Table 1). The effectiveness of the diets was compared and reasons for differences in nutritional value sought.

2. Materials and methods

2.1. Oyster culture systems

Hatchery-reared juvenile Pacific oysters (Crassostrea gigas) were used for feeding experiments at a major oyster nursery at Pipe Clay Lagoon, 30 km southeast of Hobart,

Australia (42° 58' S, 147° 32' E). The juveniles (500 to 700 μm) were grown in upweller systems, 1/20th the scale of commercially used upwellers. Each upweller consisted of a 10 l bucket with a 110 mm diameter, mesh-bottomed upweller chamber suspended inside, on which the oysters were retained. Unfiltered seawater from Pipe Clay Lagoon (salinity 32 to 35 ppt) was pumped continuously into the upwellers at 700 ml min^{-1} (1000 l d^{-1}). It flowed through the upweller chamber and the bed of oysters and out through an exit pipe. Oysters and chambers were cleaned daily with a fine spray of freshwater to remove faeces and other particles. Chambers were also given a weekly rinse with 1% sodium hypochlorite.

2.2. Feeding experiments

Five feeding experiments (2 preliminary and 3 main experiments) of either 17 or 18 d were completed over the 1995-6 nursery season (Table 2). Control oysters in all experiments fed on naturally occurring seston provided by the continuously flowing seawater. The experiments tested six species of live cultured algae, a two-species mix and two algal pastes as food supplements, added to the flowing seawater.

Preliminary feeding experiments 1 and 2 (P1 and P2) enabled us to identify a local isolate to use as a reference supplementary diet in all subsequent experiments and to determine suitable illumination. Australian species of the genus Pavlova (Pavlova sp. and Pavlova pinguis)were tested in P1 because the biochemical composition of its members contain high levels of essential PUFAs (Brown et al., 1997). P2 compared the use of P. pinguis grown under light regimes of 12:12 h L:D and 24:0 h L:D.

Experiment 1 (E1) tested Australian isolates of Rhodomonas salina and P. pinguis, chosen from P1, and a 1:1 mixed diet. Experiment 2 (E2) tested algal pastes of Chaetoceros calcitrans and Skeletonema costatum and fresh cultures of C. calcitrans and P.pinguis. In experiment 3 (E3), we tested Isochrysis sp. (T ISO), Dunaliella tertiolecta and P. pinguis,

which have different composition of essential polyunsaturated fatty acids (PUFAs) (Volkman et al., 1989, 1991). The content of PUFAs in juvenile oysters reflects their diet (Langdon and Waldock, 1981); a comparison of fatty acids (FAs) in the diets and the oysters was made to determine uptake and assimilation and give some insight into the oysters' FA requirements during supplementary feeding.

For all but one one experiment, 19 ml (~ 25 g wet weight; ~ 40 000 oysters) of oysters was placed into each upweller; the exception was P2, where 13 ml was used because of a shortage of oysters at the nursery (Table 2). The oysters were acclimated in flowing seawater overnight before supplementary feeding began the next day. They were grown under photoperiods that ranged from 6:18 h to 8:16 h light:dark (L:D). Position effects were minimised by randomly changing the positions of upweller buckets 3 to 4 times during each experiment.

Supplementary feed was added at a ration based on dry weight (DW) (Table 2) to the upwellers twice each day (at midday and midnight) over 2 h. No supplementary feed was added at weekends. The cultured algae (0.3 to 5 l) were dispensed into buckets and diluted to 9 l with seawater. The suspension was pumped into upwellers at 75 ml min^{-1} with small submersible aquarium pumps (Aquarium Powerhead 480, Second Nature, NJ, USA). For E2, pastes were resuspended in 2 l of seawater by mixing for 30 s with a hand-held blender. Rations (Table 2) of the resuspended pastes were also diluted to 9 l with seawater and added to upwellers in the same way as the live algae.

Oyster mortality was estimated from three counts (each of 200 individuals) at the start and end of each experiment. Their growth-rates were determined from measurements made at the start and finish of each experiment measured by three different methods: (a) volumetric (the packed volume of the oyster population within each upweller); (b) DW (a subsample of 200 oysters from each upweller was rinsed with distilled water, dried at 100°C for 72 h, then

weighed); (c) organic weight (OW) (the subsample of 200 oysters used for DW analysis was heated in a muffle furnace (450°C; 24 h), then reweighed to determine the OW by weight loss). In each of these methods the formula used to determine the instantaneous growth-rate (k) in d^{-1} was:

$$k = \ln (M_t / M_0) / t \quad (1)$$

where, ln = natural logarithm (base 2.718), M_t = measurement at day t, M_0 = measurement at day zero.

Apparent growth efficiency (%AGE) is a measure of the efficiency with which food was used and was determined for each diet by the equation:

$$\%AGE = [(\Delta OW_{sf} - \Delta OW_c) / DW_{feed}] \times 100 \quad (2)$$

where ΔOW_{sf} was the increase in OW of supplement-fed oysters, ΔOW_c was the increase in the OW of control oysters, and DW_{feed} was the dry weight of supplementary food. The OW of a known volume (1 to 4 ml) of oysters was measured at the start and end of an experiment to calculate the OW per upweller. This was used to calculate ΔOW_{sf} and ΔOW_c.

In E3 we compared changes in the fatty acid content per oyster with the total fatty acid presented or fed. The retention of fatty acids was calculated as:

$$\% \text{ retention} = FA_{incr} / FA_{diet} \times 100 \quad (3)$$

where FA_{incr} was the increase in fatty acid per oyster and FA_{diet} was the fatty acid available in the diet per oyster.

2.3. Algal cultures

The microalgae were obtained from the CSIRO Collection of Living Microalgae (Table 1). They were cultured in medium f/2 (Guillard and Ryther, 1962), except R. salina, which was cultured in medium f_E (Jeffrey, 1980). Starter cultures (150 ml in mid- to late-

logarithmic phase) were inoculated into 1.4 l of seawater enriched with nutrients in 2 l Erlenmeyer flasks. The flasks were illuminated with white fluorescent light (Philips daylight tubes) at 100 µmol photons m^{-2} s^{-1} at 20 ± 2°C. The cultures were bubbled with air enriched with 0.5 to 1% CO_2 at a flow-rate of 0.4 l min^{-1}. They received different light regimen according to the experiment: 12:12 h L:D, 18:6 h L:D or 24:0 h L:D (Table 2). At mid- to late-logarithmic phase, the flask contents were transferred to 10 l polycarbonate carboys containing 8 l of seawater enriched with nutrients and grown-on under identical conditions. At mid- to late-logarithmic phase, the carboy contents (except C. calcitrans) were transferred to sterile polyethylene bags containing 85 l of 0.2 µm filtered seawater enriched with nutrients. In the bags, the cultures were grown semi-continuously under the same temperature and photoperiods as before; however the light intensity was 50 to 75 µmol photons m^{-2} s^{-1} for continuously illuminated cultures and 100 to 150 µmol photons m^{-2} s^{-1} for 12:12 h L:D cultures. The cultures were mixed with air enriched with 1 to 2% CO_2, at a flow rate of 20 l min^{-1}, maintaining pH between 7.0 and 7.5. Chaetoceros calcitrans carboy cultures were transferred into glass carboys (as this species grows better in glass than polyethylene) containing 20 l of 0.2 µm filtered seawater enriched with nutrients, and grown in otherwise identical conditions. Replicate (3 to 6) cultures (bags or carboys) of each species were used throughout the experiments.

2.4. Algal pastes

Algal pastes were supplied by New South Wales Fisheries, Port Stephens Research Centre. Skeletonema costatum and C. calcitrans were grown in f/2 medium in 1000 l tubs at 23 ± 1°C and aerated with CO_2-enriched air. Skeletonema costatum was grown under 24:0 h L:D illumination and C. calcitrans under 18:6 h L:D. The algae were harvested at late-logarithmic phase by super-centrifuging in a Sharples (Type MV-35-32Y-22KY-32)

centrifuge modified to channel the effluent through one outlet and to reduce shear forces during concentration at 13000 g. The pastes were collected, transferred to 250 ml polycarbonate sample jars and dispatched in an insulated container with ice-packs by overnight courier to the CSIRO Hobart laboratory. The pastes were refrigerated at 4°C upon arrival. The pastes were received 3 d before the start of E2 and again 1 week later, so that the pastes used within the experiment were between 4 and 14 d old (average age, 8 d) on any feeding day.

2.5. Biochemical analysis of microalgae and oysters

Aliquots (1 l) of the microalgal cultures were taken every 2 to 4 d for biochemical analyses. For carbohydrate and protein determination, subsamples (10 to 80 ml) were filtered through 25 mm glass-fibre filters (Whatman GF/C). For total lipid determination, subsamples (150 to 600 ml) were filtered through 47 mm glass-fibre filters (Whatman GF/C). All filters were stored at -20°C and analysed within 6 months. For fatty acid analysis, subsamples (80 to 300 ml) were filtered through 47 mm filters and stored in liquid nitrogen until analysed. Subsamples for dry and ash weight (50 to 400 ml) were filtered through precombusted (450°C; 24 h), preweighed, 47 mm filters. For dry weight, filters were washed with 30 ml of 0.5 M ammonium formate to remove residual salts, dried at 80° C overnight and reweighed. Ash weight was determined by reheating the filters in a muffle furnace (450°C; 24 h) and reweighing. Pastes (0.21 to 0.42 g) were resuspended in 200 ml of 1 µm-filtered seawater, and subsamples filtered for later analysis of carbohydrate and protein (5 ml), lipid (80 to 100 ml), dry and ash weight (35 to 60 ml) in the same way.

Carbohydrate was analysed after the filters were hydrolysed with 3.9 ml of 0.5 M H_2SO_4 at 100°C for 4 h in polypropylene centrifuge tubes by the phenol-sulphuric acid method (Dubois et al., 1956). Protein was analysed after homogenising filters with 6% TCA,

with a modified Lowry et al. (1951) technique (Clayton et al., 1988). Lipid was determined gravimetrically (Whyte, 1987). Filters were repeatedly extracted in chloroform-methanol-water (2:4:1; 7 to 8 × 5 ml). The supernatants were combined, and chloroform and water added to bring the ratio to 1:1:0.9 for phase separation and extraction of lipids in the lower chloroform phase. The chloroform layer was concentrated under vacuum and weighed to determine total lipid.

For fatty acid analysis, samples of microalgae were extracted overnight with chloroform-methanol-water (1:2:0.8, v/v/v) (Bligh and Dyer, 1959). Chloroform and water were added to bring the ratio to 1:1:0.9 for phase separation and extraction of lipids in the lower chloroform phase. The lipid extracts were saponified and the liberated fatty acids were acidified and extracted (Volkman et al., 1989). Fatty acids were transesterified to methyl esters and analysed on GC polar and non-polar capillary columns and GC-mass spectrometry. At the end of E3 the oysters were collected, ground with a mortar and pestle, freeze-dried and analysed for fatty acids in the same way.

2.6. Analysis of seawater flowing through upwellers

The minimum and maximum temperatures of the seawater flowing into the upwellers were recorded daily. Water samples (filtered through a 20 μm nylon screen), taken weekly from Pipe Clay Lagoon. They were analysed for salinity and also multiple samples filtered through 47 mm filters (1.5 to 4 l) for analysis of chlorophyll a, total particulate matter (TPM) and particulate organic matter (POM) and fatty acids. Filter samples for fatty acids were stored and analysed as previously described; filter samples for chlorophyll a analysis were stored at -20° C for 1 to 2 months and later extracted in 90% acetone (Jeffrey and Humphrey, 1975). The concentration of chlorophyll a was determined spectrophotometrically using Jeffrey and Humphrey's (1975) equation 4. TPM filters were washed with 30 ml of 0.5 M

ammonium formate to remove residual salts, dried overnight at 80° C, and reweighed to determine the mass of the TPM. The mass of POM was measured by heating these filters in a muffle furnace (450°C, 24 h) and then reweighing them.

2.7. Statistical analysis

The different measures (volumetric, dry weight, organic weight) of oyster instantaneous growth-rate (k) were compared by regression analysis. Growth-rates from individual experiments were compared by analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) for pairwise comparisons where the F statistic was significant ($P < 0.05$). For E1, E2 and E3, ANOVA was used for between-experiment comparison of the instantaneous growth-rates of control oysters and those fed the reference P. pinguis supplement (common to all experiments), to test for experiment × treatment interactions. Such an interaction was found ($P < 0.02$), as the difference between the growth-rate of P. pinguis- supplemented and unsupplemented control oysters in E3 was significantly lower than E1 and E2, indicating that the oysters did not respond as well to supplementary food in E3. As a result, further cross-experiment comparative analyses of growth were not performed.

Differences in the gross composition (lipid, carbohydrate, protein) of diets were examined by ANOVA, and pairwise comparisons of means tested by Fisher's PLSD. Linear regression of oyster growth increase against the gross composition of diets was performed to determine its influence in a supplementary diet. All statistical tests were at a significance level of $P < 0.05$.

3. Results

3.1. Assessment of oyster growth

Instantaneous growth-rates, calculated by volume of oysters, were linearly related to instantaneous growth-rates calculated by DW ($r^2 = 0.62$) and OW ($r^2 = 0.74$) (Fig. 1). The relationships are defined by the regression equations:

$$k_{DW} = 1.004\ k_{vol} - 0.005 \quad (4)$$

$$k_{OW} = 1.104\ k_{vol} - 0.001 \quad (5)$$

where k_{vol}, k_{DW}, and k_{OW} are the instantaneous growth-rates from measurements of oyster volume, dry weight and organic weight, respectively. k_{vol} is not only simpler to measure than k_{DW} and k_{OW} but also is not biased by sub-sampling (<1% of population measured for k_{DW} and k_{OW}). For these reasons, k_{vol} oyster growth-rate was adopted for comparing growth-rate data in all experiments.

3.2. Preliminary experiments (P1 and P2)

Supplementary diets tested in the preliminary experiments all produced significantly faster growth-rates than those of control oysters (Fig. 2). Oyster mortality was < 5% for all treatments. In P1, P. pinguis produced significantly better growth in oysters ($k = 0.058\ d^{-1}$) than Pavlova sp. ($k = 0.053\ d^{-1}$) (P1; Fig. 2a). In P2, P. pinguis produced better growth in oysters when it was grown under 24:0 h L:D regime ($k = 0.066\ d^{-1}$) than when grown under a 12:12 h L:D regime ($k = 0.061\ d^{-1}$) (P2; Fig. 2b). On the basis of these results and because the alga divides more rapidly at 24:0 h L:D, P. pinguis grown under 24:0 h L:D was selected as the reference supplementary diet for subsequent experiments.

3.3. Main experiments (E1 to E3)

Oysters fed supplements grew 44 to 87 % faster than control oysters (Fig. 3a-c). Their mortality was between 5 and 10%, with no significant differences between treatments. The growth-rates of oysters fed R. salina ($k = 0.074\ d^{-1}$) or the R. salina/P. pinguis mixture ($k = 0.071\ d^{-1}$) were not significantly different (E1; Fig 3a). However, the rates for both diets were significantly higher than for the P. pinguis reference ($k = 0.066\ d^{-1}$) (Fig. 3a). All treatment oysters grew significantly faster than control oysters ($k = 0.043\ d^{-1}$).

Algal pastes were effective supplementary diets (E2; Fig 3b). Oysters fed S. costatum paste ($k = 0.064\ d^{-1}$) grew significantly faster than oysters fed other diets, except for the C. calcitrans live culture ($k = 0.062\ d^{-1}$). Oysters fed C. calcitrans paste ($k = 0.059\ d^{-1}$) or culture showed no significant difference in their growth-rates. Oysters fed the P. pinguis reference had significantly lower growth-rates ($k = 0.055\ d^{-1}$) than the oysters in all other treatments. All treatment oysters grew significantly faster than control oysters ($k = 0.034\ d^{-1}$).

Isochrysis sp. (T. ISO) and D. tertiolecta were equally effective in enhancing oyster growth-rates (75 %, $k = 0.059\ d^{-1}$) and were more effective than P. pinguis ($k = 0.049\ d^{-1}$) (E3; Fig. 3c). All treatment oysters grew significantly faster than control oysters ($k = 0.034\ d^{-1}$).

The % apparent growth efficiency (%AGE) of the diets provided a measure of how efficiently the supplement-fed microalgae were converted to oyster (organic) biomass. Values ranged from 21% (P. pinguis, E3) to 67% (R. salina/P. pinguis mix, E1) and were directly correlated to the oyster growth-rates ($r^2 = 0.83$).

3.4. Control and supplement-fed oyster growth-rates and water quality

There were no statistically significant relationships between control oyster growth-rates and the water quality parameters (Table 3). However, water temperature may have influenced growth-rates as temperatures were higher during E1, and E1 control oysters ($k = 0.043\ d^{-1}$) grew better than E2 and E3 control oysters (each, $k = 0.034\ d^{-1}$). Similarly, the low

water temperature during E3 may have contributed to the slower growth of oysters in this experiment (< E1 and seasonal average), even though the concentrations of the nutritional indicators chlorophyll a, TPM, POM and 20:5(n-3) and 22:6(n-3) were high compared to E1 and E2.

Estimates of the DW of phytoplankton in the flowing seawater were made from chlorophyll a measurements based on published data for cultured microalgae (Brown, 1991) (chlorophyll a ≅ 1.1% DW of microalgae). The supplementary diets contributed an additional 50 to 207 % to the background phytoplankton DW in the incoming seawater (<20 μm) (Table 2). Growth-rates of P. pinguis-supplemented oysters across the experiments showed a similar pattern to the control oysters. Highest growth was in E1 ($k = 0.066\ d^{-1}$; 54% increase over control), followed by E2 ($k = 0.055\ d^{-1}$; 61% increase over control), and lowest growth was in E3 ($k = 0.049\ d^{-1}$; 44% increase over control) (Table 3). Supplementary feeding was significantly less effective in E3. This effect may have been related to the lower water temperature during this experiment.

3.5. Gross composition of the microalgae

The microalgae assessed as supplementary food were significantly different in their gross composition (Table 4). R. salina contained the most protein (59%). Pavlova pinguis, Isochrysis sp. (T. ISO) and D. tertiolecta had significantly more carbohydrate (28 to 31%) than C. calcitrans and S. costatum pastes and R. salina. Species did not differ significantly in lipid content ($P > 0.05$), ranging from 13% (P. pinguis during E3) to 24% (Isochrysis sp. (T. ISO)). Some of the species had significant differences in their ash content: the highest levels were in the diatom C. calcitrans culture and paste (22 and 23%) and the lowest in the flagellates R. salina, D. tertiolecta and Isochrysis sp. (T. ISO) (10 to 13%). The gross

composition of the microalgae did not correlate to their effectiveness as supplementary diets for oysters.

3.6. Fatty acid composition of microalgae and oysters from E3

Pavlova pinguis contained a high percentage of 20:5(n-3) (24.7% of total fatty acids) and 22:6(n-3) (7.5%) (Table 5). Isochrysis sp. (T. ISO) also had a high percentage of 22:6(n-3) (11.7%) but a low percentage of 20:5(n-3) (0.7%). Dunaliella. tertiolecta contained neither of these PUFAs. Other PUFAs contained in high percentages (i.e. > 10%) were 16:4(n-3) and 18:3(n-3) in D. tertiolecta, and 18:2(n-6) and 18:4(n-3) in Isochrysis sp. (T. ISO). All microalgae had higher percentages of total PUFAs than the control diet, which had predominantly saturated and monounsaturated fatty acids (71.6%).

Oysters fed Isochrysis sp. (T. ISO) or D. tertiolecta contained high percentages of 18:2(n-6) and 18:3(n-3) respectively, and a low percentage of 20:5(n-3). Oysters supplemented with D. tertiolecta were also low in 22:6(n-3) (Table 5). This reflected the composition of these fatty acids in these diets. P. pinguis-fed oysters did not show significant changes in composition. Concentrations of total fatty acids (μg mg^{-1} DW oyster) were about 1.5 times as great in oysters fed D. tertiolecta and Isochrysis sp. (T.ISO) (i.e. the fastest-growing oysters) than P. pinguis-fed or control oysters (Table 5).

Seston in the flowing, unfiltered seawater supplied an approximate total of 0.24 and 0.32 μg $oyster^{-1}$ of 22:6(n-3) and 20:5(n-3), respectively, during E3. This constituted ~2% each of the total fatty acids supplied in the (D. tertiolecta with seawater seston) diet (Table 6). Oysters fed D. tertiolecta received the least 20:5(n-3) and 22:6(n-3) and they showed the greatest retention of these acids (estimated at 43% for 20:5(n-3) and 58% for 22:6(n-3) of the amount presented to them). Retention of 20:5(n-3) and 22:6(n-3) in Isochrysis sp.

(T.ISO)-fed oysters was 26% and 19% respectively, and in P. pinguis-fed oysters 4% and 11%, respectively.

4. Discussion

The diets tested as supplementary food for juvenile Pacific oysters enhanced growth by 44 % (P. pinguis, P2 and E3) to 87 % (S. costatum paste). This indicated that food availability was otherwise limiting the growth-rates of non-supplemented oysters at the Pipe Clay Lagoon nursery.

The nutritional value of the strains tested has previously been assessed in single or mixed species diets in recirculation systems. Isochrysis sp. (T. ISO), R. salina, C. calcitrans, S. costatum and P. pinguis are of high nutritional value, in either single or multi-species diets for juvenile oysters (Enright et al., 1986a; Laing and Millican, 1986; O'Connor et al., 1992; Brown et al., 1998). Dunaliella tertiolecta is a poor single-species diet (Walne, 1970; Langdon and Waldock, 1981; Enright et al., 1986a; O'Connor et al., 1992) because of its lack of the essential fatty acids 20:5(n-3) and 22:6(n-3) (Volkman et al., 1989). However, it has previously been successful as part of a mixed diet with S. costatum (O'Connor et al., 1992) or other phytoplankton that provide sufficient amounts of the essential fatty acids 20:5(n-3) and 22:6(n-3). In general, our results of microalgal nutritional value from testing in a flow-through supplementary feeding system were similar to those reported above.

Mixed-species diets generally produce greater oyster growth than monospecific diets, (Romberger and Epifanio, 1981; Enright et al., 1986a; Laing and Millican, 1986; Tan Tiu et al., 1989). This has been attributed to these diets providing a balanced mix of essential nutrients (Webb and Chu, 1983). However, in our supplementary feeding system, there was no benefit in providing a mixed supplementary diet – the growth response of oysters fed a

mixture of R. salina and P. pinguis was intermediate between that gained using either species alone. In fact, the background natural phytoplankton and the supplementary food represent a mixed diet for all supplementary feeding treatments. The background phytoplankton probably provided some energy and contributed to the oysters' need for micronutrients, while the supplementary algae provided extra protein and energy to sustain additional growth.

Little nutritional loss occurred during the pasting, transportation and storage of the S. costatum and C. calcitrans algal pastes compared to live microalgal diets. Nell and O'Connor (1991) showed that pastes of these species were effective for Saccostrea commercialis larvae. However, their study and ours used algal pastes that were stored for less than 14 d before use. Further research is warranted to assess the applicability of pastes, particularly those older than 14 d.

Rhodomonas salina was the most effective supplementary diet of the new Australian isolates tested. Our previous work also found this isolate to be an excellent single-species diet for C. gigas juveniles (Brown et al., 1998). Similarly, other researchers have found isolates of R. salina (clone 3C, University of Delaware) (Laing and Millican, 1986) and members of this genus, Rhodomonas sp. (Enright et al., 1986a), to be of high nutritional value as single-species diets.

Juvenile oyster growth has been correlated with the percentage of protein (Flaak and Epifanio 1978; Utting 1986) and carbohydrate (Enright et al., 1986b) in uni- and multi-species diets. We found no (statistically significant) relationship between oyster growth and any of the gross composition parameters in the supplementary diet. However, as the composition and availability of protein, carbohydrate and lipid in the natural seston (not measured) may have fluctuated significantly, any relationship between supplementary diet composition and oyster growth may have been masked. Differences in the environmental parameters between

experiments may also have affected interactions between diet composition and growth response.

In E3, growth-rates and total fatty acid concentrations in the oysters fed D. tertiolecta or Isochrysis sp. (T. ISO) were substantially higher than P. pinguis -fed oysters (Table 5). This was most likely due to the higher total fatty acid content of D. tertiolecta and Isochrysis sp. (T. ISO). Similarly, Laing and Millican (1986) found that faster growing, healthier juvenile oysters contained greater lipid (and consequently fatty acid) reserves.

The fatty acid composition of C. gigas juveniles generally reflected that of their diet, as previous work has found both with C. gigas juveniles (Langdon and Waldock, 1981) and larvae (Waldock and Nascimento, 1979; Thompson and Harrison, 1992) and also with rock scallop (Crassadoma gigantea) larvae (Whyte et al., 1990). The composition of fatty acids in oysters was influenced by their initial composition, the composition of their diets (from supplementary food and seston) and their overall nutritional condition (Table 5). The supplementary microalgae had a significant effect on the final oyster fatty acid composition by contributing 52 to 61% of total particulate fatty acids presented to supplement-fed oysters.

Thompson et al. (1993) suggested that the essential PUFAs 20:5(n-3) and 22:6(n-3) are required at a low threshold level ($\leq$ 2% of total FAs for each) for C. gigas larvae, above which no significant improvement in growth is observed. Our work suggests that this may also be true for C. gigas juveniles, as the requirements of essential PUFAs for oysters fed D. tertiolecta (which lacks 20:5(n-3) and 22:6(n-3)) were met by the seston in the flowing unfiltered seawater (providing ~ 2% of FAs). The percentage of dietary 20:5(n-3) and 22:6(n-3) retained in all oysters (control and supplement-fed) was higher than the retention of total fatty acids and is suggestive of the greater need for these fatty acids (Langdon and Waldock, 1981; Chu and Webb, 1984; Whyte et al., 1990). The retention of 20:5(n-3) and 22:6(n-3) in control oysters was much lower than those fed D. tertiolecta, which received the

same quantity of these FAs. Extra energy reserves provided by supplementary D. tertiolecta is thought to have allowed a greater retention of these essential FAs for incorporation into membranes, whereas control oysters would probably have had a significant proportion of the PUFAs degraded by β-oxidation to satisfy their maintenance energy requirements (Whyte et al., 1990).

The apparent growth efficiency (%AGE) is a measure of the supplied supplementary food converted to organic biomass. Values of %AGE ranged from 21 to 67 % between feeding experiments and treatments. Maximum gross growth efficiency (%GGE or K_1) values have been reported as 22.6% for Crassostrea virginica (Urban et al., 1983) and 59% for Ostrea edulis (Beiras et al., 1994) juveniles. The %GGE is a common measure of growth efficiency, calculated as the ingested portion of food that is converted to organic biomass, and as a result gives higher values than the %AGE. Therefore, the efficiency with which supplementary food was used for growth was similar or higher than in previous work (Urban et al., 1983; Beiras et al, 1994) where single-species diets were used. The growth efficiency of control oysters (i.e. non supplementary-fed) for organic material from in-flowing seston was much lower, and ranged from 3 to 26%. The major reason responsible for this is the low "scope for growth" of control animals because of their lower dietary intake. Hence, a higher proportion of their feed (energy) intake is required for their basal metabolism (Warren and Davis, 1967). Also, a significant proportion of this organic material may have been of poor nutritional quality (e.g. detritus, yeast) or unavailable to the oysters because of its low digestibility and/or wrong size range.

To be of practical use, supplementary feeding must be cost-effective. Costs associated with the production of mass algal cultures include labour, heating and lighting, water filtration and the chemicals for enrichment media (Persoone and Claus, 1980) as well as large capital costs for establishing a large-scale culture facility (de Pauw and Persoone, 1988). Initial

estimates (M.R. Brown and M.A. McCausland, unpublished data) of the cost of supplementary feeding at Pipe Clay Lagoon are based on a mean microalgal cost per kg (dry weight) of U.S. $250 (Coutteau and Sorgeloos, 1992), and consider the quantity required to adequately boost food concentrations at the site. These indicate that supplementary feeding of oysters between 500 μm to 3 mm adds an additional U.S. $0.23 per thousand oysters, which is an additional 2 to 3% to the production cost of U.S. $8.50 - 10.00 per thousand oysters (M.John, pers. comm.). However, supplementary feeding would significantly shorten the land-based nursery phase. Calculations based on the growth-rates of control and the best performing supplemented oysters in this work indicate that a reduction of growing time in the nursery of 42 to 47 % can be achieved for oysters between 500 μm and 3 mm. This figure may even be as high as 70% during periods of very low natural productivity (Brown and McCausland, unpublished data). Such reductions would result in a reduction in labour costs and allow for steady turnover, which could more than offset the feed costs. Also, supplementary feeding would improve the predictability of production , and increase turnover, which in turn should reduce the costs. Oysters larger than 3 mm require much greater quantities of supplementary algae to achieve significant increases in growth-rate, at which stage costs may become prohibitive. Thus, it is unlikely that supplementary feeding of oysters larger than 3 mm would be more cost-effective than being grown-out in the natural environment.

Supplementary feeding is best used at sites where oyster growth-rates are slowed by low or variable availability of seston. We have demonstrated this by significantly enhancing juvenile oyster growth-rates at such a site (Pipe Clay Lagoon). Background levels of seston in seawater provided adequate levels of some micronutrients (e.g. long chain PUFAs, as in E3), so that additional macronutients and energy from supplementary microalgae were utilised to increase growth. All supplementary diets were effective, though some produced greater

increases than others. Important findings from our study are that algal pastes are as effective as live supplementary diets and that low-PUFA diets were as effective PUFA-rich microalgal supplementary diets.

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Table 1
Microalgae used as supplementary diets for juvenile Pacific oysters Crassostrea gigas

Algal Class and Species	CSIRO Culture No.	Deposition or origin code(s)	Axenic	Culture medium	Cell size (µm)	Australian isolate
Bacillariophyceae						
Chaetoceros calcitrans (Paulsen) Takano[a]	CS-178	C.CAL. CCMP1315	Yes	f/2	3-6	No
Skeletonema costatum (Greville) Cleve[a]	CS-181	SKEL. CCMP1332	Yes	f/2	5 x 10	No
Chlorophyceae						
Dunaliella tertiolecta Butcher[a]	CS-175	WHOI1. DUN. CCMP1320	Yes	f/2	10-12	No
Cryptophyceae						
Rhodomonas salina [b]	CS-24	—	No	f_E	5 x 12	Yes
Prymnesiophyceae						
Isochrysis aff. galbana Parke (T. ISO)[a]	CS-177	T. ISO. CCMP1324	Yes	f/2	3 x 5	No
Pavlova sp.[c]	CS-63	SPECK 16.3	No	f/2	5	Yes
Pavlova pinguis Green[d]	CS-375	PRPL01	No	f/2	5	Yes

[a] R. R. L. Guillard, Bigelow Laboratory for Ocean Science, West Boothbay Harbor, Maine, USA.
[b] CSIRO Algal Culture Collection, isolated from Port Hacking, New South Wales, Australia.
[c] D. Frood, isolated from Port Philip Bay, Victoria, Australia.
[d] CSIRO Algal Culture Collection, isolated from Pipe Clay Lagoon, Tasmania, Australia.

Table 2
Feeding experiments undertaken with juvenile Pacific oysters, Crassostrea gigas during the 1995/6 season

	Preliminary experiments		Main experiments		
	P1	P2	E1	E2	E3
Period of trial	6 Nov. - 24 Nov. 1995	4 Dec. - 22 Dec. 1995	8 Jan. - 26 Jan. 1996	19 Feb. - 8 Mar. 1996	19 Mar. - 4 Apr. 1996
Initial volume oysters (ml)	19	13	19	19	19
Control replicates	3	4	3	3	3
Mean background[a] phytoplankton (mg d^{-1} upweller^{-1})	42	64	63	58	90
Supplementary microalgae Treatment (replicates)	P. pinguis (5) Pavlova sp. (5)	P. pinguis 12:12 L:D (6) 24:0 L:D (6)	P. pinguis (5) R. salina (5) 1:1 mixture (5)	P. pinguis culture (5) C. calcitrans paste (4) culture (4) S. costatum paste (4)	P. pinguis (5) D. tertiolecta (5) Isochrysis sp. (5)
Algal illumination (L:D)	12:12	see above	24:0	P. pinguis - 24:0 C. calcitrans - 18:6 S. costatum - 18:6	24:0
Weekday algal ration (mg weekday^{-1} upweller^{-1})	180	140	190	180	190
Mean daily algal ration (mg d^{-1} upweller^{-1})	130	103	137	130	134
Mean daily algal ration[b] (mg d^{-1} ml^{-1} oysters)	6.8	7.9	7.2	6.8	7.1
Increase over background phytoplankton (%)	207	63	117	125	50
Experiment duration (d)	18	18	18	18	17
No. days fed (d)	13	13	13	13	12

[a] Calculation based on percentage background chlorophyll a levels (Brown, 1991)
[b] Figure based on packed volume of oysters on Day 0 of feeding trial.

Table 3
Mean (± 1 s.d.) physical and chemical properties of seawater during feeding experiments E1 to E3 (n = 3) and in the 1995/6 season; n = 25, other than for temperature (n =62) and fatty acids (n = 10).

	Feeding experiment			1995/6 season mean (Nov. - Apr.)
	E1	E2	E3	
Control oyster growth-rate (d^{-1})	0.043 ± 0.003	0.034 ± 0.003	0.034 ± 0.005	0.038 ± 0.006[a]
P. pinguis growth-rate (d^{-1})	0.066 ± .002	0.055 ± 0.001	0.049 ± 0.001	0.059 ± 0.007[a]
Minimum daily temperature (°C)	17.9 ± 1.2	17.3 ± 1.4	15.4 ± 1.1	16.0 ± 1.9
Maximum daily temperature (°C)	22.2 ± 1.7	20.6 ± 2.2	17.8 ± 1.3	19.4 ± 2.4
Chlorophyll a (µg l^{-1})	0.69 ± 0.17	0.63 ± 0.40	0.98 ± 0.64	0.68 ± 0.29
TPM (mg l^{-1})	2.15 ± 0.45	2.41 ± 0.97	3.45 ± 2.34	2.19 ± 1.43
POM (mg l^{-1})	0.19 ± 0.08	0.59 ± 0.36	0.85 ± 0.49	0.48 ± 0.34
POM (% TPM)	9.2 ± 4.6	23.5 ± 11.2	25.7 ± 2.5	23.2 ± 10.8
Salinity (‰)	33.8[b]	33.9 ± 0.7	34.2 ± 0.2	34.0 ± 0.5
Polyunsaturated fatty acids (µg l^{-1})				
20:5(n-3)	416[c]	380[c]	825 ± 494	719 ± 432
22:6(n-3)	528[c]	431[c]	602 ± 316	1013 ± 1007

[a] Average of preliminary and main experiments run throughout the 1995/6 season.
[b] Only one sample taken during experiment. Range of salinity throughout the year is 33-35 $g.kg^{-1}$.
[c] One analysis performed on combined weekly samples collected during month in which experiment was run.

Table 4
Mean (± 1 s.d.) gross biochemical composition of diets tested in supplementary feeding experiments of juvenile Pacific oysters Crassostrea gigas.

Feeding experiment and species	Protein (n ≥ 4) (%)	Carbohydrate (n ≥ 4) (%)	Lipid (n ≥ 2) (%)	Ash (n ≥ 4) (%)
E1				
P. pinguis	36 ± 7 [b]	28 ± 5 [a]	18 ± 4 [a]	14 ± 4 [c.d]
R. salina	59 ± 20 [a]	19 ± 5 [b]	19 ± 3 [a]	10 ± 3 [d]
1:1 mixture	48 ± 18 [a.b]	24 ± 7 [a.b]	19 ± 5 [a]	12 ± 5 [c.d]
E2				
P. pinguis	39 ± 11 [b]	31 ± 8 [a]	18 ± 4 [a]	15 ± 5 [b.c.d]
C. calcitrans culture	47 ± 8 [a.b]	23 ± 3 [a.b]	19 ± 4 [a]	23 ± 7 [a]
C. calcitrans paste	29 ± 3 [b]	17 ± 4 [b]	18 ± 3 [a]	22 ± 2 [a.b]
S. costatum paste	31 ± 9 [b]	19 ± 2 [b]	19 ± 4 [a]	19 ± 2 [a.b.c]
E3				
P. pinguis	35 ± 15 [b]	29 ± 6 [a]	13 ± 1 [a]	18 ±10 [a.b.c]
D. tertiolecta	39 ± 12 [b]	28 ±10 [a]	23 ± 1 [a]	12 ± 4 [c.d]
Isochrysis sp. (T. ISO)	34 ± 7 [b]	30 ± 3 [a]	24 ± 5 [a]	13 ± 6 [c.d]

Means in columns sharing a common superscript letter were not significantly different (P > 0.05).

Table 5
The fatty acid composition (% weight of total fatty acids) and total fatty acids (μg mg^{-1}) of dietary sources, control oysters, and supplement-fed oysters fed during E3. Error values are ± range for algae and oysters (n=2), and ± 1 s.d. for the control diet (n=3).

	Dietary sources of fatty acids				Oysters fed the corresponding diets			
Fatty acid	Unfiltered seawater	P. pinguis	Isochrysis sp.	D. tertiolecta	Control	P. pinguis	Isochrysis sp.	D. tertiolecta
Saturates	44.9 ± 3.5	23.9 ± 0.3	25.6 ± 0.1	14.0 ± 0.0	24.9 ± 0.9	24.7 ± 0.1	24.1 ± 0.5	23.3 ± 0.2
Branched-chain fatty-acids	2.9 ± 1.1	2.1 ± 0.3	2.4 ± 0.2	5.2 ± 0.4	3.1 ± 0.4	2.5 ± 0.1	1.8 ± 0.1	1.9 ± 0.0
Monounsaturates	26.7 ± 3.7	15.8 ± 1.0	18.5 ± 0.8	6.9 ± 0.0	16.4 ± 0.3	16.5 ± 0.1	17.5 ± 0.9	15.5 ± 0.2
Polyunsaturates								
16:4(n-3)	0.0	0.0	0.0	19.6 ± 0.1	0.0	0.0	0.0	0.0
18:2(n-6)	3.1 ± 1.0	0.7 ± 0.0	11.4 ± 3.0	5.2 ± 0.7	1.0 ± 0.0	0.9 ± 0.1	5.8 ± 0.0	2.4 ± 0.0
18:3(n-3)	1.8 ± 0.6	1.6 ± 0.2	6.4 ± 0.0	39.7 ± 0.4	0.8 ± 0.0	0.5 ± 0.0	2.3 ± 0.1	16.2 ± 0.1
18:3(n-6)	2.7 ± 1.1	0.0	1.8 ± 0.1	3.3 ± 0.0	0.0	0.0	0.6 ± 0.0	1.0 ± 0.0
18:4(n-3)	2.5 ± 0.9	9.0 ± 0.1	16.3 ± 2.6	0.6 ± 0.0	1.3 ± 0.0	1.1 ± 0.0	3.6 ± 0.1	1.5 ± 0.0
20:4(n-6)	0.8 ± 0.2	3.9 ± 0.7	0.4 ± 0.0	0.0	3.4 ± 0.0	4.6 ± 0.2	2.7 ± 0.1	2.6 ± 0.0
20:5(n-3)	5.4 ± 0.8	24.7 ± 0.0	0.7 ± 0.1	0.0	15.3 ± 0.4	14.9 ± 0.4	9.5 ± 0.3	11.0 ± 0.1
22:6(n-3)	5.3 ± 2.0	7.5 ± 0.0	11.7 ± 0.6	0.0	21.8 ± 0.3	17.4 ± 0.6	19.4 ± 0.6	12.3 ± 0.3
Others	3.6 ± 0.7	1.1 ± 0.1	0.9 ± 0.2	0.0	1.9 ± 0.1	1.5 ± 0.1	3.0 ± 0.8	2.4 ± 0.0
Total polyunsaturates	25.2 ± 3.6	57.9 ± 1.1	53.4 ± 0.9	73.7 ± 0.4	47.9 ± 1.0	49.2 ± 0.1	50.5 ± 0.0	53.7 ± 0.4
Non-methylene interrupted	0.0	0.0	0.0	0.0	7.5 ± 0.1	6.9 ± 0.1	6.0 ± 0.3	5.5 ± 0.1
Others	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total fatty acids (μg mg^{-1} dry weight)	4.34 ± 1.89	75.2 ± 20.1	107 ± 6	111 ± 15	2.00 ± 0.08	2.07 ± 0.16	3.16 ± 0.15	3.15 ± 0.03

Table 6
The concentrations of the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) in juvenile oysters and in their diet over the E3 experimental period.

	20:5(n-3)		22:6(n-3)		Total fatty acids	
	Total (µg oyster^{-1})	Retention (%)	Total (µg oyster^{-1})	Retention (%)	Total (µg oyster^{-1})	Retention (%)
Diets [a]						
Control (unfiltered water)	0.32	—	0.24	—	4.82	—
P. pinguis fed	1.69	—	0.65	—	10.0	—
Isochrysis sp. fed	0.38	—	1.16	—	12.3	—
D. tertiolecta fed	0.32	—	0.24	—	12.5	—
Oysters						
Estimated pre-E3 content [b]	0.042	—	0.06	—	0.27	—
Calculated post-E3 content						
Control	0.073	10	0.11	21	0.48	4
P. pinguis fed	0.11	4	0.13	11	0.77	5
Isochrysis sp. fed	0.14	26	0.28	19	1.44	10
D. tertiolecta fed	0.18	43	0.20	58	1.62	11

[a] µg of the respective fatty acids in the particulate fraction presented to each oyster within the study period. Values for the supplementary diets include the contribution from the background unfiltered water flowing into the upwellers.

[b] Fatty acid analyses were not undertaken on pre-experiment spat: this data is estimated based on the assumption that pre-experiment control oysters has a similar concentration of fatty acids and % composition of the PUFAs as contol oysters at the end of the experiment.

Fig. 1. Linear relationships of volumetrically measured oyster growth rates with (a) organic weight (OW) and (b) dry weight (DW) measured growth rates.

Fig. 2. Mean instantaneous growth rates (± 1 s.d.) of oysters in preliminary experiments (a) P1 and (b) P2, based on a packed volume. Both experiments had a reference diet of P. pinguis. Histogram bars with different letters above are significantly different ($P < 0.05$).

Fig. 3. Mean instantaneous growth rates (± 1 s.d.) of oysters in main experiments (a) E1, (b) E2 and (c) E3, based on packed oyster volume. All experiments had a reference diet of P. pinguis. Histogram bars with common letters above are not significantly different ($P > 0.05$).

Figure 1

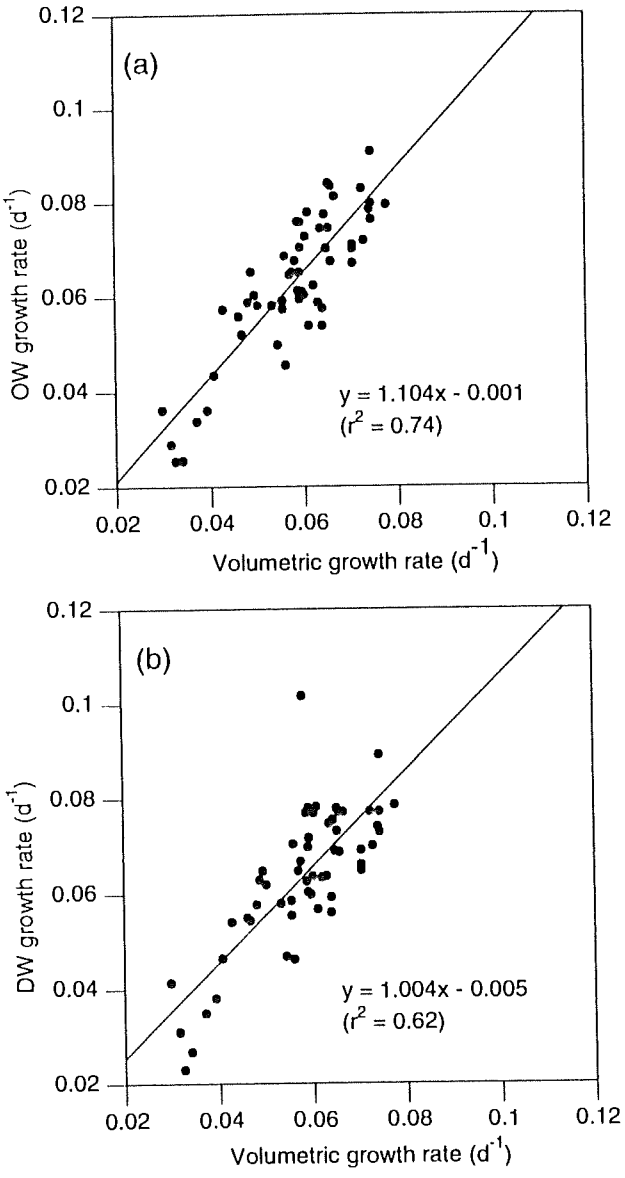

(a)
OW growth rate (d^{-1})
0.12
0.1
0.08
0.06
0.04
0.02
y = 1.104x - 0.001
(r^2 = 0.74)
0.02 0.04 0.06 0.08 0.1 0.12
Volumetric growth rate (d^{-1})
(b)
DW growth rate (d^{-1})
0.12
0.1
0.08
0.06
0.04
0.02
y = 1.004x - 0.005
(r^2 = 0.62)
0.02 0.04 0.06 0.08 0.1 0.12
Volumetric growth rate (d^{-1})

Figure 2

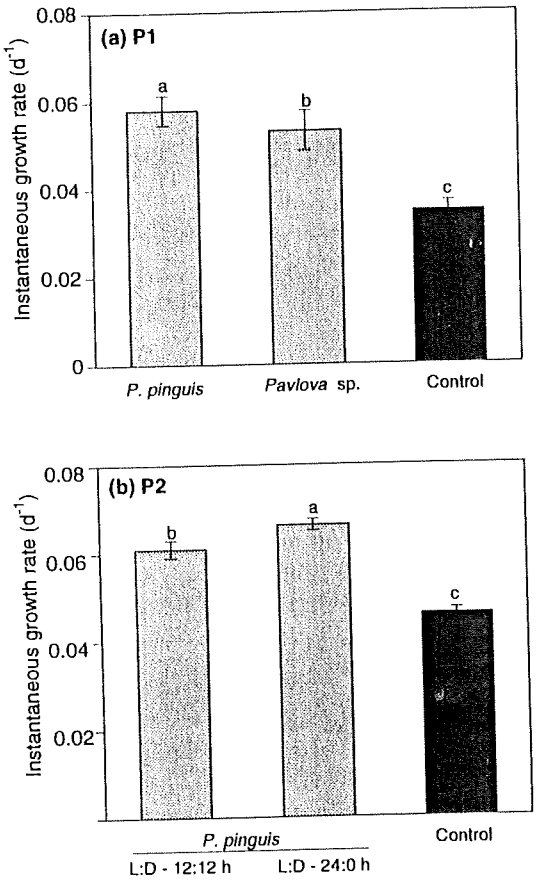
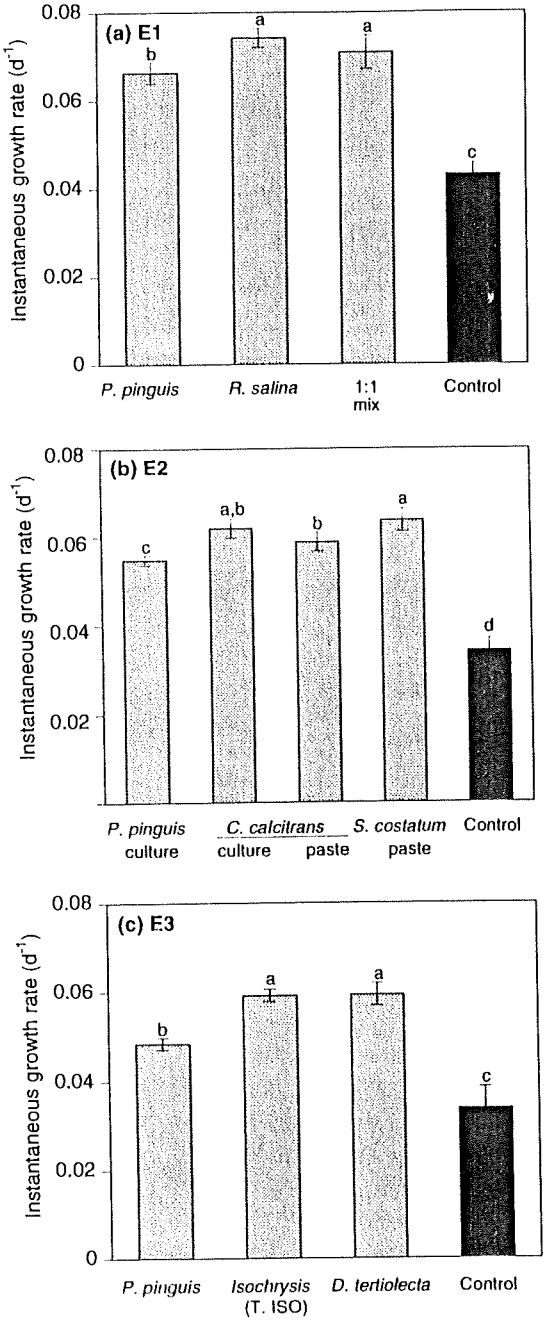

(a) P1
Instantaneous growth rate (d-1)
0.08
0.06
0.04
0.02
0
a
b
c
P. pinguis
Pavlova sp.
Control
(b) P2
Instantaneous growth rate (d-1)
0.08
0.06
0.04
0.02
b
a
c
P. pinguis
L:D - 12:12 h
L:D - 24:0 h
Control

Figure 3


(a) E1
Instantaneous growth rate (d-1)
0.08
0.06
0.04
0.02
0
a
a
b
c
P. pinguis
R. salina
1:1 mix
Control
(b) E2
Instantaneous growth rate (d-1)
0.08
0.06
0.04
0.02
c
a,b
b
a
d
P. pinguis culture
C. calcitrans culture
C. calcitrans paste
S. costatum paste
Control
(c) E3
Instantaneous growth rate (d-1)
0.08
0.06
0.04
0.02
0
b
a
a
c
P. pinguis
Isochrysis (T. ISO)
D. tertiolecta
Control

Abbreviations: chl - chlorophyll, TPM = total particulate matter, POM = particulate organic matter, DOC = dissolved organic carbon, k = instantaneous growth rate

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
24-Mar-92	15.0	35.45	3.94	0.34	0.25	2.44					0.030
31-Mar-92	16.5	34.73	3.40	0.17	0.32	1.89					0.014
7-Apr-92	14.5	34.80	5.37	0.34	0.38	2.56					0.034
15-Apr-92	15.8	34.78	3.22	0.17	0.28	2.15					0.029
22-Apr-92	11.0	34.91	3.94	0.17	0.29	0.96					0.020
29-Apr-92	13.2	34.54	0.90	0.00	0.26	1.44					0.033
6-May-92	12.6	34.80	2.86	0.17	0.34	1.31					
13-May-92	13.3	34.68	2.86	0.17	0.31	1.22					
20-May-92	11.2	34.34	3.94	0.17	0.42	1.94					0.010
27-May-92	11.1	34.56	5.01	0.17	0.38	1.05					0.024
3-Jun-92	11.1	34.40	4.83	0.17	0.37	1.01					0.016
10-Jun-92	9.9	34.52	4.83	0.17	0.37	1.61					0.007
17-Jun-92	9.2	34.25	6.62	1.36	0.44	1.88					0.003
24-Jun-92	8.8	33.79	6.09	1.02	0.41	1.12					0.005
1-Jul-92	8.0	34.25	5.55	1.36	0.44	1.17					0.017
8-Jul-92	8.1	33.62	5.91	1.19	0.41	1.14					0.004
15-Jul-92	9.0	33.63	6.62	1.19	0.44	1.49					0.019
22-Jul-92	7.8	33.45	5.01	0.51	0.35	1.21					0.026
29-Jul-92	8.5	33.54	4.12	0.17	0.41	3.07					0.025
12-Aug-92	9.2	33.56	3.58	0.51	0.42	3.34					0.015
19-Aug-92	9.2	33.53	2.33	1.52	0.53	3.00					0.026
26-Aug-92	9.2	33.31	2.86	0.51	0.35	2.11	1.07	4.89			0.013
2-Sep-92	9.5	33.97	2.69	1.19	0.40	1.18	0.71	6.04			0.004
9-Sep-92	9.8	32.55	6.98	0.34	0.35	1.57	0.91	5.82			0.017
16-Sep-92	10.3	33.60	5.01	1.02	0.38	1.55	1.20	7.35			
23-Sep-92	11.0	33.39	5.70	0.51	0.33	1.39	1.28	5.64			
30-Sep-92	11.2	33.47	4.80	0.17	0.31	0.58	0.33	5.84			
7-Oct-92	11.3	33.84	3.80	0.34	0.31	1.59	1.00	5.01			
15-Oct-92	13.3	33.61	4.00	0.34	0.62	1.14	0.37	6.22			
21-Oct-92	11.9	33.51	3.80	0.17	0.31	0.93	0.71	4.32			
29-Oct-92	15.6	33.58	2.20	0.17	0.28	0.63	0.52	5.67			
5-Nov-92	16.1	33.74	2.30	0.17	0.28	1.36	0.86	3.49			
12-Nov-92	14.7	33.92	1.50	0.34	0.28	1.87	1.15	5.82			
19-Nov-92	16.6	33.74	1.50	0.17	0.26	1.59	1.32	4.96			
26-Nov-92	13.8	33.54	1.20	0.17	0.28	2.15	1.95	8.44			
3-Dec-92	15.2	34.77	3.20	0.34	0.34	1.30	1.03	5.21			0.009
10-Dec-92	17.3	34.64	2.50	1.20	0.42	2.12	1.87	5.88			0.020
17-Dec-92	17.0	35.91	3.30	0.34	0.34	1.77	1.64	5.51			0.025
23-Dec-92	16.4	34.43	2.00	0.17	0.31	1.13	0.96	4.86			0.026
30-Dec-92	16.3	34.84	2.80	0.17	0.33	2.31	1.76	5.23			0.047
7-Jan-93	17.2	34.62	2.30	0.17	0.30	0.86	0.47	4.26			0.010
14-Jan-93	17.8	34.52	2.80	0.34	0.30	1.21	0.88	5.61			0.022
21-Jan-93	18.0	34.54	1.70	0.34	0.34	1.74	1.41	5.17			0.035
28-Jan-93	18.9	34.77	2.70	0.34	0.39	1.21	0.86	6.04			0.044
4-Feb-93	16.6	35.17	3.30	0.17	0.36	1.32	1.05	6.87			0.028
11-Feb-93	18.3	34.84	3.00	0.17	0.34	1.03	0.79	5.24			0.020
18-Feb-93	18.4	36.28	5.30	0.17	0.28	0.96	0.65	4.97			0.017
25-Feb-93	17.7	34.53	2.20	0.17	0.36	1.14	1.04	5.69			0.022
3-Mar-93	14.0	34.59	2.30	0.17	0.34	1.16	0.99	6.38			0.028
11-Mar-93	16.6	34.72	1.70	0.17	0.36	0.98	0.63	5.94			0.014
18-Mar-93	17.0	34.51	1.90	0.13	0.36	1.19	0.87	5.43			0.018
25-Mar-93	16.9	34.77	2.80	0.13	0.39	1.25	1.11	6.57			0.020
1-Apr-93	15.8	34.56	1.80	0.20	0.34	1.06	0.94	6.41			0.019
8-Apr-93	15.4	34.48	2.10	0.03	0.35	1.29	1.28	5.83			0.017
15-Apr-93	14.8	34.73	2.40	0.11	0.38	0.91	0.86	5.20			0.014
23-Apr-93	16.1	34.59	2.10	0.12	0.35	0.78	0.69	4.37			0.005
29-Apr-93	12.6	35.15	2.50	0.13	0.41	0.52	0.38	4.08			0.007
7-May-93	14.6	34.60	1.70	0.08	0.36	0.87	0.65	4.63			0.004
13-May-93	13.2	34.84	1.50	0.06	0.38	1.35	1.13	5.66			0.011
20-May-93	14.4	33.98	1.30	0.16	0.28	1.22	1.11	5.39			
27-May-93	13.8	34.18	1.80	0.11	0.38	1.46	1.23	4.51			
3-Jun-93	12.7	34.32	1.30	0.20	0.55	1.64	1.38	4.92			
10-Jun-93	11.6	34.33	1.80	0.21	0.45	1.83	1.45	5.33			
17-Jun-93	8.5	34.43	2.60	0.52	0.44	2.14	1.77	7.56			
24-Jun-93	8.8	34.17	2.30	0.43	0.49	1.92	1.63	10.32			
1-Jul-93	8.9	33.69	4.00	0.94	0.49	1.53	1.34	9.36			
15-Jul-93	9.6	33.92	1.70	0.46	0.40	1.69	1.38	6.24			
21-Jul-93	10.3	33.87	2.60	0.65	0.45	1.57	1.31	4.25			
29-Jul-93	9.3	34.03	1.10	0.46	0.30	2.04	1.69	3.65	1.15		
5-Aug-93	10.1	34.02	1.30	0.22	0.34	1.58	1.52	3.81	1.09		
12-Aug-93	10.2	34.19	2.30	0.79	0.59	1.64	1.57	4.09	1.36		
19-Aug-93	10.8	34.20	3.20	0.44	0.37	1.49	1.31	4.21	1.49		

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
26-Aug-93	10.7	33.78	3.50	0.72	0.42	1.39	1.27	3.62	1.08		
2-Sep-93	11.3	34.21	1.90	0.48	0.36	1.87	1.54	2.42	0.67		
8-Sep-93	11.7	33.90	2.60	0.92	0.42	1.69	1.61	3.84	1.09		
16-Sep-93	11.4	34.04	2.40	0.06	0.37	1.95	1.74	3.57	0.87		
23-Sep-93	12.0	34.03	1.80	0.00	0.34	1.40					
30-Sep-93	14.4	33.74	2.40	0.35	0.38	0.65					
6-Oct-93	13.8	34.13	2.10	0.00	0.35	0.65		2.87	0.91		
15-Oct-93	13.9		2.70	0.30	0.35	1.33		3.52	0.93		
22-Oct-93	14.7	34.92	2.30	0.40	0.41	0.77					
29-Oct-93	15.0	34.30	1.90	0.00	0.26	0.58		2.52	0.82		
5-Nov-93	13.2	34.38	2.50	0.00	0.36	0.71					
12-Nov-93	14.5	34.08	1.20	0.60	0.37	0.51					
19-Nov-93	14.9	34.21	0.00	0.00	0.16	0.54		3.17	1.18		
25-Nov-93	15.1	33.89	1.30	0.40	0.19	1.28	0.89	1.93	0.55		
2-Dec-93	15.1	34.21	1.70	0.00	0.31	1.39	1.26	2.36	0.69		
9-Dec-93	16.8	34.21	1.20	0.00	0.23	1.94	1.37	2.07	0.66		0.005
16-Dec-93	13.4	34.77	1.50	0.00	0.42	1.68	1.24	3.19	0.96		0.012
23-Dec-93	15.0	34.73	2.50	0.60	0.41	0.84	0.59	1.84	0.51		0.007
30-Dec-93	14.5	34.43	1.50	0.00	0.31	1.37	0.98	1.51	0.52		0.003
6-Jan-94	13.0	34.40	2.30	0.00	0.23	0.94	0.64	2.66	0.79		
13-Jan-94	16.0	33.86	0.80	0.00	0.26	1.19	1.95	1.36	0.41		-0.025
20-Jan-94	15.5	34.59	1.20	0.20	0.28	0.92	0.78	7.58	1.02		-0.014
27-Jan-94	16.5	34.34	1.20	0.40	0.29	1.07	1.00	6.93	0.95		0.012
3-Feb-94	15.0	34.14	2.30	0.00	0.32	1.00	0.28	11.16	1.65		0.027
10-Feb-94	16.0	33.89	1.00	0.00	0.31	2.15	1.26	6.94	1.08		0.053
17-Feb-94	15.0	34.31	2.10	0.00	0.31	1.66	1.07	8.01	1.38		0.017
24-Feb-94	16.0	34.20	1.30	0.00	0.29	1.71	1.04	7.21	1.19		0.006
3-Mar-94	15.0		2.70	0.00	0.39	3.87	2.01	22.10	4.19		-0.010
10-Mar-94	16.0	33.67	1.70	0.00	0.37	2.13	1.73	7.90	1.18		0.011
17-Mar-94	14.0	32.67	2.90	0.00	0.36	0.86	1.24	10.71	2.02		0.021
24-Mar-94	15.0	34.19	1.30	0.00	0.29	0.76	0.41	6.99	1.30		-0.019
31-Mar-94	16.0	34.27	2.70	0.00	0.36	1.59	0.69	7.32	0.90		0.005
7-Apr-94	13.5	33.68	3.70	0.20	0.36	1.21	0.55	8.96	1.37		-0.003
14-Apr-94	13.5	34.88	2.30	0.00	0.31	1.31	1.08	6.21	0.50		0.029
21-Apr-94	11.0	34.16	4.40	0.00	0.39	1.01	0.95	7.65	1.09		0.011
28-Apr-94	13.0	32.93	4.08	0.29	0.34	1.41	1.25	6.99	1.00		0.023
5-May-94	11.0	34.29	3.81	0.19	0.32	0.92	0.88	6.28	0.74		0.033
12-May-94	11.0	33.41	4.72	0.18	0.33	0.98	1.16	6.44	0.88		0.019
19-May-94	8.5	34.13	5.33	0.24	0.33	0.98	0.92	9.63	1.34		
26-May-94	11.0	34.26	6.18	0.24	0.37	1.31	1.16	7.20	0.91		
2-Jun-94	10.0	33.60	6.79	1.01	0.39	1.04	1.18	8.20	1.11		0.026
9-Jun-94	10.0	34.09	5.71	1.26	0.39	1.54	1.07	8.98	1.27		
16-Jun-94	8.0	34.57	6.04	1.73	0.52	1.37	1.37	8.24	1.13		
23-Jun-94	9.0	33.77	6.34	1.49	0.39	1.20	0.77	7.44	1.02		
30-Jun-94	7.5	33.79	5.79	0.75	0.42	1.55	1.31	7.36	1.02		
7-Jul-94	9.0	33.74	6.84	0.44	0.35	1.89	1.47	8.48	1.08		
14-Jul-94	8.5	33.80	5.46	0.51	0.34	2.21	1.51	5.28	1.00		
21-Jul-94	8.0	33.93	5.26	0.00	0.35	1.90	1.45	16.80	3.04		
28-Jul-94	8.0	33.80	3.31	0.22	0.31	3.19	2.30	8.11	1.47		
4-Aug-94	8.0	33.51	4.15	0.15	0.32	3.98	1.27	10.81	1.94		
11-Aug-94	8.0	33.42	3.40	0.19	0.32	2.86	0.57	15.06	2.56		
18-Aug-94	7.5	33.25	4.80	1.00	0.34	2.12	1.60	9.30	1.84		
25-Aug-94	8.0	33.82	2.11	0.27	0.35	4.30	2.75	9.67	1.72		
1-Sep-94	9.0	33.73	1.46	0.27	0.22		1.62	3.60	0.86		
8-Sep-94	8.0	33.00	1.14	0.09	0.26		0.73	4.17	1.61		
15-Sep-94	9.0	33.64	1.47	0.15	0.25		1.83	3.95	2.21		
22-Sep-94	10.5	33.65	1.47	0.07	0.26		1.71	2.21	0.78		
29-Sep-94	8.5	32.23	1.98	0.09	0.19		1.11	2.08	0.71		
6-Oct-94	10.5	32.49	2.30	0.08	0.20		0.32	1.29	0.36		
13-Oct-94		32.94	2.88		0.20		0.88	1.06	0.39		
20-Oct-94	12.7	32.65	28.30	<0.10	0.36	0.43	0.39	2.25	0.50	2.10	
27-Oct-94	13.7		15.10	0.10	0.25	1.02	0.99	1.74	0.39		
3-Nov-94	13.3	33.61	10.50	<0.10	0.24	1.03	0.63	1.28	0.36	1.10	
10-Nov-94	12.5	33.90	8.10	<0.10	0.23	1.14	1.31	1.11	0.27		
17-Nov-94	13.2	33.23	13.30	<0.10	0.21	0.80	0.38	1.80	0.39	1.10	
24-Nov-94	13.5	33.44	17.00	<0.10	0.23	0.70	0.24	1.58	0.40		
30-Nov-94	14.8	33.96	8.60	<0.10	0.25	1.06	1.19	3.94	0.90	1.30	
8-Dec-94	16.0	34.49	9.60	0.20	0.23	1.44	0.81	2.39	0.51		
14-Dec-94	18.0	34.75	10.50	<0.10	0.23	0.80	0.47	1.61	0.47	1.20	
22-Dec-94	16.5	34.34	7.90	<0.10	0.23	0.47	0.38	1.59	0.49		
30-Dec-94	18.5	34.46	11.90	<0.10	0.30	0.45	0.69	4.54	1.05	1.10	
5-Jan-95	18.0	34.54	8.20	<0.10	0.26	0.59	0.82	1.17	0.36		0.030
12-Jan-95	17.0	34.95	12.10	<0.10	0.26	0.87	1.56	3.05	0.83	1.80	0.005
19-Jan-95	20.0	34.65	10.30	<0.10	0.27	0.99	0.91	3.18	0.66		0.028
26-Jan-95	16.0	35.36	13.80	<0.10	0.16	1.27	0.62	1.63	0.52	1.70	0.016
3-Feb-95		34.67	15.00	<0.10	0.29	0.69	0.48	1.09	0.42		0.009

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
9-Feb-95	16.0	34.81	8.90	0.80	0.28		1.01	1.94	0.49	1.80	0.039
16-Feb-95	18.5	34.60	6.60	0.30	0.27	1.28	1.04	1.13	0.50		0.050
23-Feb-95	16.0	35.54	14.10	<0.10	0.26	0.53	0.55	1.98	0.70	1.80	0.035
2-Mar-95	18.0	34.76	6.60	<0.10	0.24	1.01	0.33	1.13	0.38		0.062
10-Mar-95	17.0	35.62	12.50	<0.10	0.24	0.73	0.66	2.46	0.82	1.50	0.008
16-Mar-95	14.5	35.04	25.40	<0.10	0.33	1.21	1.07	2.41	0.73		0.013
23-Mar-95	14.5	35.09	14.00	0.10	0.34	0.87	0.94	3.96	0.71	0.90	0.031
29-Mar-95		35.31	16.10	0.10	0.56	0.74	0.67	2.13	0.52		0.030
6-Apr-95	11.5	35.02	10.60	0.10	0.33	0.98	1.49	2.24	0.68	1.10	0.012
13-Apr-95	11.5	34.15	11.80	<0.10	0.39	1.22	1.56	2.88	0.74		0.017
20-Apr-95	11.0	34.29	17.90	0.10	0.47	2.10	2.04	5.74	1.60	1.00	-0.009
27-Apr-95	11.0	34.18	16.60	<0.10	0.47	1.22	1.36	1.78	0.42		0.011
4-May-95	12.0	34.36	16.20	<0.10	0.44	0.91	1.70	2.63	0.56	0.90	0.018
11-May-95	11.2	34.12	18.00	0.10	0.39	2.19	1.87	3.64	0.86		
18-May-95	11.5	34.30	3.43	6.74	0.38	1.95	1.79	2.00	0.51	5.50	0.018
25-May-95	10.0	34.41	2.57	0.38	0.32	1.39	1.02	1.35	0.36		0.023
1-Jun-95	11.0	34.15	2.91	0.76	0.38	2.41	1.71	2.37	0.74	1.40	0.008
8-Jun-95	11.0	34.35	2.91	1.61	0.36	2.69	2.13	2.50	0.73		0.046
15-Jun-95	11.0	34.12	1.23	1.33	0.34	3.60	3.10	2.41	0.85	2.50	0.010
22-Jun-95	6.0	33.77	2.91	1.80	0.47	0.93	0.95	2.47	0.52		0.022
29-Jun-95	8.5	33.49	3.69	3.23	0.52	2.33	2.25	2.91	0.82	1.90	0.011
6-Jul-95	6.5	33.45	2.83	1.42	0.36	0.93	0.94	2.10			-0.003
13-Jul-95		33.04	4.83	3.42	0.43	1.57	1.23	1.81	0.56	1.70	0.012
20-Jul-95	8.0	33.23	3.77	2.28	0.79		6.86	32.06	6.13		0.014
27-Jul-95	9.0	33.21	3.69	4.36	0.38	2.11	1.92	1.20	0.51	1.70	0.026
3-Aug-95	8.0	33.68	2.23	2.85	0.38	1.00	0.99	1.83	0.54		0.031
10-Aug-95	9.0	33.42	0.82	0.47	0.29	3.75	2.45	1.84	0.64	2.00	0.014
17-Aug-95	9.5	33.71	1.14	0.76	0.36	2.64	1.43	2.04	0.72		0.004
24-Aug-95	9.0	33.59	1.39	0.85	0.32	1.43	1.22	2.97	0.85	1.40	0.013
31-Aug-95	9.0	33.79	0.65	1.23	0.41	2.68	2.19	4.76	1.28		0.008
7-Sep-95	9.5	33.54	1.56	0.76	0.31	1.16	1.09	2.49	0.77	1.90	-0.001
14-Sep-95	10.0	33.70	2.15	0.85	0.31	1.33	1.04	2.04	0.64		0.020
21-Sep-95	9.5	34.34	1.89	1.61	0.38	1.73	0.95	3.47	0.89	1.30	
28-Sep-95	10.0	33.99	1.31	0.28	0.23	0.88	0.89	1.42	0.55		
5-Oct-95		33.78	0.82	0.19	0.20		0.49	1.82	0.61	1.40	
12-Oct-95		33.89	0.98	0.19	0.22	0.59	0.28	1.78	0.50		
19-Oct-95		34.03	1.48	0.19	0.20	0.64	0.51	2.37	0.66		
26-Oct-95		34.07	2.15	0.19	0.25	0.30	1.62	2.01	0.61		
2-Nov-95		33.24	1.14	0.19	0.18	0.30	0.58	1.46	0.50	1.30	
9-Nov-95	14.5	34.10	1.64	0.19	0.20	0.36	0.34	1.31	0.47		
16-Nov-95		34.10	2.40	0.19	0.18	2.26	0.56	1.33	0.29	1.10	
23-Nov-95	15.0	34.08	1.56	0.19	0.25	0.96	0.50	1.70	0.24		
29-Nov-95		34.51	1.23	0.09	0.22	1.11	0.44	1.11	0.45	1.10	
6-Dec-95	16.0	34.23	1.06	0.19	0.23		0.59	1.24	0.26		
13-Dec-95		34.58	2.06	0.09	0.25	0.96	0.74	2.39	0.53	1.10	
21-Dec-95	13.0	33.54	1.56	0.19	0.25	0.70	0.76	8.05	1.68		
28-Dec-95	12.5		2.40	0.19	0.18	1.06	0.75	2.97	0.46	1.40	
4-Jan-96	16.0	33.38	2.79	0.21	0.12	0.27	0.41	1.70	0.14		
11-Jan-96	16.0	33.80	6.21	0.42	0.12	0.54	0.66	2.56	0.24	1.60	0.009
18-Jan-96	19.5		2.61	0.21	0.12	0.36	0.54	1.67	0.23		0.030
25-Jan-96	17.5		2.79	0.21	0.14	0.80	0.87	2.22	0.10		0.021
1-Feb-96			1.51	0.21	0.11	0.76	0.68	1.91	0.30	1.50	0.012
8-Feb-96		34.44				1.30	0.71	1.62	0.62		0.006
15-Feb-96		33.35	10.80	0.00	0.25	0.68	0.55	1.63	0.64	2.40	0.004
22-Feb-96		33.14	6.86	0.10	0.11	0.66	0.33	1.62	0.19		0.020
29-Feb-96	16.0	34.17	5.09	0.10	0.14	0.61	0.47	2.12	0.72	1.40	0.010
7-Mar-96	17.0	34.41	3.52	0.21	0.14	1.36	1.08	3.49	0.88		0.050
14-Mar-96	17.0	34.57	2.42	0.31	0.14	0.78	0.60	1.89	0.56	1.40	0.026
21-Mar-96	15.0	34.37	3.07	0.21	0.12	1.19	0.69	1.88	0.53		0.008
28-Mar-96	17.0	34.04	2.97	0.21	0.16	0.67	0.55	2.33	0.60	1.20	0.027
3-Apr-96	15.0	34.07				2.18	1.71	6.14	1.42		0.026
11-Apr-96	12.5	32.98				0.77	0.73	1.82	0.52	1.30	0.007
19-Apr-96	13.0	32.81	10.30	0.00	0.33	1.37	1.05	2.53	0.84		0.007
26-Apr-96	13.5	33.63	9.70	0.00	0.39	0.85	0.92	2.59	0.75	1.30	0.009
2-May-96	13.5	33.14	13.00	0.00	0.32	2.63	1.95	4.35	1.26		0.002
9-May-96	15.0	33.59	14.10	0.00	0.33	1.55	1.21	1.80	0.56	1.40	0.024
16-May-96	11.0	33.82	8.40	0.00	0.33	2.59	2.07	2.36	0.74		
23-May-96	9.5	33.71	8.10	0.00	0.32	1.21	1.02	1.77	0.64	1.50	-0.019
30-May-96	9.5	33.73	8.10	0.00	0.29	1.02	0.92	1.26	0.55		0.019
6-Jun-96	9.0	33.81	9.70	0.00	0.32	1.65	1.28	1.58	0.64	1.30	0.014
13-Jun-96	9.0	33.67	10.30	1.00	0.45	1.02	1.18	1.57	0.60		0.011
20-Jun-96	9.5	33.35	12.40	1.00	0.44	0.88	0.77	1.22	0.62		-0.007
27-Jun-96	8.0	33.11	9.20	0.30	0.24	1.02	0.92	1.78	0.68		0.008
4-Jul-96	10.0	33.54	10.80	1.40	0.41	1.16	1.09	0.89	0.55		-0.002
11-Jul-96	10.0	33.71	8.70	1.30	0.41					1.20	-0.011
18-Jul-96	9.5	33.79	7.10	0.80	0.35	1.07	1.33	0.54	0.38		0.017

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
25-Jul-96	7.5	33.63	4.50	0.00	0.29	1.12	0.74	0.94	0.41	1.30	0.009
1-Aug-96	10.0	34.33	5.50	1.40	0.41	1.23	1.00	1.39	0.48		-0.003
8-Aug-96	7.5	34.21	3.70	0.00	0.25	1.31		0.82	0.40		0.023
15-Aug-96	8.0	33.75	2.20	0.00	0.24	1.45	0.97	1.07	0.43		0.005
22-Aug-96	9.0	32.57	12.70	0.00	0.25	0.82	0.69	0.60	0.35		0.069
29-Aug-96	9.5	33.36	4.50	0.00	0.22	1.20	1.03	0.83	0.43		0.077
5-Sep-96	10.0	33.33	3.50	0.00	0.21	0.85		0.93	0.78	1.10	0.012
12-Sep-96	11.0	33.52	3.50	0.00	0.26	1.09	0.73	2.26	1.22		0.006
19-Sep-96	12.0	33.57	4.00	0.20	0.24	0.73	0.56	1.05	0.90	0.60	-0.003
26-Sep-96	13.0	33.94	3.70	0.20	0.24	0.86	0.84	1.57	0.80		0.027
3-Oct-96	13.5	33.79	5.00	0.00	0.19	1.18	0.96				0.030
10-Oct-96		33.58	3.50	0.20	0.22	0.45	0.36	1.28	0.66	0.80	0.010
17-Oct-96	12.5	34.14	3.70	0.00	0.24	0.81	0.45	1.30	0.59		-0.004
24-Oct-96		33.82	3.50	0.00	0.18	0.41	0.33	2.07	0.59	0.70	0.037
31-Oct-96	12.0	34.01	4.80	0.10	0.22	0.35	0.37	1.11	0.34		-0.010
7-Nov-96	15.0	34.21	5.50	0.10	0.21	0.28	0.28	1.10	0.39	0.90	0.001
14-Nov-96	14.5	34.20	16.90	0.10	0.14	0.26		1.58	0.45		
21-Nov-96	13.0	33.40	7.60	0.10	0.18	0.37		1.67	0.47		
28-Nov-96	15.0	33.30	6.10	0.10	0.16	0.41	0.38	0.93	0.31		
5-Dec-96		33.88	6.60	0.10	0.22	0.61	1.00	1.94	0.62	1.10	
19-Dec-96		34.25	4.00	0.00	0.18	1.55	0.88	2.81	0.96		
24-Dec-96	22.0	34.29				0.95	0.57	1.87	0.57	0.90	
2-Jan-97	17.0	34.54	6.80	0.00	0.22	0.24	0.19	0.78	0.70		
9-Jan-97	17.0	34.43	4.50	0.00	0.22	0.61	0.37	1.21	0.57		
16-Jan-97	14.5	34.67	5.50	0.00	0.23	0.32	0.38	1.22	0.78		-0.008
28-Jan-97	17.0	34.52	7.10	0.00	0.33	0.35	0.32			0.90	0.002
30-Jan-97	16.0	34.33	9.20	0.00	0.33	0.43	0.20	0.74	0.62		0.004
6-Feb-97	18.0	34.68	4.00	0.00	0.22	0.37	0.24	1.30	0.47	1.70	0.004
13-Feb-97	18.0	34.90	10.30	0.00	0.30	0.45	0.35	1.59	0.42		-0.007
20-Feb-97		34.76	7.10	0.00	0.26	0.42	0.21	1.37	0.43		0.021
27-Feb-97		35.14	3.25	0.14	0.29	0.44	0.39	1.04	0.34		-0.032
6-Mar-97		35.00	3.74	0.21	0.25	0.48	0.38	2.01	0.55		0.037
13-Mar-97		34.77	1.85	0.21	0.27	0.50	0.26	0.87	0.30		
20-Mar-97		35.02	2.79	0.21	0.32	0.65	0.41	2.24	0.53	1.60	0.014
27-Mar-97	15.5	34.39	1.85	0.14	0.27	0.37	0.41	0.87	0.46		0.013
3-Apr-97	12.5	34.37	2.81	0.14	0.29		0.39	0.89	1.11	1.40	-0.011
10-Apr-97	12.0	34.30	4.25	0.21	0.34	0.38	0.60	1.22	0.50		-0.010
17-Apr-97	13.5		3.27	0.14	0.34	0.58	0.42	0.96	0.46	1.50	-0.042
24-Apr-97	12.5	33.95	3.26	0.14	0.32	0.93		1.10	0.46		-0.010
1-May-97	13.0		3.74	0.21	0.35	0.68	0.45	0.72	0.30	1.60	-0.003
9-May-97	11.5	34.36	4.21	0.64	0.35	1.67	1.04	1.47	0.51		-0.004
15-May-97	11.0	33.91	2.30	0.28	0.30	0.62	0.43	0.75	0.35	1.40	0.014
22-May-97	10.5		2.77	0.28	0.32	0.85	0.53	1.37	0.38		0.021
29-May-97			3.23	0.64	0.39	0.64	0.61	1.09	0.38	1.40	0.025
5-Jun-97	10.0	34.31	1.36	0.21	0.25	0.77	0.57	0.57	0.33		0.014
12-Jun-97	9.5	34.15	1.82	0.22	0.30	0.65	0.54	0.59	0.24	1.40	0.060
19-Jun-97	9.5	34.10	3.69	0.22	0.37	0.55	0.46	0.58	0.25		-0.025
26-Jun-97	9.0		3.68	0.65	0.35	1.02	0.40	0.63	0.35	1.40	
Average	12.7	34.1	4.8	0.38	0.32	1.26	1.01	3.79	0.79	1.45	0.015
(± s.d.)	(± 3.2)	(± 0.6)	(± 4.2)	(± 0.68)	(± 0.09)	(± 0.71)	(± 0.65)	(± 3.49)	(± 0.63)	(± 0.65)	(± 0.017)

For statistical compilation (see below) nitrate levels reported as <0.10 have been assumed to have a value of 0.05.

Average (± s.d.) annual values of data - calendar year basis

Year	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
From May 1992	12.2 (± 3.0)	34.1 (± 0.7)	3.8 (± 1.6)	0.47 (± 0.43)	0.36 (± 0.08)	1.6 (± 0.6)	1.1 (± 0.5)	5.6 (± 1.1)			0.019 (± 0.011)
1993	13.9 (± 2.8)	34.4 (± 0.5)	2.2 (± 0.8)	0.27 (± 0.25)	0.37 (± 0.08)	1.3 (± 0.4)	1.1 (± 0.4)	4.7 (± 1.8)	0.92 (± 0.29)		0.017 (± 0.010)
1994	12.1 (± 3.3)	33.8 (± 0.6)	5.3 (± 5.0)	0.24 (± 0.39)	0.31 (± 0.07)	1.5 (± 0.9)	1.1 (± 0.5)	6.4 (± 4.2)	1.1 (± 0.7)	1.3 (± 0.4)	0.012 (± 0.020)
1995	12.21 (± 3.5)	34.21 (± 0.6)	6.21 (± 6.2)	0.80 (± 1.29)	0.32 (± 0.11)	1.36 (± 0.8)	1.25 (± 1.0)	2.92 (± 4.3)	0.75 (± 0.82)	1.6 (± 0.9)	0.019 (± 0.015)
1996	12.6 (± 3.5)	33.7 (± 0.4)	6.5 (± 3.7)	0.23 (± 0.38)	0.24 (± 0.10)	1.0 (± 0.5)	0.8 (± 0.4)	1.8 (± 1.0)	0.58 (± 0.27)	1.2 (± 0.4)	0.014 (± 0.019)
Jp to June 1997	13.4 (± 3.0)	34.5 (± 0.3)	4.2 (± 2.2)	0.19 (± 0.19)	0.30 (± 0.05)	0.6 (± 0.3)	0.4 (± 0.2)	1.1 (± 0.4)	0.47 (± 0.19)	1.4 (± 0.2)	0.003 (± 0.023)

Average (± s.d.) annual values of data - production season basis (July-June)

Year	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	chl. a (µg/L)	chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)	Oyster k (day^{-1})
1992/93	13.6 (± 3.4)	34.2 (± 0.7)	3.0 (± 1.5)	0.37 (± 0.37)	0.37 (± 0.07)	1.4 (± 0.6)	1.0 (± 0.4)	5.6 (± 1.2)			0.019 (± 0.010)
1993/94	12.8 (± 2.6)	34.1 (± 0.4)	2.7 (± 1.6)	0.33 (± 0.42)	0.35 (± 0.08)	1.3 (± 0.6)	1.2 (± 0.4)	6.0 (± 3.6)	1.1 (± 0.6)		0.011 (± 0.018)
1994/95	12.4 (± 3.7)	34.1 (± 0.8)	8.9 (± 6.4)	0.43 (± 1.06)	0.31 (± 0.09)	1.5 (± 1.0)	1.2 (± 0.7)	3.6 (± 3.4)	0.85 (± 0.60)	1.7 (± 1.0)	0.022 (± 0.016)
1995/96	12.2 (± 3.4)	33.7 (± 0.5)	4.3 (± 3.8)	0.57 (± 0.91)	0.27 (± 0.12)	1.2 (± 0.7)	1.1 (± 1.0)	2.8 (± 4.3)	0.72 (± 0.83)	1.5 (± 0.3)	0.013 (± 0.013)
1996/97	12.5 (± 3.3)	34.1 (± 0.5)	5.0 (± 3.0)	0.22 (± 0.35)	0.27 (± 0.07)	0.7 (± 0.4)	0.6 (± 0.3)	1.2 (± 0.5)	0.52 (± 0.21)	1.2 (± 0.3)	0.009 (± 0.024)

Abbreviations: chl - chlorophyll, TPM = total particulate matter, POM = particulate organic matter, DOC = dissolved organic carbon,

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	Chl a (µg/L)	Chl a (<20 µm) (µg/L)	TPM (<20 µm) (mg/L)	POM (<20 µm) (mg/L)	DOC (mg/L)
1-Oct-92	12.3		33.50	0.30	0.31	1.05				
15-Oct-92	13.0		18.50	0.30	0.31	1.22				
29-Oct-92	15.9		7.80	0.20	0.31	1.18				
12-Nov-92	16.0		10.00	0.30	0.40	1.73				
26-Nov-92	17.0		10.30	0.20	0.28	2.14				
10-Dec-92	16.2		13.50	0.20	0.20	2.86				
23-Dec-92	16.8		17.50	0.20	0.33	2.08				
7-Jan-93	18.0		18.50	0.20	0.48	1.95				
14-Jan-93	16.5		4.30	0.20	0.26	2.36				
21-Jan-93	17.5		7.80	0.20	0.26	2.17				
28-Jan-93	16.4		10.80	0.30	2.01	1.84				
5-Feb-93	17.3		13.70	0.20	0.25	1.69				
11-Feb-93	20.1		11.80	0.20	0.37	2.54				
18-Feb-93	17.6		9.70	0.20	0.37	2.26				
3-Mar-93	16.4		14.30	0.20	0.28	1.64				
18-Mar-93	17.2		21.40	0.10	0.25	1.94				
25-Mar-93	16.8		15.70	0.00	0.22	2.07				
8-Apr-93	15.8		10.40	0.10	0.22	3.14				
23-Apr-93	16.5		4.70	0.00	0.16	2.49				
13-May-93	14.8		1.90	0.00	0.53	2.70				
27-May-93	14.9		1.90	0.00	0.18	2.85				
3-Jun-93	14.2		1.40	0.00	0.08	2.24				
10-Jun-93	13.8		0.40	0.00	0.15	2.03				
24-Jun-93	11.6		1.40	0.00	0.13	2.70				
1-Jul-93	12.0		1.70	0.00	0.11	1.89				
15-Jul-93	11.4		13.80	0.10	0.10	1.97				
29-Jul-93	12.9		3.40	0.00	0.08	2.08				
26-Aug-93	13.0		3.40	0.00	0.11	2.34				
29-Sep-93	17.0		9.10	0.20	0.11	2.07				
15-Oct-93	14.0		6.70	0.00	0.07					
19-Oct-93	15.0									
3-Nov-93	14.5		5.40	0.40	2.16	1.08				
12-Nov-93	15.0		5.40	1.40	0.37					
16-Nov-93	20.0		4.00	1.20	0.34					
25-Nov-93	16.0		11.70	0.80	0.03	2.11				
9-Dec-93	20.0		7.50	0.40	0.19	2.26				
23-Dec-93	18.0		9.60	0.00	0.19	1.95				
20-Jan-94	17.0		3.50	0.00	0.23	3.51				
2-Feb-94	19.0		10.20	0.00	0.19	2.07				
9-Feb-94	21.0		4.00	0.00	0.21	0.76				
23-Feb-94	20.0		9.80	0.00	0.19	3.04				
6-Apr-94	17.0		8.30	0.00	0.19	3.46				
13-Apr-94						3.65				
20-Jul-94		35.19	2.94	0.08	0.09					
10-Aug-94	7.5	32.71	4.21	0.27	0.08	3.63				
17-Aug-94	7.5	33.98	4.28	0.06	0.07	1.63				
31-Aug-94	8.0	34.95	4.23	0.05	0.07	0.97		2.65	0.79	3.30
14-Sep-94	8.0	34.87	4.58	0.28	0.08	1.09		1.46	0.55	3.20
5-Oct-94	9.5	31.11	12.99	0.09	0.08	1.00		2.73	0.71	
12-Oct-94		33.75	5.63	0.00	0.07	1.49		2.46	0.74	
19-Oct-94	16.0	34.44	16.70	<0.05	0.35	2.36	1.27	1.88	0.69	2.10
26-Oct-94			11.10	<0.05	0.26	1.34		2.40	0.68	
2-Nov-94	16.0	35.06	13.40	<0.05	0.28	2.53	2.71	2.68	0.73	1.70
9-Nov-94	12.5	33.63	10.10	<0.05	0.25	1.01	0.92	2.66	0.73	
16-Nov-94	18.0	34.28	9.30	0.55	0.25	0.62	1.10	2.76	0.67	1.50
23-Nov-94	16.0	34.66	9.90	<0.05	0.37	2.12	2.15	3.40	0.99	
30-Nov-94		34.73	18.10	0.33	0.60	3.35	2.99	14.31	4.56	1.90
14-Dec-94	20.0	36.42	19.20	<0.05	0.32	3.00	2.05	3.35	1.07	1.90
21-Dec-94	21.0	36.49	18.00	<0.05	0.37	3.90	3.68	3.22	1.01	
28-Dec-94	20.0	36.60	13.60	<0.05	0.27	2.46	2.34	4.49	1.19	1.70
4-Jan-95	20.0	36.72	13.50	0.05	0.39	3.72	2.58	3.98	1.32	
11-Jan-95		36.04	9.50	0.07	0.50	4.99	1.89	3.86	1.10	2.60
25-Jan-95	19.0	35.76	16.90	0.19	0.78	6.50	4.37	4.13	1.33	2.00
8-Feb-95	18.0	27.40	41.60	0.08	0.40	3.39	2.62	3.15	1.06	4.00
15-Feb-95	23.0	31.55	15.40	<0.05	0.65	5.89	3.61	7.83	1.99	
22-Feb-95		33.26	13.80	0.06	0.47	1.92	1.46	3.22	0.57	3.00
1-Mar-95			14.80	<0.05	0.42	4.82	2.65	4.42	1.61	
8-Mar-95	22.0	35.24	9.00	0.10	0.30	5.07	2.34	3.37	1.20	2.20
15-Mar-95	17.5	35.74	11.50	0.08	0.53	5.72	2.47	9.76	2.94	
22-Mar-95		35.98	10.80	0.08	0.38	2.65	1.43	5.75	1.33	1.60
29-Mar-95	14.5	35.98	10.30	0.05	0.26	1.83	0.75	1.65	0.54	
5-Apr-95	13.5	35.70	7.40	0.11	0.28	2.17	1.39	2.91	1.02	1.20

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	Chl a (µg/L)	Chl a (<20 µm) (µg/L)	TPM (<20 µm) (mg/L)	POM (<20 µm) (mg/L)	DOC (mg/L)
11-Apr-95		35.48	4.50	0.15	0.47	4.55	1.58	4.48	1.31	
19-Apr-95	13.5	34.98	19.90	0.14	0.73	5.46	2.49	9.14	2.59	1.50
26-Apr-95	12.5	34.16	21.90	0.14	0.43	1.81	1.80	4.47	1.02	
3-May-95		33.64	23.30	0.52	0.31	1.35	1.77	2.84	0.76	1.50
17-May-95	8.5	34.91	9.58	7.89		2.50	2.00	4.50	1.09	1.80
24-May-95		35.08	8.64	0.62	0.21	2.73	2.16	2.72	0.77	
7-Jun-95	8.0	35.32	5.00	1.97	0.12	2.88	1.68	5.58	1.63	1.20
21-Jun-95	7.0									
5-Jul-95	8.0	34.17	7.98	0.62	0.21	2.11	2.67	3.09	0.96	3.10
11-Jul-95		34.34	4.26	0.31	0.16	1.29	0.98	2.24	0.70	1.60
19-Jul-95		26.70	61.60	5.30	0.14	0.87	0.88	6.88	1.84	
2-Aug-95	9.0	32.48	8.92	0.62	0.12	1.28	0.92	2.45	0.88	2.00
9-Aug-95	11.0	34.45	57.87			2.72	2.60	17.38	4.29	
16-Aug-95	13.0	26.66	46.69	1.56	0.11	2.25	2.21	5.34	1.63	4.10
6-Oct-95		35.61	18.45	0.31	0.12	2.22	1.72	3.63	1.18	2.20
11-Oct-95	13.5					1.47	0.70	2.06	0.65	
18-Oct-95		33.55	11.67	0.10	0.16	1.75	1.14	3.68	1.13	
25-Oct-95		34.01	10.25	0.10	0.16	1.56	1.39	3.42	1.11	
31-Oct-95		33.59				0.74	0.75	2.10	0.82	1.90
7-Nov-95	13.5	30.25	46.69	0.93	0.14	1.66	1.85	3.56	0.49	
15-Nov-95		31.25	46.69	1.14	0.20	1.93	1.84	3.16	0.45	2.60
23-Nov-95		34.18	15.33	0.21	0.14	1.81	2.83	5.61	1.10	
29-Nov-95		33.71	8.73	0.10	0.32	2.87	1.91	5.81	1.89	1.50
6-Dec-95	19.0		11.67	0.10	0.16	2.94	2.43	6.79	1.62	
13-Dec-95		35.07	9.11	0.21	0.09	1.57	1.42	2.11	0.60	1.60
27-Dec-95	16.0	25.28	72.80	0.52	0.20	1.95	1.86	6.49	1.25	5.30
3-Jan-96	16.0	1.39	200.77	6.13		1.94	1.43	17.78	3.66	
24-Jan-96	16.0	31.85	334.52	0.21	0.20	3.27	2.34	4.93	0.76	2.60
31-Jan-96		5.82	208.36	0.62		2.55	2.27	14.56	3.07	
7-Feb-96		28.68	8.26	0.10	0.07	1.99	1.64	2.01	0.85	3.90
21-Feb-96		31.25	501.63	0.52	0.14	2.23	1.66	2.39	0.34	3.10
6-Mar-96		31.68	11.29	0.10	0.12	3.10	3.02	4.17	1.12	2.60
13-Mar-96			10.72	0.10	0.09	2.36	1.74	2.58	0.92	
20-Mar-96	16.0	34.26	7.70	0.10	0.11	3.95	2.65	3.88	1.13	1.70
27-Mar-96		33.81	8.83	0.10	0.11	2.27	1.83	3.36	1.01	
3-Apr-96		33.52	16.30	0.21	0.12	3.87	3.12	8.28	2.44	2.00
10-Apr-96	12.5	33.02	11.39	0.10	0.07	1.53	1.38	3.31	1.05	
19-Apr-96		27.73	112.20	0.00	0.17	1.60	1.34	3.56	1.23	3.60
24-Apr-96	13.5	2.99		1.70	0.19	3.41	3.41	13.39	3.77	
1-May-96		27.35	109.60	0.30	0.21	1.97	1.97	2.51	0.99	4.10
8-May-96	13.0	31.90	32.40	0.30	0.21	2.92	2.92	2.79	0.90	
15-May-96	14.0	32.67	19.80	0.20	0.19	0.77	0.77	0.99	0.50	1.80
22-May-96	13.0	33.55	16.30	0.20	0.17	2.26	2.26	2.39	0.84	
29-May-96	10.0	32.61	20.30	0.20	0.14	1.46	1.46	2.10	0.86	1.90
5-Jun-96	11.0	32.75	17.50	0.20	0.15	1.83	1.83	2.86	1.07	
12-Jun-96	10.0	33.90	13.50	0.30	0.19	1.53	1.53	1.79	0.79	1.40
19-Jun-96	11.0	34.51	15.80	0.70	0.32	1.68	1.68	2.07	0.99	
26-Jun-96	11.0	33.64	17.70	0.50	0.25	1.69	1.69	1.62	0.88	1.40
3-Jul-96	9.0	33.51	14.60	0.30	0.23	1.35	1.15	1.14	0.85	
17-Jul-96	9.0	10.22				1.64	1.34	4.38	1.40	
24-Jul-96	7.5	21.81		0.20	0.11	2.77	2.38	3.95	1.25	4.90
31-Jul-96	10.0	0.47				3.69	3.48	14.10	3.47	13.00
7-Aug-96	10.5	13.49				2.03	2.18	9.18	2.63	
14-Aug-96	10.0	28.93	41.80	0.30	0.16	4.06	2.23	7.70	2.21	3.10
21-Aug-96	10.0	24.80	81.30	0.10	0.11	1.71	1.44	4.15	1.29	
28-Aug-96	12.0	30.78	14.60	0.10	0.14	1.64	1.57	2.16	0.83	2.60
11-Sep-96	12.0	33.20	11.90	0.00	0.13	3.16	1.95	3.34	1.79	1.00
18-Sep-96	13.0	32.49	18.00	0.00	0.14	2.35	1.71	4.78	2.23	
2-Oct-96	13.0	6.62				2.86	1.59			
9-Oct-96	16.0	15.75				3.83	2.96	11.11	3.72	
16-Oct-96	11.0	27.13	33.70	0.00	0.10	2.07	1.78	2.40	1.05	
30-Oct-96	13.0	33.16	21.50	0.00	0.09	2.14	2.15	2.35	0.80	1.20
6-Nov-96	21.0	17.88				2.45				11.10
13-Nov-96	18.0	33.21	5.00	0.00	0.24	1.97	3.68	7.16	1.88	1.50
20-Nov-96		33.95	20.90	0.00	0.08	1.95	1.27	2.84	1.14	
27-Nov-96	19.0	34.17	31.20	0.00	0.13	1.82	1.60	4.03	1.30	1.60
18-Dec-96		34.95	22.10	0.00	0.11	3.52	2.08	3.50	0.95	1.60
31-Dec-96		36.19	20.90	0.00	0.11	3.02	1.43	3.31	1.20	1.90
8-Jan-97		36.39	24.50	0.00	0.11	2.45	1.65	2.45	1.34	
15-Jan-97		36.64	19.20	0.00	0.12	3.54	1.94	3.47	1.18	2.20
22-Jan-97		35.76	21.50	0.00	0.09	3.37	1.32	3.20	1.73	
29-Jan-97		35.51	7.10	0.00	0.09	6.74	1.21	3.06	1.44	
5-Feb-97	22.0	35.88	10.80	0.00	0.17	8.47	2.55	5.28	1.67	
12-Feb-97		34.62	18.60	0.00	0.20	4.76	1.62	1.40	0.63	2.60
19-Feb-97		34.79	26.90	0.00	0.22	2.76	2.10	2.39	0.93	
26-Feb-97		35.31	6.07	0.21	0.39	7.81	3.78	8.91	2.17	2.00
5-Mar-97		35.33	5.10	0.21	0.10	3.50	2.20	3.61	1.12	
12-Mar-97		35.84	6.57	0.33	0.20	4.96	1.88	5.87	1.45	2.30

Date	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	Chl a (µg/L)	Chl a (<20 µm) (µg/L)	TPM (<20 µm) (mg/L)	POM (<20 µm) (mg/L)	DOC (mg/L)
19-Mar-97		36.01	4.14	0.22	0.14	4.42	1.71	2.83	0.94	
26-Mar-97	16.0	35.94	5.59	0.56	0.39	15.26	3.89	6.14	2.15	
2-Apr-97	15.0	33.66	11.14	0.17	0.10	3.80	2.24	2.26	2.09	
9-Apr-97	14.0	33.91	7.56	0.29	0.22		2.38	2.89	1.08	2.00
16-Apr-97		34.13	8.06	0.24	0.12		1.78	2.65	1.11	
23-Apr-97	13.0	34.79	6.57	0.06	0.12	4.60	2.25	1.71	0.80	
30-Apr-97	14.5	34.86	7.06	0.06	0.10	2.33	2.02	1.70	0.73	2.00
7-May-97	12.5	34.86	8.56	0.06	0.12	2.49	2.22	1.33	0.65	1.60
14-May-97	11.0	34.14	10.62	0.06	0.08	2.50	2.19	1.30	0.68	
21-May-97	9.0	34.60	6.59	0.07	0.10	3.27	1.95	1.35	0.63	1.60
28-May-97		34.40	6.11	0.07	0.08	2.39	1.91	1.36	0.62	
4-Jun-97	9.0	34.41	5.63	0.13	0.12	2.50	2.09	1.60	0.76	1.60
11-Jun-97	10.0	34.34	5.64	0.07	0.07	1.70	1.11	0.77	0.48	
18-Jun-97	9.0	34.35	4.67	0.07	0.08	1.47	0.96	0.77	0.39	1.60
25-Jun-97	8.0	33.88	4.68	0.07	0.05	1.53	0.94	1.78	0.62	
average (±s.d.)	14.3 (± 3.8)	31.5 (± 7.5)	23.3 (± 54.2)	0.35 (± 0.93)	0.24 (± 0.26)	2.66 (± 1.65)	1.98 (± 0.74)	4.21 (± 3.30)	1.29 (± 0.82)	2.56 (± 1.98)

For statistical compilation (see below) nitrate levels reported as <0.05 have been assumed to have a value of 0.025.

Average (± s.d.) annual values of data - calendar year basis

Year	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	Chl. a (µg/L)	Chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)
From Oct 92	15.3 (± 1.9)		15.9 (± 8.7)	0.24 (± 0.05)	0.31 (± 0.06)	1.75 (± 0.66)				
1993	15.8 (± 2.4)		8.0 (± 5.5)	0.22 (± 0.35)	0.35 (± 0.50)	2.17 (± 0.42)				
1994	15.2 (± 5.0)	34.6 (± 1.4)	9.7 (± 5.2)	0.09 (± 0.14)	0.22 (± 0.13)	2.23 (± 1.09)	2.13 (± 0.92)	3.60 (± 3.16)	1.08 (± 1.02)	2.16 (± 0.69)
1995	14.3 (± 4.8)	33.5 (± 3.0)	20.2 (± 18.0)	0.72 (± 1.59)	0.31 (± 0.19)	2.78 (± 1.54)	1.92 (± 0.79)	4.69 (± 2.90)	1.29 (± 0.75)	2.31 (± 1.07)
1996	12.7 (± 3.2)	26.6 (± 10.6)	59.8 (± 105.5)	0.39 (± 1.03)	0.15 (± 0.06)	2.39 (± 0.82)	2.00 (± 0.68)	4.87 (± 4.07)	1.48 (± 0.93)	3.20 (± 2.99)
Up to June 1997	12.5 (± 3.9)	35.0 (± 0.8)	10.0 (± 6.6)	0.12 (± 0.14)	0.14 (± 0.09)	4.20 (± 3.05)	2.00 (± 0.71)	2.80 (± 1.93)	1.10 (± 0.53)	1.95 (± 0.35)

Average (± s.d.) annual values of data - seasonal basis (July-June)

Year	T (°C)	Salinity (ppt)	Silicate (µM)	Nitrate (µM)	Phosphate (µM)	Chl. a (µg/L)	Chl. a (<20µm) (µg/L)	TPM (<20µm) (mg/l)	POM (< 20 µm) (mg/l)	DOC (mg/L)
1992/93	15.9 (± 1.9)		10.9 (± 7.8)	0.15 (± 0.11)	0.35 (± 0.37)	2.12 (± 0.54)				
1993/94	16.3 (± 3.0)		6.9 (± 3.4)	0.26 (± 0.45)	0.29 (± 0.49)	2.28 (± 0.83)				
1994/95	14.5 (± 5.2)	34.6 (± 1.8)	12.4 (± 7.4)	0.40 (± 1.33)	0.33 (± 0.19)	2.93 (± 1.61)	2.15 (± 0.84)	4.19 (± 2.64)	1.22 (± 0.81)	2.10 (± 0.77)
1995/96	12.9 (± 2.7)	29.8 (± 8.4)	57.7 (± 101.8)	0.68 (± 1.28)	0.16 (± 0.06)	2.08 (± 0.77)	1.85 (± 0.69)	4.73 (± 4.11)	1.29 (± 0.92)	2.55 (± 1.08)
1996/97	12.6 (± 3.8)	30.6 (± 8.7)	15.4 (± 14.4)	0.10 (± 0.13)	0.14 (± 0.07)	3.41 (± 2.43)	2.00 (± 0.70)	3.76 (± 2.87)	1.33 (± 0.75)	3.00 (± 3.00)

APPENDIX iv. Fatty acid compositions (% of total acids) in monthly-integrated samples (4-5 samples taken at weekly intervals) in seawater at Pipe Clay Lagoon.

Fatty acid	Nov-94	Dec-94	Jan-95	Feb-95	Mar-95	Apr-95	May-95	Jun-95	Jul-95	Aug-95	Sep-95	Oct-95	Nov-95	Dec-95	Jan-96	Feb-96	7-Mar-96	14-Mar-96	21-Mar-96	28-Mar-96	3-Apr-96	Apr-96	Monthly average
Saturated Fatty Acids																							
14:0	6.7	4.4	4.4	4.5	5.4	2.6	4.3	5.6	6.9	5.9	5.9	7.4	5.7	4.4	6.9	5.4	6.5	5.0	8.5	4.9	6.5	5.7	5.5 ± 1.2
15:0	1.3	1.3	1.2	1.2	1.3	1.0	1.1	0.1	1.2	1.4	1.3	1.7	1.6	1.0	1.5	1.4	3.4	1.5	1.4	1.7	2.1	1.7	1.3 ± 0.4
16:0	22.2	21.0	17.5	21.0	20.0	21.2	20.4	19.6	19.4	19.7	20.6	21.9	23.8	20.8	23.3	23.0	29.6	22.5	24.1	24.8	24.7	23.1	21.3 ± 1.9
17:0	0.7	0.7	0.6	0.6	0.7	0.7	0.6	0.6	0.7	0.6	0.7	0.8	1.0	0.7	0.8	0.9	1.3	0.8	0.8	1.0	1.1	1.0	0.7 ± 0.1
18:0	5.7	5.1	6.2	5.4	5.1	5.5	7.5	4.7	6.4	5.8	5.8	7.4	8.0	5.6	7.7	8.2	16.1	9.2	9.4	12.4	13.6	10.6	6.8 ± 1.9
19:0	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.4	0.3	0.4	0.2	0.3	0.3	0.6	0.6	0.3	0.3	0.3 ± 0.1
20:0	0.7	0.8	1.0	0.9	0.9	0.6	0.6	0.9	0.9	0.6	0.8	0.8	0.6	0.5	0.9	0.7	0.9	0.8	1.1	1.1	1.1	1.0	0.8 ± 0.1
22:0	0.5	0.7	0.6	0.5	0.6	0.7	0.5	0.6	0.7	0.6	0.7	0.8	0.6	0.4	0.7	0.6	0.4	0.6	0.6	0.8	0.9	0.9	0.6 ± 0.1
Total Saturated	38.1	34.2	31.8	34.4	34.3	32.7	35.2	32.5	36.7	35.0	36.0	40.9	41.7	33.6	42.1	40.5	58.4	40.8	46.5	47.3	50.3	44.2	37.3 ± 4.6
Branched Fatty Acids	2.2	2.1	2.7	2.4	2.8	2.0	4.5	1.8	1.8	2.9	2.1	2.4	2.8	1.7	2.5	2.6	3.5	4.1	2.0	2.6	2.5	3.3	2.5 ± 0.7
Monoenoic Fatty Acids																							
16:1(n-9)	2.8	2.6	0.3	0.4	0.9	1.2	1.5	0.4	0.6	1.3	1.2	1.4	2.7	0.5	2.2	2.6	10.9	3.8	0.9	1.5	3.2	2.4	1.6 ± 1.1
16:1(n-7) & 16:2(n-4)	9.3	9.4	11.2	13.9	13.1	7.5	9.0	9.0	13.1	13.5	9.5	13.9	8.1	8.4	7.7	8.8	2.0	9.1	9.3	7.7	9.6	10.0	10.1 ± 2.4
16:1(n-5)	0.8	0.9	0.9	0.9	0.9	0.4	0.4	0.7	0.5	0.4	0.7	0.9	0.3	0.3	0.7	0.4	0.3	0.4	0.4	0.6	0.4	0.6	0.6 ± 0.2
16:1(n-13)t	1.1	1.2	1.0	1.1	1.4	1.2	1.2	1.6	1.3	1.6	1.4	1.1	0.9	0.3	0.9	0.8	0.2	0.5	1.2	1.3	1.0	1.5	1.1 ± 0.3
18:1(n-9)	5.8	6.2	7.6	5.7	5.7	7.2	6.0	5.1	7.7	4.8	8.9	7.0	7.7	28.0	15.9	7.0	12.7	6.9	6.2	8.7	4.8	6.3	8.4 ± 5.5
18:1(n-7)	4.0	6.2	5.4	4.9	5.4	5.7	3.3	3.2	3.9	4.4	4.1	4.4	5.2	4.1	5.1	5.6	1.5	7.8	3.5	6.9	4.2	6.6	4.8 ± 0.9
20:1(n-9)	0.2	0.1	1.3	0.0	0.3	0.2	0.2	0.3	0.3	0.1	0.3	0.3	0.2	3.3	1.4	0.2	0.1	0.3	0.4	0.7	0.1	0.4	0.5 ± 0.8
other	0.6	0.7	1.2	0.5	0.4	0.4	0.1	0.3	0.3	0.4	0.6	0.5	0.3	0.6	0.7	0.3	0.3	0.7	0.6	0.7	0.5	0.6	0.5 ± 0.2
Total Monoenoic	24.5	27.3	28.9	27.5	28.0	23.7	21.5	20.7	27.8	26.6	26.7	29.5	25.3	45.7	34.7	25.8	28.1	29.5	22.5	28.1	23.8	28.4	27.8 ± 5.4
Polyenoic Fatty Acids																							
16:4(n-1)	1.8	2.1	1.3	1.9	2.5	2.3	2.4	3.6	3.1	3.2	2.4	1.7	1.5	0.7	1.2	1.5	0.2	1.4	2.2	1.3	1.8	2.2	2.0 ± 0.8
16:2(n-7) & 16:3(n-4)	0.6	0.7	0.6	0.9	0.8	0.6	0.8	1.0	1.2	1.7	0.8	0.7	0.4	0.3	0.5	0.5	0.2	0.5	0.7	0.5	0.7	1.1	0.8 ± 0.3
18:2(n-6)	2.3	2.5	2.6	2.4	2.1	2.5	2.2	2.4	2.8	1.7	3.1	2.3	2.4	1.2	2.0	2.7	1.5	2.8	2.3	4.2	1.3	1.9	2.3 ± 0.4
18:3(n-3)	4.1	2.9	2.2	2.8	2.7	4.1	3.3	2.9	2.1	1.7	3.5	2.2	2.5	0.9	1.5	2.7	0.7	1.3	2.4	1.6	1.2	1.8	2.5 ± 0.9
18:3(n-6)	4.5	5.1	3.4	5.2	4.4	7.1	6.4	7.3	4.1	3.9	5.3	2.4	3.5	1.0	1.8	4.6	2.0	3.1	3.5	1.5	2.5	2.2	4.2 ± 1.8
18:4(n-3)	5.7	5.3	4.2	0.2	4.8	7.6	6.5	7.6	4.9	5.2	6.2	3.4	3.8	1.4	2.1	4.0	0.8	2.1	3.5	1.9	2.4	3.3	4.4 ± 2.1
20:4(n-6)	0.7	0.8	1.0	0.8	0.9	0.7	0.6	0.0	0.0	0.0	0.6	0.8	0.7	1.1	0.7	0.7	0.2	0.8	0.6	1.0	0.7	0.8	0.6 ± 0.3
20:5(n-3)*	6.7	7.3	9.0	9.3	8.4	7.8	8.4	9.4	9.6	10.8	6.5	8.1	6.1	2.9	4.0	6.2	1.1	5.6	6.1	4.6	7.2	6.4	7.3 ± 2.1
22:5(n-6)	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.1	0.4	0.2	0.3	0.1 ± 0.2
22:6(n-3)#	7.4	8.1	9.4	10.7	6.9	7.7	7.3	9.5	4.5	6.4	5.4	4.3	6.1	6.3	5.1	7.0	2.9	6.8	6.0	3.0	4.4	3.2	6.7 ± 2.0
other	1.2	1.5	2.5	1.3	1.2	1.4	0.8	1.6	1.0	0.9	1.1	1.2	2.9	2.8	1.3	0.9	0.3	1.1	1.4	1.6	0.6	0.8	1.4 ± 0.7
Total Polyenoic	35.1	36.4	36.6	35.5	34.8	41.6	38.8	45.1	33.6	35.5	35.1	27.1	30.0	19.0	20.6	31.0	9.9	25.5	28.9	21.7	23.0	24.0	32.3 ± 7.3
Fatty acids (µg/L)	11.7	9.0	12.4	9.1	8.3	8.1	7.8	21.4	2.9	6.0	8.3	7.1	7.3	53.3	10.3	6.1	66.6	9.0	12.3	8.0	18.8	5.3	12.1 ± 11.5

* includes 20:4(n-6) if "0" listed in table for this fatty acid # includes 22:5(n-6) if "0" listed in table for this fatty acid other = fatty acids =<0.5% in all samples

APPENDIX v. Fatty acid compositions (% of total acids) in monthly-integrated samples (4-5 samples taken at weekly intervals) in seawater at Little Swanport.

Fatty acid	Nov-94	Dec-94	Jan-95	Feb-95	Mar-95	Apr-95	May-95	Jun-95	Jul-95	Aug-95	Oct-95	Average
Saturated Fatty Acids												
14:0	1.8	5.4	4.5	3.9	2.1	4.6	4.3	4.8	4.2	3.7	3.2	3.9 ± 1.1
15:0	0.7	1.2	1.0	1.3	0.9	1.4	1.3	1.6	1.5	1.1	1.2	1.2 ± 0.3
16:0	17.1	18.5	15.2	19.4	19.0	20.6	18.0	24.1	18.3	18.2	19.8	18.9 ± 2.2
17:0	0.5	0.5	0.5	0.6	0.6	0.7	0.7	0.9	0.8	0.7	0.8	0.7 ± 0.1
18:0	6.6	3.8	3.6	4.6	5.6	6.2	5.2	8.5	7.1	6.7	5.5	5.7 ± 1.5
19:0	0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.4	0.2	0.2	0.2	0.3 ± 0.1
20:0	0.7	0.5	0.4	0.7	0.6	0.8	0.8	1.1	0.8	1.0	0.7	0.7 ± 0.2
22:0	0.7	0.6	0.5	0.8	0.7	1.0	0.7	1.1	0.9	1.3	0.9	0.8 ± 0.3
Total Saturated	28.3	30.6	25.9	31.6	29.7	35.6	31.2	42.7	33.7	33.0	32.3	32.2 ± 4.4
Branched Fatty Acids	3.6	3.5	2.1	3.1	3.1	2.7	2.8	3.0	3.3	3.9	3.3	3.1 ± 0.5
Monoenoic Fatty Acids												
16:1(n-9)	0.8	1.0	0.9	1.0	0.5	1.4	1.0	2.3	3.1	0.9	1.5	1.3 ± 0.8
16:1(n-7) & 16:2(n-4)	6.0	10.8	9.8	9.4	7.6	9.3	10.1	7.9	7.7	8.5	6.3	8.5 ± 1.6
16:1(n-5)	0.4	0.5	0.4	0.7	0.6	0.7	0.4	0.5	0.7	1.3	0.5	0.6 ± 0.3
16:1(n-13)t	1.5	1.3	1.1	1.8	1.2	1.3	1.7	0.9	1.6	1.1	1.3	1.4 ± 0.3
18:1(n-9)	4.6	3.9	6.9	4.0	5.3	7.0	6.3	9.6	8.0	8.1	9.1	6.6 ± 2.0
18:1(n-7)	7.9	6.4	5.3	8.8	7.2	7.0	8.6	6.0	6.1	7.0	5.9	6.9 ± 1.1
20:1(n-9)	0.2	0.1	1.4	0.4	0.1	0.2	0.2	0.5	0.2	0.4	0.3	0.4 ± 0.4
other	0.5	0.5	0.3	0.9	0.5	1.2	0.3	0.2	0.5	0.7	0.4	0.5 ± 0.3
Total Monoenoic	21.8	24.6	26.2	27.0	23.0	28.1	28.7	27.8	28.1	28.0	25.2	26.2 ± 2.3
Polyenoic Fatty Acids												
16:4(n-1)	2.5	2.1	1.7	2.1	1.6	2.1	1.9	1.8	2.5	2.2	1.8	2.0 ± 0.3
16:2(n-7) & 16:3(n-4)	0.6	1.4	1.0	1.4	0.6	1.0	0.8	0.8	1.1	0.7	0.5	0.9 ± 0.3
18:2(n-6)	1.9	2.3	2.3	2.1	2.2	2.6	2.7	4.1	2.6	2.4	2.1	2.5 ± 0.6
18:3(n-3)	5.9	4.8	2.7	5.5	5.3	3.7	3.2	1.6	1.6	2.5	3.6	3.7 ± 1.5
18:3(n-6)	4.8	2.4	2.7	2.5	3.8	3.3	4.1	2.2	3.3	4.5	3.4	3.4 ± 0.9
18:4(n-3)	9.9	6.1	5.2	5.9	7.8	6.0	7.5	2.8	5.3	6.3	8.3	6.5 ± 1.9
20:4(n-6)	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	0.0	0.2 ± 0.3
20:5(n-3)*	9.5	11.8	12.6	10.0	10.8	8.5	8.4	6.9	9.7	8.4	10.2	9.7 ± 1.6
22:5(n-6)	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.1
22:6(n-3)#	9.3	9.2	13.5	6.8	10.3	5.5	7.4	5.4	7.3	6.5	7.9	8.1 ± 2.4
other	1.2	1.1	3.0	1.6	1.5	0.9	1.1	0.8	1.0	0.9	1.3	1.3 ± 0.6
Total Polyenoic	45.6	41.2	45.7	38.2	44.1	33.5	37.2	26.4	34.8	35.0	39.1	38.3 ± 5.8
Fatty acids (µg/L)	9.5	18.6	23.0	14.3	11.1	13.8	8.7	72.1	8.2	10.5	9.9	18.2 ± 18.5

* includes 20:4(n-6) if "0" listed in table for this fatty acid # includes 22:5(n-6) if "0" listed in table for this fatty acid other = fatty acids =<0.5% in all samples

APPENDIX vi Concentrations of major pigments (ng L^{-1}) in monthly-integrated samples (4-5 samples/month taken at weekly intervals) in seawater at Pipe Clay Lagoon and Little Swanport. Value of 0 = not detected. Pigment abbreviations: c2 + c1 = chlorophyll c2 + c1; fuco-lk = fucoxanthin-like; fuco = fucoxanthin; 19-hex = 19'hexanoyloxyfucoxanthin; diad-lk = diadinoxanthin; diad = diadinoxanthin; allo = alloxanthin; zea = zeaxanthin; chl b = chlorophyll b; chl a = chlorophyll a; ββ-car = β,β-carotene.

Pipe Clay Lagoon

Date/Site	c2+c1	fuco-lk	fuco	19 hex	diad-lk	diad	allo	zea	chl b	chl a	ββ-car
Nov-94	65	78	100	14	0	31	12	46	82	653	33
Dec-94	38	25	25	0	7	13	0	16	39	437	10
Jan-95	0	45	57	22	0	19	13	15	39	481	14
Feb-95	42	104	104	16	13	32	11	22	78	769	27
Mar-95	0	84	90	0	0	15	10	27	78	639	29
Apr-95	0	78	74	9	0	36	0	20	63	727	20
May-95	0	172	219	64	0	71	24	43	104	1131	36
Jun-95	0	205	150	0	0	28	11	8	39	953	17
Jul-95	90	170	160	0	0	54	34	32	159	1194	50
Aug-95	0	0	500	0	0	75	12	19	79	1065	39
Sep-95	71	91	116	18	0	32	23	46	122	833	37
Oct-95	13	50	52	0	0	18	5	18	23	343	11
Nov-95	58	0	140	23	0	26	11	31	58	471	29
Dec-95	0	67	59	0	13	17	7	22	72	594	29
Jan-96	9	71	65	0	0	24	0	17	34	443	3
Feb-96	60	72	72	31	0	43	20	28	86	686	39
Mar-96	44	74	65	0	10	36	6	38	59	591	21
Apr-96	0	72	53	0	0	10	6	5	23	422	2
May-96	97	109	148	0	0	29	19	39	96	896	39
Jun-96	0	60	54	0	0	9	18	17	45	533	22
Jul-96	67	114	108	22	0	32	17	39	83	825	31
Aug-96	69	66	102	0	0	23	15	25	59	664	28
Sep-96	0	69	59	0	0	16	4	15	53	461	18
Oct-96	0	34	28	0	0	8	0	6	8	183	6
Nov-96	0	5	24	0	0	6	0	10	16	205	7
Dec-96	27	69	50	0	14	19	0	16	22	447	19
Jan-97	20	0	55	0	0	9	0	15	33	227	99
Feb-97	0	28	22	0	0	4	0	11	13	179	7
Mar-97	18	48	46	0	0	7	0	24	51	374	21
Average	27	71	96	8	2	26	10	23	59	601	26
s.d.	(± 32)	(± 50)	(± 91)	(± 14)	(± 5)	(± 18)	(± 9)	(± 12)	(± 35)	(± 277)	(± 19)

Little Swanport

Nov-94	0	77	110	0	0	10	53	44	260	956	7
Dec-94	13	376	370	0	53	54	45	75	195	3994	115
Jan-95	13	251	185	0	35	24	0	42	127	1620	72
Feb-95	34	215	178	0	0	9	75	7	182	1755	58
Mar-95	0	110	119	0	19	14	70	54	145	1272	50
Apr-95	21	141	99	0	19	16	18	13	95	1105	5
May-95	0	117	93	0	13	9	36	6	156	1058	9
Jun-95	0	40	271	0	31	41	38	64	488	2832	143
Jul-95	0	88	57	0	8	10	7	7	137	462	12
Aug-95	0	108	86	0	16	11	19	8	137	751	16
Oct-95	0	76	85	0	17	21	66	32	111	979	43
Nov-95	0	131	120	0	34	19	52	89	126	1396	8
Dec-95	0	68	66	0	11	11	29	34	147	1116	42
Jan-96	0	59	101	0	0	20	0	39	74	878	45
Feb-96	33	105	97	0	15	12	65	9	87	1282	49
Mar-96	0	200	210	0	32	26	76	67	157	1939	64
Nov-96	13	34	36	0	12	9	14	33	112	766	32
Dec-96	0	25	73	0	0	11	66	25	240	1327	53
Jan-97	0	107	132	0	7	17	39	100	91	1368	45
Feb-97	0	126	101	0	49	17	24	60	187	1282	67
Mar-97	0	424	419	0	54	131	49	83	249	2956	140
Average	6	137	143	0	20	23	40	42	167	1481	51
s.d.	(± 11)	(± 105)	(± 100)	(± 0)	(± 17)	(± 27)	(± 25)	(± 29)	(± 90)	(± 843)	(± 40)

AMSA, July 1996, Hobart

SUPPLEMENTARY FEED INCREASES PRODUCTION OF JUVENILE PACIFIC OYSTERS

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The juvenile Pacific oyster industry, which supplies growers with spat, is presently valued at $1.5 m p.a. Hatchery-produced spat are grown-on in land-based nurseries, often by pumping local seawater through upweller systems. This technique has been used by nurseries for over a decade as it is effective and saves on food costs. However, growth is limited in this system due to the reliance on the productivity of the local waterway to supply the nutritional requirements of the spat.
We are undertaking a 3 year FRDC-funded project to cost-effectively increase growth rates of juvenile Pacific oysters by supplementing their natural diet with cultured microalgae. After 2 years we have completed 10 feeding trials, testing 7 species of algae and 2 algal pastes. We have investigated the effect of the diet composition, animal size, food quantity and methods of feeding.
Our results show:

- Oyster growth rates increased with higher supplementary food concentration.
- *Isochrysis* sp. (T ISO), *Dunaliella tertiolecta, Chaetoceros calcitrans* and *Chroomonas* sp. were the most effective live diets. Increases in growth rates ranged from 73-81% when oysters were fed a supplementary ration of 7.5mg dry wt. algae/g wet oysters
- Supplementary algal diet lacking polyunsaturated fatty acids (*D. tertiolecta*) gave similar increases in growth rates to an algal diet rich in the essential 22:6(n-3) (*Isochrysis* sp.).
- Algal pastes were effective supplementary food sources. *Skeletonema costatum* and *C. calcitrans* pastes gave increases comparable to those of the fresh *C. calcitrans* culture.
- Low water temperature reduced the effectiveness of supplementary feeding.
- Two rations/day were more effective than one ration/day.

Supplementary feeding may be a cost-effective method of increasing production of juvenile Pacific oysters. Our future work will examine other supplementary foods such as yeasts, and will compare the effectiveness of supplementary feeding between nursery sites.

ASPAB, Jan 1997, Hobart

SUPPLEMENTARY FEED INCREASES THE GROWTH OF JUVENILE PACIFIC OYSTERS

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- Algal pastes were effective supplementary food sources. *Skeletonema costatum* and *C. calcitrans* pastes gave increases comparable to those of the fresh *C. calcitrans* culture.
- Low water temperature reduced the effectiveness of supplementary feeding.
- Two rations/day were more effective than one ration/day.

Supplementary feeding may be a cost-effective method of increasing production of juvenile Pacific oysters. Preliminary results from our recent work, which examines other supplementary foods such as dried yeast and algal products, will also be presented.

AMSA July, 1997, Auckland, New Zealand

Improvement in feeding techniques for the production of juvenile Pacific oysters (*Crassostrea gigas*)

Brown, Malcolm[1], McCausland, Malcolm[1] and Knuckey, Richard[2]
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Juvenile Pacific oysters (*Crassostrea gigas*) are commonly cultured in land-based systems of flowing seawater. These systems are effective, but the food abundance and quality can be limiting in the inflowing water and therefore a constraint to oyster growth.

Our research has aimed to develop cost-effective feeding techniques to supplement the natural levels of food in seawater, to improve oyster production. Diets have included conventional Northern hemisphere strains of microalgae used in aquaculture, new Australian microalgae held in the CSIRO Culture Collection and isolated during the project, microalgal pastes, and "off-the-shelf" artificial diets. They have been added directly to the inflowing seawater, and their effects on oyster growth evaluated over 2-3 weeks. Factors examined, and to be discussed in this presentation include:

- diet type and concentration
- food presentation (frequency and duration)
- size class of oysters (from 500–1300μm)
- seasonality, and growth at an alternative nursery site

Supplementary feeding with live microalgae has been the most effective. Growth rates of oysters have increased from 200 to 500% (over 2 to 3 weeks) at the Pipeclay Lagoon site, leading to a potential reduction in the production time at the nursery by more than half. The cost-benefit of supplementary feeding will be discussed.

Tas. Aquaculture Exchange, July 1997, Hobart

INCREASING THE PRODUCTION OF JUVENILE OYSTERS (*C. GIGAS*) THROUGH SUPPLEMENTARY FEEDING

M. Brown[1], M. McCausland[1], R. Knuckey[2], M. John[3] and
G. Hollingsworth[3]

[1] CSIRO Division of Marine Research, Hobart Tas.; [2] CRC for Aquaculture; [3] Shellfish Culture Ltd., Sandford, Tas.

Juvenile Pacific oysters (*Crassostrea gigas*) are commonly cultured in land-based systems of flowing seawater. These systems are effective, but the food abundance and quality can be limiting in the inflowing water and therefore a constraint to oyster growth.

Our research has aimed to identify cost-effective diets to supplement the natural levels of food in seawater, to improve oyster production. Diets have been added directly to the inflowing seawater, and their effects on oyster growth evaluated over 2-3 weeks. Factors examined, and to be discussed in this presentation include:

- diet type (live, pasted or dried microalgae, yeast)
- diet concentration
- food presentation (frequency, duration)
- seasonality
- size class of oysters (from 500–1300μm)
- site

Supplementary feeding with live microalgae has been the most effective. Growth rates of oysters have increased from 200 to 500% (over 2 to 3 weeks) at the Pipeclay Lagoon site, leading to a potential reduction in the production time at the nursery by more than half. The cost-benefit of supplementary feeding will be discussed.

AMSA (poster), Adelaide, July 1998

IMPROVEMENTS IN GROWTH RATES OF JUVENILE OYSTERS THROUGH SUPPLEMENTARY FEEDING

BROWN, MALCOLM AND MCCAUSLAND, MALCOLM

CSIRO Division of Marine Research, Hobart, Tas, 7000

Juvenile Pacific oysters (*Crassostrea gigas*) are commonly cultured in land-based systems of flowing seawater. These systems are effective, but the food abundance and quality can be limiting. An initial survey showed that the growth rate of oysters at a nursery at Pipeclay Lagoon, Tasmania, was being restricted by inadequate food. We assessed whether the oyster's growth rates could be increased at the site by supplementing their diet. Juvenile *C. gigas* were grown in experimental upwellers. Each trial compared a control (receiving only flowing seawater) with supplementary-fed oysters (flowing seawater, plus diet). In 14 trials we tested 8 different algal diets, 2 algal pastes and 2 dried diets as supplementary feed – and also different methods of feed presentation. Diets were typically fed so as to increase the background food concentration by 150 to 225%

Supplementary feeding varied in effectiveness, depending on diet and season. Typically, it increased oyster's growth rates by ≥ 100%. The most effective diets included *Isochrysis* sp. (T.ISO), *D. tertiolecta, Rhodomonas salina, C. calcitrans* (live or paste) and *Skeletonema costatum* (paste). Supplementary feeding was cost-effective when natural productivity was otherwise low. We estimate that growing microalgae for feeding would only add ≈2–3% onto the production cost of juvenile oysters. However, as supplementary feeding would significantly shorten production, there would be a reduction in labour costs that could more than offset the feed costs.

SCIENCE AND TECHNOLOGY

EDITED B

Marine nutrition . . . CSIRO researcher Dr Malcolm Brown checks cultured algae which will be fed to baby oysters — Picture: BRUCE MONTGOMERY

Diet of algae makes oysters grow faster

By CLAIRE HARVEY

MALCOLM Brown likes his oysters to be large, covered in algae — and served in a 10 litre bucket.

Dr Brown and his colleagues at the CSIRO have been able to triple the growth rate of juvenile Pacific oysters through their three-year research project, which involves feeding different types of algae to the creatures and measuring their weight.

"We put lots of oysters in a 10 litre plastic bucket and pump in different mixes of cultured algae," said Dr Brown, senior research scientist at the CSIRO's fisheries division.

"By getting the right strain of algae for each species, we can in some cases triple the growth rate."

Oysters naturally feed on algae, but CSIRO's researchers have discovered that different strains can increase the oysters' growth.

A bottleneck in the oyster industry a few years ago meant that companies producing juvenile oysters couldn't keep up with demand from farmers waiting to mature the oysters in estuaries.

"They had slow growth rates in the early juvenile stage, and reduced survival — it appeared from our background research that it was inadequate nutrition," Dr Brown said.

"This shortage of juvenile oysters threatened the whole industry."

Funded by a $300,000 federal Fisheries Research and Development Corporation grant, this project also has health-food applications.

"Beta-carotene, which is becoming very popular in the health-food industry, is one of the natural products that people are busily extracting from these algae," he said.

The greater cost-effectiveness which Dr Brown claims for the research will do little to improve the healthiness of oysters, however, particularly in terms of human consumption.

"Our research concentrates on the juvenile stage, but after about six months they leave that environment and go to the local estuary for a couple of years," he said.

"After that, the water quality is crucial — there are some toxic algaes which can be passed up the food chain to the human consumer."

After leaving the juvenile stage, oyster growth is at the mercy of nature.

"The farmers try to pick a site that has good concentratons of algae, and they leave them for a couple of years — basically it's at the whim of nature and if they're in a good site the oysters grow well," Dr Brown said.

"Once the oysters get to a certain size, it's easier and less costly to select a natural environment suitable for the oysters and then let nature take over.

"This research will mean a cost benefit to seed producers — their seed will be cheaper and faster to grow, so they may increase their overall production. That causes greater development of the larger industry."

with a view to full commercial production.

Scallop trials during the late 1980s and early 1990s have been recently hindered by a lack of availability of scallop spat, but if hatchery-produced scallop becomes a reality, large-scale scallop culture could well be on the books.

For more information contact Bill Fotheringham, Sea Farms, PO Box Middleton, Tas, 7163. Tel : (03) 6293 1314, fax : (03) 6293 2020.

Do oysters go well with *Pavlova?*

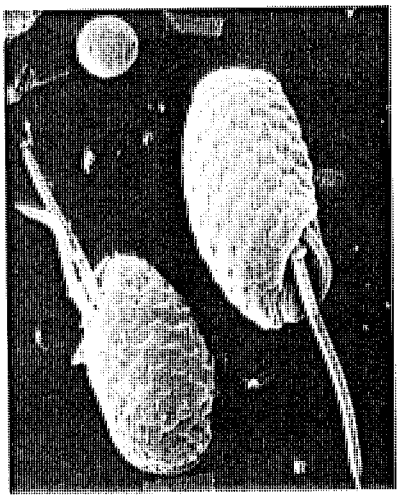
CSIRO scientists at the Division of Fisheries in Hobart fed cultures of the microalga Pavlova pinguis *to juvenile oysters – and doubled the oyster spat's growth rate! It was a clear demonstration of the remarkable nutritional qualities of microalgae, the tiny marine organisms from which all land plants evolved.*

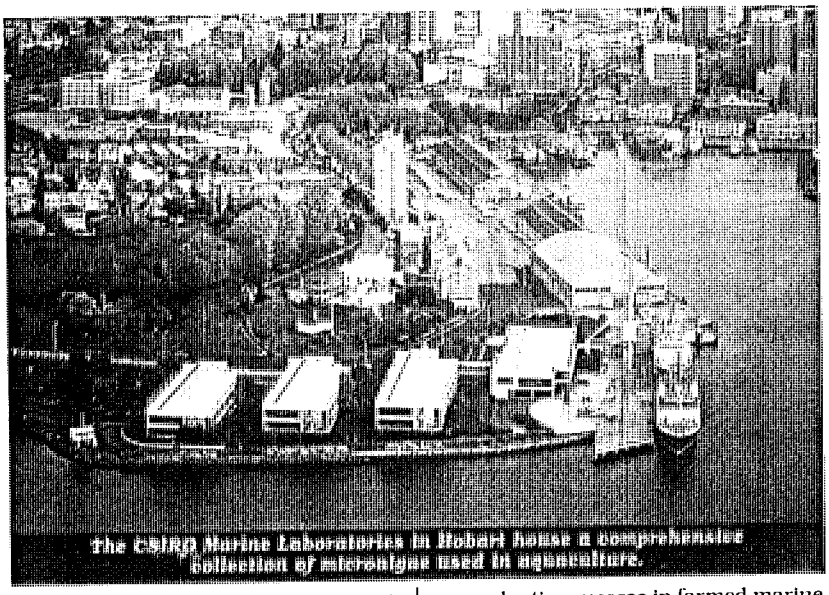
The *Pavlova* so enjoyed by the Tasmanian oysters were cultured in the growing rooms of the CSIRO's Microalgae Culture Collection. In this living information bank, representatives of every known family of marine microalgae live and grow in carefully controlled laboratory conditions. In glistening ranks, flasks of sterilised sea water contain the balance of nutrients that each species prefers. Timing devices change darkness to daylight, and the ambient temperature is held at the level to suit the collection's billions of inhabitants.

CSIRO senior research scientist Dr Sue Blackburn is the director of the collection. "Hundreds of the smallest microalgae could fit on the head of a pin, and a litre of sea water may contain a billion organisms of these single-celled plants, which are the first links in the marine food chain," Dr Blackburn says. Algae are among the most diverse life forms on the planet. The giant kelp plants that grow into towering underwater forests belong to the same plant family as the microscopic phytoplankton that drift with the currents of the world's oceans.

Solar powered pastures of the sea

Like green plants on land, algae use photosynthesis to create new living tissue. Plant pigments absorb sunlight and convert it into carbohydrates from the molecules of carbon dioxide and water. In the process, they absorb carbon dioxide and release large amounts of oxygen. It's the microalgae that make the world's oceans a 'carbon sink,' and play a critical role in maintaining balance in the Earth's atmosphere. These wandering pastures of the sea are grazed by marine animals including zooplankton, fish larvae and filter-feeding molluscs such as oysters, scallops, mussels and clams. A single adult oyster may consume 50 billion algal cells each day!





The CSIRO Marine Laboratories in Hobart house a comprehensive collection of microalgae used in aquaculture.

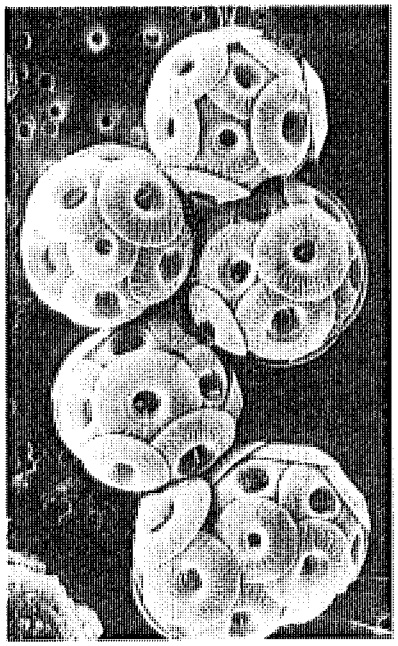
Dr Blackburn says that aquaculture operations use thousands of litres of algal culture daily, to feed the larval and juvenile stages of farmed species including oysters, prawns, abalone, barramundi and giant clams. The CSIRO supplies starter cultures of selected algal strains, from which operators grow the larger quantities needed for feeding. "In any farming operation, the nutritional quality of feed determines the production rates and quality of the finished product," she says. "Better feed means better productivity, and improved returns. That's where the CSIRO's algal collection is of particular value to the industry, because the starter cultures we supply are of the highest quality, and are grown under the stringent scientific conditions generally not found in operations 'on the ground.'"

Targeted nutrition boosts growth rates

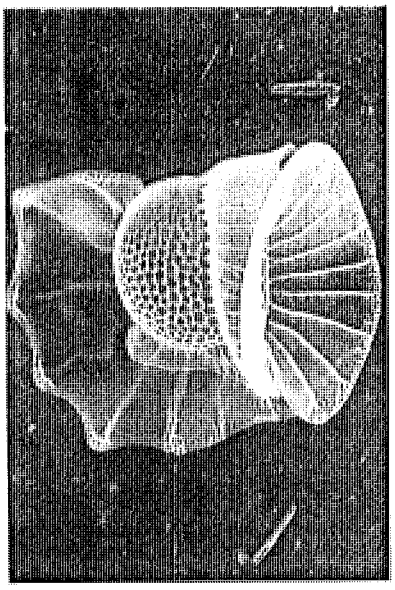
The scientist behind the Pavlova-oyster studies is Dr Malcolm Brown. Along with colleagues at the CSIRO Marine Laboratories in Hobart, Dr Brown has shown the links between the specific nutritional content of algal strains and the particular feeding requirements of farmed species. The knowledge gained means that aquaculture operators can take a very finely targeted approach to nutrition. "The days of 'this green one works OK' should be over," Dr Brown says. "Now, the industry can select an algal strain with the right chemical composition, then adapt its culturing and harvesting techniques to maximise the algae's nutritional quality, boosting growth rates and improving the productivity of the operation."

Proteins, carbohydrate, fats and vitamins – microalgae pack a lot of nutrition into a very tiny parcel. Over the past 10 years, scientists at the CSIRO Division of Oceanography examined 56 strains of microalgae for the presence of long-chain polyunsaturated fatty acids (PUFAs), which are essential for high growth rates and reproductive success in farmed marine animals. The study showed that all strains except green algae contained high proportions of the essential PUFAs.

In another finding with particular relevance to the aquaculture industry, CSIRO research has shown that the nutritional quality of certain microal-



gae changes according to culture method and harvest time. Two methods were studied. In batch culture, the entire crop is harvested at once, at a young or mature growth stage. In the semi-continuous culture method, one-tenth to one-half is removed every one or two days, and replaced with fresh growth medium. Replicating industry practice, 85 litre bag cultures of three different microalgae were grown in batch and semi-continuous mode, and the nutritional composition tested at different growth stages. Results showed that young cultures are highest in protein and lowest in carbohydrates and lipid (fats), while old cultures have less protein but higher carbohydrate and fat levels. So, by understanding how changed culture conditions affect microalgae, nutritional quality can be adjusted to suit the growth needs of particular aquacultured animals.



Studies also proved that different light conditions can change the nutritional composition of microalgae. When cultures of the diatom *Thalassiosira pseudonana* were exposed to continuous light, carbohydrate and saturated fats increased, while polyunsaturated fats were highest in day-night cycles.

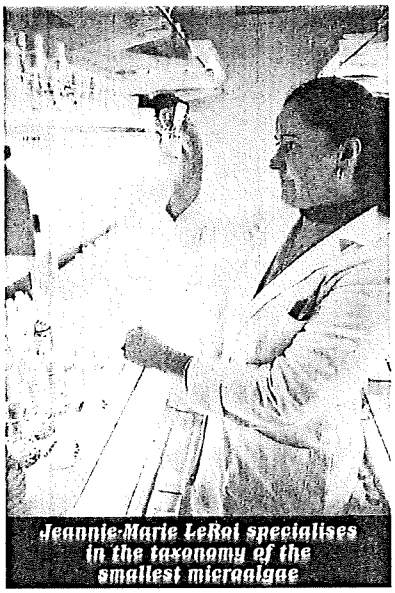
Research shows that although microalgae are rich sources of most vitamins, in some strains the content can be variable, suggesting that mixed algal diets are the most effective way to provide high concentrations of vitamins for aquacultured animals. When these findings are linked to the knowledge that farmed species prefer different nutritional regimes – for example, scallop larvae grow better with high proportions of saturated fatty acids – aquaculture operators have some powerful tools to maximise growth and productivity.

High quality cultures give best results

CSIRO's close association with industry in Australia and overseas means that 'tailor-made' algae suitable for local conditions can be requested. Cultures are quality-assured and supplied in a condition of active, healthy growth. This means that when the industry grows the cultures up to feeding quantities, rapid growth rates can be expected. A range of strains are also available guaranteed bacteria-free.

Dr Blackburn has a word of warning for operators who push their algal cultures too long. "In hatchery conditions, bacterial contamination of feed cultures is hard to avoid, and eventually, the health of the culture suffers," she says. "That's why it's important that fresh starter cultures from high-quality laboratory stock are used at regular intervals."

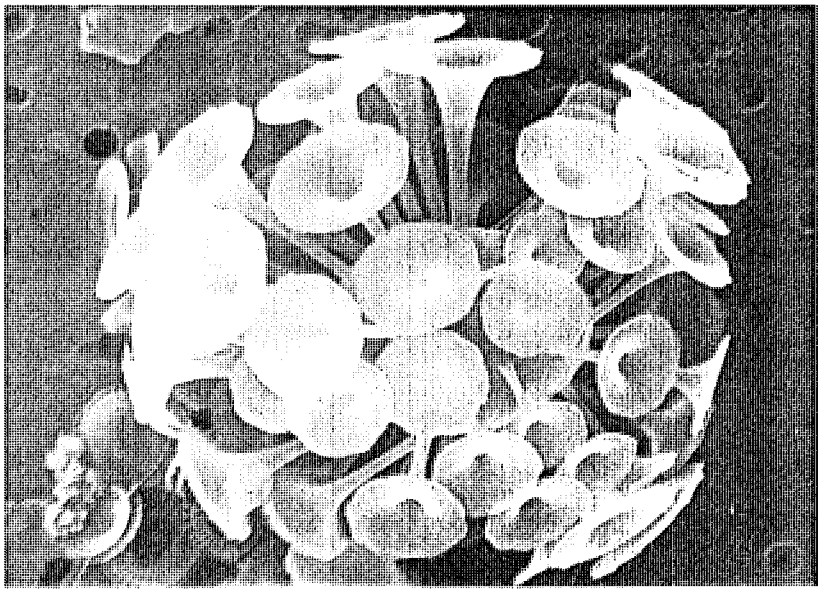
And the cost? It's surprisingly low – a CSIRO-endorsed, quality-assured bacteria-free 20 ml tube of selected algal culture costs only $70, while the price of a 250 ml flask is $120. An air freight delivery service ensures that the living cultures arrive in good con-



Jeannie-Marie LeRoi specialises in the taxonomy of the smallest microalgae

The CSIRO has considerable depth and breadth of expertise in algal culture. The Microalgae Culture Collection was developed through the 1960s and 1970s by Dr Shirley Jeffrey to support the CSIRO's program of research into ocean phytoplankton. The collection contains over 700 strains representing almost 300 species, and includes microalgae from tropical to Antarctic waters. Over 80% are Australian in origin. In the collection are strains of Australian harmful algal bloom species, including toxic cyanobacteria (blue-green algae) and red-tide forming dinoflagellates.

Dr Jeffrey realised the potential value of a microalgal supply service to the aquaculture industry, and the service was established with the assistance of a grant from the Fisheries Research and Development Corporation in 1986. The algal culture facility, within the CSIRO Marine Laboratories in Hobart, Tasmania was built in 1984. The service supplies aquaculture clients and research institutions in Australia and in overseas countries including New Zealand, Japan, Hong Kong, South Africa, Singapore, Germany, USA, Vietnam, the Solomon Islands and the United Kingdom.



dition, within a maximum time of 72 hours.

"Considering the benefits to be gained from finely-targeted nutrition for commercial aquaculture species, the outlay for these cultures is minimal," Dr Blackburn says.

For further information, including a list of available strains and details on their nutritional qualities, contact Cathy Johnston at the CSIRO Microalgae Culture Collection, CSIRO Marine Laboratories, GPO Box 1538 Hobart Tasmania 7001 Australia, phone (03) 62 325 316, fax (03) 62 325 300 or email microalgae@ml.csiro.au

Freshwater Farmer

Continued from page 82

Disadvantages

- Rooted plants have a much longer life cycle than phytoplankton. Thus, the rate of nutrient flow is slower.
- Rooted plants are relatively large and cannot be readily used by zooplankton unless they become partly decomposed.
- Rooted plants and filamentous algae, unlike phytoplankton, are not always uniformly distributed in a pond. The fixed location and large size of rooted plants interfere with crayfish farming practices, such as feeding and trapping.
- Rooted plants and filamentous algae both encourage mosquito production by providing suitable habitat.
- Die-offs of rooted plants and filamentous algae may result in rapid decomposition and oxygen depletion.
- Reeds and other rooted plant[illegible]nds provide habitats for birds and [illegible]pecies which may prey upon crayfish.
- When a pond surrounded by reeds is drained, a considerable number of crayfish remain amongst the plants.
- If a crayfish pond containing a heavy infestation of filamentous algae is drained, the algae settles, covering the crayfish making it extremely difficult to recover them.
- Rooted plants slow the drainage of ponds and thus prolong the dry-out period.
- Reeds can infest supply canals, interfere with water flow and cause siltation.

Conclusion

There are many advantages of maintaining phytoplankton communities in ponds. However, farmers should be aware of the sometimes disastrous consequences associated with the incorrect application of fertilisers when stimulating phytoplankton blooms. Applying organic materials to ponds should only be undertaken if oxygen levels can be maintained at an acceptable level.

The obstructions and interference to trapping, draining and maintenance of ponds and canals over-rides any desirable aspects of encouraging the growth of rooted plants. It is difficult to achieve satisfactory control of problem emergent and submerged plants in an aquatic habitat. In attempting to manage invasive plants, repeated and expensive action may be necessary to maintain the required level of control.

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Increasing the growth of juvenile Pacific oysters (Crassostrea gigas) by supplementary-feeding with microalgal and dried diets

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Abstract

The growth rates of juvenile Pacific oysters (Crassostrea gigas) were assessed between 1992 and 1997 at a commercial nursery, at Pipe Clay Lagoon, Australia. The instantaneous growth rates of oysters based on organic weight, k_{OW}, ranged from $k_{OW} = 0.003 \pm 0.023$ d^{-1} (January to June 1997) to 0.020 ± 0.011 d^{-1} (December 1992 to May 1993). Reduced growth rates between January and June 1997, was associated with lower concentrations of chlorophyll a (0.43 $\mu g\ L^{-1}$) and total particulate matter (TPM; 1.1 mg L^{-1}) than in the previous seasons (chlorophyll a = 0.86–1.5 $\mu g\ L^{-1}$; TPM = 1.8–6.9 mg L^{-1}). Growth rates of juvenile oysters at another site, Little Swanport, in trials conducted in February 1997 and April 1997 were also higher (k_{VOL}, based on volume = 0.14 and 0.056 d^{-1}, compared to $k_{VOL} \leq 0.005$ d^{-1} at Pipe Clay Lagoon). The higher growth rates at Little Swanport were also associated with higher concentrations of seston (eg. chlorophyll a $\geq$ 2.0 $\mu g\ L^{-1}$).

Trials were undertaken at Pipe Clay Lagoon to establish whether supplementary-feeding could improve growth rates. In four trials, supplementary feeding juvenile oysters with Isochrysis sp. (T.ISO) at concentrations that increased the ambient food concentrations by $\approx$ 2 to 3-fold, improved growth rates k_{VOL} by $\geq$ two-fold. The first two trials assessed growth rates with different oyster stocking volumes, and methods of diet presentation. In trial 3, two "off-the-shelf" products, i.e. AlgaMac 2000 and Microfeast® MB-30 proved effective as supplementary diets (k_{VOL} = 0.024 and 0.032 d^{-1}, repectively, compared to $k_{VOL} = 0.009$ d^{-1} for control), though not as good as Isochrysis sp. (T.ISO) ($k_{VOL} = 0.054$). Trial 4 established a relationship between k_{VOL} and supplementary feed concentrations.

A more detailed evaluation of the cost-benefit of supplementary feeding is necessary. Nevertheless, at sites like Pipe Clay Lagoon characterised by low and variable productivity, supplementary feeding does appear to be cost-effective, and in fact necessary for the nursery to maintain a continuity of oysters seed supply to service the needs of their customers.

Keywords: AlgaMac 2000, artificial diet, oyster, nutrition, Microfeast, Isochrysis sp. (T.ISO)

Introduction

A common method for cultivating Pacific oysters (C. gigas) from newly-set up to 3-4 mm is to hold them in upweller or downweller systems in land-based nurseries, and pump seawater through to provide food particles (Rodhouse et al., 1981; Dix, 1991). The advantage of the method is that the associated food costs are low, as the oysters feed on natural seston. However, the concentrations of inflowing phytoplankton and other food particles can vary significantly because of prevailing environmental conditions and competitive grazing from filter feeders in the waterway (Spencer and Gough, 1978; Alpine and Cloern, 1992; Gibbs et al., 1992). As the juvenile oysters' growth will be affected by food availability, this approach provides limited control over growth rates.

An alternative approach for nurseries is to supplement natural seston with cultured microalgae or other commercial feeds. Though this presents additional food costs for the nursery, the approach may be cost-effective due to a reduced holding time (less labour) of oysters within the nursery. Further, during prolonged periods of low productivity (food concentration) in the associated waterway, supplementary feeding will enable industry to maintain their production of oyster seed to service the needs of their clients.

We undertook a weekly monitoring program from 1992 to 1997 to document changes in juvenile Pacific oyster growth rates, and water quality parameters at one of the major commercial nurseries in Australia, at Pipe Clay Lagoon, Tasmania. This program aimed to study factors affecting oysters' growth at the site, and to assess whether supplementary feeding might be warranted. Though we did not establish a statistical relationship, it was apparent during the early phase of the project that food availability was a major constraint to oyster's growth (Brown and McCausland, 1998). Between November 1994 and March 1997 we completed 16 experimental trials, to assess to what extent supplementary feeding could improve oyster production, and to compare the effectiveness of different diets. In a recent publication, we reported on 5 of these trials

conducted between November 1995 and April 1996 (McCausland et al., 1999). That study demonstrated that Isochrysis sp. (T.ISO), Dunaliella tertiolecta, Chaetoceros calcitrans, Rhodomonas salina, and pastes prepared from C. calcitrans and Skeletonema costatum were effective as supplementary diets. When fed at concentrations that increased the ambient phytoplankton concentrations by 150–225% (compared to natural seawater), the diets improved oysters' growth rate by 60–100%.

Here, were report on an additional 4 supplementary-feeding trials, assessing the value of Isochrysis sp. (T.ISO) 1) using different methods of diet presentation, 2) at different oyster stocking volumes, 3) compared to dried diets, and 4) at different concentrations. We also present a summary of data from our weekly monitoring program, and compare the natural growth rates of juvenile oysters at the Pipe Clay Lagoon site, to those at another major nursery site, i.e. Little Swanport.

Material and methods

Monitoring of seawater composition and juvenile oyster growth rates at commercial nurseries

Our weekly monitoring program to compare the growth rates of oysters with the composition of seawater flowing into a commercial nursery, at Pipe Clay Lagoon, Tasmania, Australia (42° 58' S, 147° 32' E) was conducted between 24 March 1992, and 26 June 1997.

At 10 a.m. each Thursday, 1 L of juvenile oysters (1600-1800 µm size range) was transferred to a commercial-scale nursery upweller (Rodhouse et al., 1981). Oysters from this size range were chosen because they were available for a greater period of the growing season, and hence would give a better indication of growth across the entire season. The commercial-scale upweller comprised a 45 cm diameter chamber with a nylon-meshed base and was retained within a 150 L plastic tub. Unfiltered seawater (salinity 32-35 ppt) was pumped at 14 L min^{-1} into each upweller (between inner wall of tub and outer wall of inner chamber). It flowed up through the upweller chamber and the bed of oysters and out through an exit pipe. Oysters and the upweller screens were cleaned daily for 5-10 min to remove faeces and adherent particles, by gently rinsing in seawater and carefully brushing the underside of the screens with a nylon brush.

Sub-sample of 200 oysters were removed from the upweller at the commencement of the trial and after 7 d for the analysis of dry and organic (meat) weight of oysters. The sub-samples were rinsed with distilled water, dried at 100°C for 24 h, then weighed to determine dry weight (DW). Sub-samples were then heated in a muffle furnace (450°C; 24 h), and reweighed to determine the organic weight (OW) by weight loss. The instantaneous growth rate of the oysters (k) over each week was given by the formula:

$$k = \ln (M_7 / M_0) / t$$

where, M_t = measurement after 7 d, M_0 = measurement at day zero.

Water was sampled weekly, at the same time as oysters were sampled. Temperature was recorded, and 20 L of water was passed through a 20 μm nylon screen, to remove particles outside the size range readily ingested by oyster spat. For chlorophyll a analysis, water samples (2.5 L) were filtered under vacuum through glass fibre filters (Whatman GFC; 47 mm), and the filters then stored at -20°C for < 2 months prior to analysis. Filters were cut into 5 mm squares and transferred to 10 mL centrifuge tubes together with 4.5 mL of ice-cold acetone. Samples were then vortexed, sonicated, diluted with 0.5 mL water, and then left in the dark at 4°C for 30 min to complete extraction (Wright et al., 1991). Chlorophyll a in the extracts were determined spectrophotometrically (Jeffrey and Humphrey, 1975).

For analysis of particulate matter, water samples (2.5 L) were filtered under vacuum through preweighed, precombusted glass fibre filters (47 mm). Filters were rinsed with 10 mL of water to remove residual seawater, dried (100°C, 16 h) then weighed to determine the total particulate matter. Organic material on filters was combusted by heating at 450°C for 16 h. Filters were reweighed, and the change in weight calculated to estimate the particulate organic matter in the original sample.

Samples were analysed for pigments, particulate fatty acids, dissolved organic carbon and nutrients (phosphate, silicate and nitrate). Full details of the methods used and the results of these analyses have been reported elsewhere (Brown and McCausland, 1998) and are not discussed in detail here.

For logistical reasons, the routine monitoring of both oyster growth rates and water quality was only undertaken at the Pipe Clay Lagoon site. However, we did assess water quality at Little Swanport, another nursery site on the east coast of Tasmania (Brown and McCausland, 1998). We undertook two short-term trials trials to compare the natural growth rates of oyster at the two sites, the first during February 1997 (10 d duration) and the second during April 1997 (13 d duration). Prior to the trials the stock had been maintained at the Pipe Clay Lagoon site. A volume of 1.5 L of

oysters (800-900 μm) was divided; half of the stock was translocated to the Little Swanport site and the other half retained at Pipe Clay Lagoon. After 2 days of acclimation to normal nursery conditions, duplicate upwellers were stocked at 300 mL of oysters. Oysters were then subjected to normal nursery maintenance until the completion of the experiment. The instantaneous growth rate of oysters was calculated on the basis of changes in dry and organic weight of sub-samples of 300 oysters, and also by a volumetric measurement of the entire oyster population (thus eliminating errors associated with sub-sampling).

Supplementary feeding trials – general

Within the first year of the monitoring program we observed that the natural growth rate of juvenile oysters at the Pipe Clay lagoon site was highly variable and at times (apparently) limited by an inadequate food supply (Brown and McCausland, 1998). Consequently, the program developed to evaluate whether oyster growth rates could be enhanced significantly through supplementary feeding.

Shellfish Culture Ltd provided hatchery-reared juvenile Pacific oysters (C. gigas) for feeding trials at their oyster nursery at Pipe Clay Lagoon. The early juvenile stages (i.e. from 400 to 1300 μm) were targeted in the growth trials, as industry indicated that these stages were most prone to mortality and poor growth. The oysters were grown in experimental upweller systems, 1/20th the scale of commercially used upwellers and stocked at an initial volume of 9–30mL. Each upweller consisted of a 10 L bucket with a 110 mm diameter, mesh-bottomed chamber suspended inside, on which the oysters were retained. Unfiltered seawater from Pipe Clay Lagoon (salinity 32-35 ppt) was pumped continuously into the upwellers at 700 mL min^{-1} (1000 L d^{-1}), flowing up through the chamber and the bed of oysters and out through an exit pipe. An estimation of the (background) concentrations of natural phytoplankton flowing through upwellers was calculated using chlorophyll a measurements, and an assumption that chlorophyll a represented 1.0% of

phytoplankton dry weight (Brown, 1991; Brown and Jeffrey, 1992). Oysters and chambers were cleaned daily with a fine spray of freshwater to remove faeces and other particles. Chambers were also given a weekly rinse with 1% sodium hypochlorite.

Oysters were acclimated within the upweller units overnight before commencing each trial. The photoperiod under which the oysters were grown ranged from 6:18 h to 8:16 h light:dark (L:D). The positions of the upwellers were randomly altered 3 to 4 times throughout each trial. Oyster mortality was estimated from three counts, each of 200 individuals, at the start and end of each trial. The instantaneous growth rate of oysters was calculated on the basis of changes in dry and organic weight of sub-samples of 300 oysters, and volumetrically. However, results from our previous study, demonstrated that instantaneous growth rates (k_{VOL}) using volumetric analysis were more reliable, because errors due to subsampling were eliminated (McCausland et al., 1999). Moreover, k_{VOL} was related to instantaneous growth rates calculated using DW (k_{DW}) and OW (k_{OW}) by the following equations:

$k_{DW} = 1.004\ k_{VOL} - 0.005$ $(R^2 = 0.62)$

$k_{OW} = 1.104\ k_{VOL} - 0.001$ $(R^2 = 0.74)$.

Therefore, only instantaneous growth rate data based on volume are presented here for the supplementary feeding trials, and the site-comparison study.

Isochrysis sp. (T.ISO) used as supplementary feed in the trials was grown in f_2 medium (Guillard and Rhyther, 1962) in 85 L polythene bags and mixed with 1 to 2% CO_2, at a flow rate of 20 L min^{-1}. The cultures were irradiated with continuous light (Philips daylight tubes; 50 to 75 µmol photon m^{-2} s^{-1}). Each culture was maintained semi-continuously in late-logarithmic growth phase (Brown et al., 1993) for 1-2 weeks, and 3-4 different cultures were used as feed within each trial.

Specific details relating to the four trials are summarised in Table 1, and given in fuller details below.

Trial 1 - different oyster stocking volumes

Oysters (500–700 μm) were stocked at 3 different volumes: 10 mL, 20 mL and 30 mL (20 mL ≈ 25 g wet weight). At each volume, there were two treatment groups: a control (n = 2 or 3) and a supplementary-fed group (n = 4). Control oysters received only the continuous flow of unfiltered seawater. Supplementary-fed oysters received, in addition, an initial ration of 220 mg (dry wt equivalent) of Isochrysis sp. (T.ISO) upweller^{-1} d^{-1}. The ration was increased weekly, in proportion to the growth of the slowest-growing supplementary-fed treatment. The feed was delivered continuously using a peristaltic pump (Cole-Palmer, Masterflex model 7519-05) from the bags (at 1 to 2 L h^{-1}; depending on culture density). The cultured algae was continuously mixed and diluted with seawater (≈ 1:50 to 1:100), also pumped using a Cole-Palmer peristaltic pump (model 7018-20). This diluted mixture was dripped into upwellers by pumping through Nylex irrigation drippers, at 4 L h^{-1}. The trial lasted 16 d.

Trial 2 – different methods of presenting the supplementary-feed

Oysters (500–700 μm) were stocked at 20 mL in all upwellers. Three different methods of presenting the food were examined. They were: a) a single-passage, flow-through, at 700 mL min^{-1} (i.e. the standard method used in trial 1), b) a single-passage, flow-through, at 230 mL min^{-1} and c) recircirculation of water within the upweller (using a submersible aquarium pump; Aquarium Powerhead 480, Second Nature, NJ, USA). In the latter treatment, additional water was dripped in to provide 8 water changes of water volume d^{-1}. For each method, there was a control (n = 2 or 3) or a supplementary-fed group (n = 4). Supplementary feed was delivered continuously (as in trial 1) at an initial ration of 200 mg upweller^{-1} d^{-1}, increasing weekly in proportion to the growth of the slowest-growing supplementary-fed treatment. The trial lasted 16 d.

Trial 3 – live versus dried diets as supplementary feed

Oysters (400–500 μm) were stocked at 9 mL in all upwellers. Three different supplementary-diets (n = 5 or 6) were assessed and compared to a non-supplementary fed control (n = 4). The diets were: a) Isochrysis sp. (T.ISO) b) AlgaMac 2000, a spray-dried thraustochytrid (Schizochytrium sp.) supplied by Aqaufauna Biomarine (Hawthorne, Cal., USA) and c) Microfeast ® MB-30, a yeast-based, dried diet. The dried products had been received 5 months previously, and stored unopened at 4°C. Immediately prior to use they were resuspended (0.5 g in 2L seawater for AlgaMac 2000; 2L of freshwater for Microfeast ® MB-30) by mixing for 30 sec with a low-speed blender.

For logistical reasons, the diets were presented differently in this trial. Rations (initially 68 mg dry weight equivalent) of the algal or dried diets were dispensed into 10 L plastic buckets, and the volume made up to 9 L with seawater. The suspension was pumped into each treatment upweller at 75 $mL.min^{-1}$ using a small aquarium pump (Aquarium Powerhead 480) over 2 h. On each weekday, a ration was fed at midday and a second equivalent ration (prepared at ≈ 5 p.m) was fed at midnight using a timer-switch to active the pumps. Hence the oysters were fed initially a total ration 135 mg $weekday^{-1}$; rations were increased weekly in proportion to the growth of the respective treatment groups. The oysters were not supplementary-fed on weekends, but received flow-though water (i.e. same as control). The trial lasted 26 d; oysters (except controls) were supplementary fed for 17 of these days.

Trial 4 – different concentrations of supplementary diet

Trial 4 was conducted immediately after trial 3. Oysters used in this trial were those supplementary-fed Isochrysis sp. (T.ISO) from trial 3. Their initials size range was 700–1300 μm and they were

stocked at 12 mL upweller^{-1}. Treatments included supplementary-fed oyster receiving either 60, 120 or 180 mg of Isochrysis sp. (T.ISO) upweller d^{-1} (n = 4 or 5) and a control (n = 3).

Supplementary-feed was delivered continuously using a peristaltic pump, as in trials 1 and 2. The ration was increased weekly, in proportion to the growth of the slowest-growing supplementary-fed treatment. The trial lasted 17 d.

In conjunction with this trial, we continued to maintain the control oysters from trial 3. The oysters were restocked at the same biomass (based on organic weight) as the oyster in trial 4, at a volume of 12 mL. This allowed us to effectively assess the effects of supplementary feeding over the duration of the two trials, i.e. 43 d.

Apparent growth efficiency of supplementary diets

Apparent growth efficiency (%AGE) was calculated for each diet within the trials, as a measure of the efficiency by which food was used:

$$\%AGE = [(\Delta OW_{sf} - \Delta OW_c) / DW_{feed}] \times 100$$

where ΔOW_{sf} is the change in OW of supplementary-fed oysters (entire upweller), ΔOW_c is the increase in the OW of control oysters (entire upweller), and DW_{feed} is the dry weight of supplementary food. The OW of a known volume (1 to 4 ml) of oysters was measured at the start and end of an trial to calculate the OW per upweller, to enable calculation of ΔOW_{sf} and ΔOW_c.

Statistical analysis

Data from the monitoring program were analysed by regression modelling approaches, with growth rate of oysters as the dependent variable, and nutrient composition as explanatory variables. Effects

were examined to see if they were linear or non-linear. Growth rates from supplementary feeding trials and between-site trials were compared using analysis of variance (ANOVA), with significant analyses followed by Fisher's protected least significant difference (PLSD) test for pairwise comparisons.

Power analyses (Searcy-Bernal, 1994) were undertaken using instantaneous growth rate (k) data (based on volumes) from previous supplementary trials (Brown and McCausland, 1998) to assess the level of replication required. For example, four replicates/treatment were required to detect a 20% difference in k between treatments, with a power value of 0.8, significance level of $P < 0.05$ and based on 4 treatments.

Results

Monitoring program of water quality and juvenile oysters

Seawater composition and the instantaneous growth rates (based on OW) of 1600-1800 µm C. gigas showed a significant variation within and between production seasons (Table 1). Average chlorophyll a concentrations (an indicator of phytoplankton biomass) ranged from 0.9 to 1.5 µg L^{-1} during the first 4 seasons. However, during January 1997 to June 1997 the average concentration reduced to 0.43 µg L^{-1}. The reduction in chlorophyll a was accompanied by significantly lower growth rates of oyster during this season ($k = 0.003\ d^{-1}$) compared to the previous 4 seasons ($k = 0.10$ to $0.20\ d^{-1}$). During the last 3 seasons (January 1995 to June 1997) there was a general trend of decreasing concentrations of chlorophyll a, TPM and POM (parameters associated with food quantity and quality) and oyster instantaneous growth rate k.

Comparison of juvenile oyster growth rates at Pipe Clay Lagoon and Little Swanport

The growth rates of 800-900 µm juvenile oysters at Pipe Clay Lagoon and Little Swanport were compared during February 1997 (Trial A) and April 1997 (Trial B) (Table 2). In both trials, growth rates were significantly greater at Little Swanport ($k = 0.14$ and $0.056\ d^{-1}$ for Trials A and B respectively) than at Pipe Clay Lagoon ($k \leq 0.005\ d^{-1}$). Higher growth rates at Little Swanport were associated with significantly higher concentrations of chlorophyll a (8 and 3.5-fold in Trials A and B, respectively), TPM, POM and also higher water temperatures, than at Pipe Clay Lagoon (Table 2).

The growth rate of oysters at Little Swanport during Trial B was less than half that observed in Trial A and corresponded to lower concentration of food (chlorophyll a, TPM, POM) and a lower water temperature. Concentrations of chlorophyll a, TPM and POM, and oyster growth rates at Pipe Clay Lagoon within the trials were typical of those observed during our weekly monitoring program at the same site, during the January to June 1997 season (Table 1).

Supplementary feeding trial 1

Across the three stocking volumes, i.e. 10, 20 and 30 mL, supplementary feeding enhanced oyster growth by approximately 6-fold (Fig. 1a). Rates were highest at 10 mL for both the supplementary-fed ($k = 0.094\ d^{-1}$) and control ($k = 0.015\ d^{-1}$) treatments ($P < 0.05$). %AGE, a measure of the efficiency of conversion of algal to oyster biomass in supplementary-fed treatments, were highest in the 20 and 30 mL volume treatments (%AGE = 24).

Supplementary feeding trial 2

Trial 2 adopted a 20 mL stocking volume for all treatments, as this volume provided a high % AGE for supplementary-fed oysters based on the feeding rations used in Trial 1. Growth rates based on volumetric analysis showed negligible growth for the control oysters ($k \leq 0.003$) (Fig. 1b). In fact,

the growth rates based on organic weight (data not shown) demonstrated that control oysters were starving during this trial as they lost weight ($k \leq -0.006$). Supplementary feeding improved growth significantly in all oyster treatments, with best growth observed in the 700 mL min^{-1} flow-through treatment ($P < 0.05$). Correspondingly, % AGE was also highest in the 700 mL min^{-1} flow-through treatment (%AGE = 23).

Supplementary feeding trial 3

We had planned to stock oysters at 20 mL volume, based on our findings of %AGE in trial 1. However, because of a limited availability of juvenile oysters when we commenced this trial and trial 4, oysters were stocked at about half that volume. Accordingly though, we reduced the supplementary-fed ration in proportion to this reduced oyster stocking volume.

The microalgal (i.e. Isochrysis sp. (T.ISO)) and two "off-the-shelf" products were all effective as supplementary diets, producing significantly faster growth rates than control oysters ($P < 0.05$) (Fig 1c). The microalgal diet produced about twice the increase in growth as the dried diets; Microfeast ® was slightly superior to AlgaMac 2000 ($P < 0.05$). Accordingly, % AGE was greatest in Isochrysis sp. (T.ISO)-fed oysters.

Supplementary feeding trial 4

Supplementary feeding with Isochrysis sp. (T.ISO) at all concentrations significantly enhanced oyster's growth ($P < 0.05$; Fig 1d). There was a trend of increasing growth rate with increasing ration. The 120 mg and 180 mg ration treatments gave significantly better growth than the 60 mg ration ($P < 0.05$); the 180 mg and 120 mg rations were not significantly different. % AGE values were identical for the 60 and 120 mg rations (31), but was lower for the 180 mg ration (26).

Combination of results from trials 3 and 4.

At the completion of Trial 3 (i.e. after 26 d), control and Isochrysis sp. (T.ISO) supplementary-fed oysters were restocked at the same volume. Maintenance of these oysters with or without supplementary feeding for a further 17 d allowed us to evaluate supplementary feeding over a longer period.

Over the 43 d of the study, supplementary feeding had a dramatic effect on growth (Fig. 2). From an initial volume of 9 mL, supplementary-fed oysters increased to 239 mL ($k = 0.076\ d^{-1}$), whereas control oyster increased to 22 mL ($k = 0.021\ d^{-1}$). Supplementary-fed oysters attained a size (hinge-tip to end of shell) of 3.2 ± 0.8 mm, sufficient for the next stage of nursery cultivation; i.e. planting out in nylon-meshed tray in local waterways.

Comparison of data between trials

During the four supplementary-feeding trials, concentrations of chlorophyll a (0.31 to 0.71 $\mu g\ L^{-1}$), TPM (1.3 to 1.9 $mg\ L^{-1}$) and POM (0.40 to 0.61 $mg\ L^{-1}$) (Table 3) were within the range of values reported for the January to June 1997 production season. However, their concentrations were generally lower than concentrations reported for previous production seasons (Table 1).

It was not possible to correlate differences in the water quality parameter between trials 1 to 4 and the growth rate of control oysters. This is because the data set was limited, the quality of the nutrients (eg. phytoplankton species composition) was not assessed and other nutrients that could affect growth were not assessed. Also, oysters in trials 3 and 4 were stocked at about half the volume of trials 1 and 2, and this could have improved their growth rate by ≈ 40% (c.f. trial 1; growth rates of oysters stocked at 20 versus 10 mL). Nevertheless, control growth rates in trial 4 (k = 0.047 d-1) were substantially higher than in other trials ($k \leq 0.015\ d^{-1}$) and this may be been related to the (apparently) higher concentration of chlorophyll a and POM during this trial.

Because of minor variations in the fixed variables of trials (eg. stocking density, food delivery and oyster size-class) it is difficult to make accurate comparisons on the effect of supplementary feeding between trials. Nevertheless, all trials included one treatment that was supplementary-fed with 10 to 11 mg Isochrysis sp. (T.ISO) d^{-1} mL^{-1} oysters, with feed delivered in in a flow-through system at 700 mL min^{-1} (Table 1). Therefore, a comparison of their % AGE values gives an indication of the effectiveness of supplementary-feeding, across the trials. %AGE values for these respective treatments were similar in trials 1 to 3 (%AGE = 20–24) but apparently higher in trial 4 (%AGE = 31).

Discussion

Our survey of growth rates of juvenile oysters at Pipe Clay Lagoon over five years demonstrated a significant intra- and inter-seasonal variation, as well as significant changes often occurring from week to week. These changes were most likely related to food-related factors (phytoplankton quantity and species composition, other particles and dissolved organics), but also abiotic factors such as water temperature and seawater nutrient composition. Another confounding factor was that each week we were assessing growth in a different oyster population and thus a different genetic stock. Hence because of the complex interaction between the many factors impacting on oyster growth rates, we were unable to statistically correlate growth with any specific nutritional factor, nor multiple factors through a multiple regression analysis. Nevertheless, during the last two years of the survey, there was a trend of decreasing concentrations of chlorophyll a (i.e. measure of phytoplankton abundance), TPM and POM and an associated reduction in oyster growth. This was particularly evident during January to June 1997, when, compared to previous seasons, average chlorophyll a concentrations were ≤ one-half, and oyster growth rates were ≤ one-third.

Brown (1988) assessed the influences of environmental factors on the growth rates of Pacific oysters (≈ 22 and 45 mm size classes) at 10 locations in British Columbia, Canada, using multivariate analysis. He assessed oysters from a common broodstock to eliminate variation due to genetics. Food supply (concentrations of POM, chlorophyll b and carotenoids), temperature and to a lesser extent, salinity were the major factors affecting oysters' growth rate. As chlorophyll b is characteristic of phytoplankton from Chlorophyceae, Euglenophyceae and Prasinophyceae (Jeffrey et al., 1997), Brown (1988) suggested that for the sites he surveyed, these algae may be important nutritional components within bivalve diets. Data from our study showed seasonal trends in chlorophyll b concentrations closely matched those of chlorophyll a, with a chlorophyll a:b of 10:1 (Brown and McCausland, 1998).

Effects of site were demonstrated in our study by comparing the growth rates of control oysters at Little Swanport and Pipe Clay Lagoon in February and April 1997. The growth at Little Swanport during February 1997 was significantly greater than ever achieved at Pipe Clay with supplementary feeding, both in this study, and previous supplementary trials (Brown and McCausland, 1998). During these trials, the availability of food (based on chlorophyll a, POM and TPM concentrations) at Little Swanport was significantly greater than at Pipe Clay Lagoon. Also Little Swanport site was characterised by higher concentrations of dissolved organic carbon (Brown and McCausland, 1998); oysters and other filter feeders are able to utilise small organic molecules such as amino acids and organic acids within this fraction (Manahan and Stephens, 1983). Despite the higher productivity of the Little Swanport site seen in this study, salinities can drop dramatically during periods of high rainfall (Brown and McCausland, 1998) making the site unsuitable for oyster cultivation for several weeks of a year (Martin John, pers. comm.). During periods of low salinity, oysters may cease feeding (even if ambient food concentrations are high) or growth may be depressed because of the metabolic cost associated with maintaining osmotic balance (Brown, 1988).

Based on the poor and variable productivity at Pipeclay Lagoon – especially during the 1997 production season – supplementary feeding trials were undertaken at the site to assess whether growth could be significantly enhanced. Across the four trials, supplementary feeding with Isochrysis sp. (T.ISO) increased daily instantaneous growth by ≥ 2-fold. It is difficult to accurately assess the ambient "food" concentration in seawater, because of differences in the availability and digestibility of the complex mixture of suspenoids (eg. bacteria, yeast, detritus, silt, as well as phytoplankton). However, assuming that phytoplankton comprises 70% available food (Brown, 1988), then we estimate that supplementary feeding increased food concentrations across the trials by between ≈ 2-fold (trials 3 and 4) and 4-fold (trials 1 and 2).

In trial 1, the growth rates of oysters reduced with increasing stocking volumes, both for supplementary fed oysters as well as control oysters. Most likely, this reduction was simply related to lower food availability (per unit of oyster biomass) with increasing stocking volume. The stocking volume of 10 mL produced the best growth, but at this volume the utilisation of algae (%AGE) was lowest. As feed production costs are the major component of supplementary feeding costs, combinations of stocking densities and feeding rations should be adjusted to maximise % AGE, yet maintaining significant improvements in growth rates.

Trial 2 found that recirculation of water reduced oyster growth. Reasons for this were not investigated, though one explanation is that this method produced a greater build-up of waste products (e.g. ammonia, faecal material) in the system which negatively impacted on growth. This may also explain why oysters maintained at a seawater flow rate of 230 mL min^{-1} $upweller^{-1}$ grew less than oysters fed at a flow rate of 700 mL min^{-1} $upweller^{-1}$. The latter flow rate (≈ 28 mL min^{-1} g^{-1} wet weight of oysters) is within the range of 20 to 50 mL min^{-1} g^{-1} of oysters suggested to be optimal for the feeding of juvenile C. gigas in upwelling systems (Spencer et al., 1986). At these flow rates, approximately 20% of suspended material is filtered by C. gigas (Spencer et al., 1986). This and other studies found that lower flow rates do produce higher levels of filtration and

therefore a better use of food (Rodhouse and O'Kelly, 1981), but they also give reduced growth. For example, Manzi et al. (1986) found that growth of mussel Mercenaria mercenaria was reduced when more than 20% of ambient chlorophyll a was removed from water passing through upwellers. Kirby-Smith (1972) found scallop growth was reduced when more than 40% of chlorophyll a was removed from inflowing water.

The two dried products, Microfeast ® MB-30 (yeast-based) and AlgaMac 2000 (algal-based) were successful as supplementary diets, though their growth performance were not as good as live algae in Trial 3. MB-30 and other Microfeast ® products, 100 and L-10, have also proven to be effective as partial diets (80% component with 20% algae) for juvenile Sydney rock oyster, Saccostrea commercialis (Nell et al., 1996). AlgaMac 2000 has also been assessed as part of a mixed diet for C. gigas (at either 80% or 40% component with algae) though it's nutritional value was not as good as a high-quality algal diet (Boeing, 1997). Nevertheless the cheaper cost of these and related products (≈$US 50 kg^{-1} or less) compared to microalgae (typically around US$200 kg^{-1} dry weight) indicates that further assessment of the cost-effectiveness of these products under commercial situations is warranted. In particular, both the control growth rates during this trial (k = 0.009 d^{-1}) and the average levels of chlorophyll a (0.55 µg L^{-1}) in inflowing water, were significantly lower than in the previous seasons. Therefore, the diets were acting more as "complete" diets, than supplementary diets and they may be even more effective under normal ambient (i.e. higher) concentrations of chlorophyll a

Trials 4 demonstrated that growth rates of oysters increased with increasing concentrations of supplementary diet. Maximum growth rates were not established, though possibly only modest growth increases could be given at higher rations. We calculated that at the highest ration, the average cell concentration of Isochrysis sp. (T.ISO) flowing through upwellers ranged from 10 cells μL^{-1} (week 1) to 30 cells μL^{-1} (week 3). This is comparable to the optimum feed concentration of 30 cells μl^{-1} of Isochrysis sp. (T.ISO) found for clam Venerupis pullastra seed (Beiras et al., 1993) and

oyster Ostrea edulis seed (Beiras et al., 1994) in flow-through systems. Also, the instantaneous growth rate at the highest ration ($k = 0.11\ d^{-1}$) was comparable to maximum growth rates of bivalve seed reported in other studies, e.g. $k = 0.13\ d^{-1}$ for C. virginica (Urban et al., 1983), $k = 0.14$ for O. edulis (Beiras et al., 1994), $k = 0.12\ d^{-1}$ for Saccostrea commercialis (O'Connor et al., 1992) and 0.070 for C. gigas (Enright et al., 1986a).

Trials 3 and 4 were run in succession to determine benefits of longer-term supplementary feeding. There was a 2.5-fold improvement of growth rate over the 43 d period. Based on a microalgal production costs of US$ 200 (eg. "average" commercial hatchery; Coutteau and Sorgeloos, 1992) the direct additional feed costs would approximate US$0.20 per thousand oysters for growth from 0.5 to 3.0 mm. This compares to an "average" production cost of ≈ US$8-10 per thousand oysters of 5 mm size (M. John, Shellfish Culture, pers. comm.) – though during this period of low growth the "effective" production costs for control oysters would have been significantly greater. Almost certainly, supplementary feeding was cost-effective in increasing production during this time. During periods when control growth rates and/or background phytoplankton concentrations are already high, the cost-effectiveness of supplementary feeding may be equivocal. Nevertheless, supplementary feeding may be an important strategy even during these periods, to increase production to meet seasonal demands by farmers (i.e. continuity of supply).

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Figure captions

Fig. 1. Specific growth rates (k_{VOL}; based on volume) of juvenile Pacific oysters with (grey bars) or without (black bars) supplementary-feeding:
A) at 3 different initial stocking volumes. Initial oyster size, 500–700 µm; initial ration 220 mg Isochrysis sp. (T.ISO) upweller^{-1} d^{-1}; trial duration 16 d,
B) using different methods of presenting the diet. Initial oyster size 500–700 µm; initial ration 200 mg Isochrysis sp. (T.ISO) upweller^{-1} d^{-1}; trial duration 16 d,
C) fed different diets. Initial oyster size, 400–500µm; initial ration 135 mg diet upweller^{-1} weekday^{-1}, trial duration 27 d (17 feeding days),
D) fed different concentrations of Isochrysis sp. (T.ISO).

Fig. 2. Growth of oysters, with or without supplementary-feeding, over 43 d. During the first 26 d, oysters (400-500 µm initial size) were initially fed 135 mg Isochrysis sp. (T.ISO) upweller^{-1} weekday^{-1} (17 feeding days). After 26 d, oysters were restocked at 12 mL upweller^{-1}. Data from days 26 to 43 has been calculated assuming no restocking of upwellers. From days 26 to 43, oysters (700–1300 µm) were initially fed 180 mg Isochrysis sp. (T.ISO) upweller^{-1} d^{-1}.

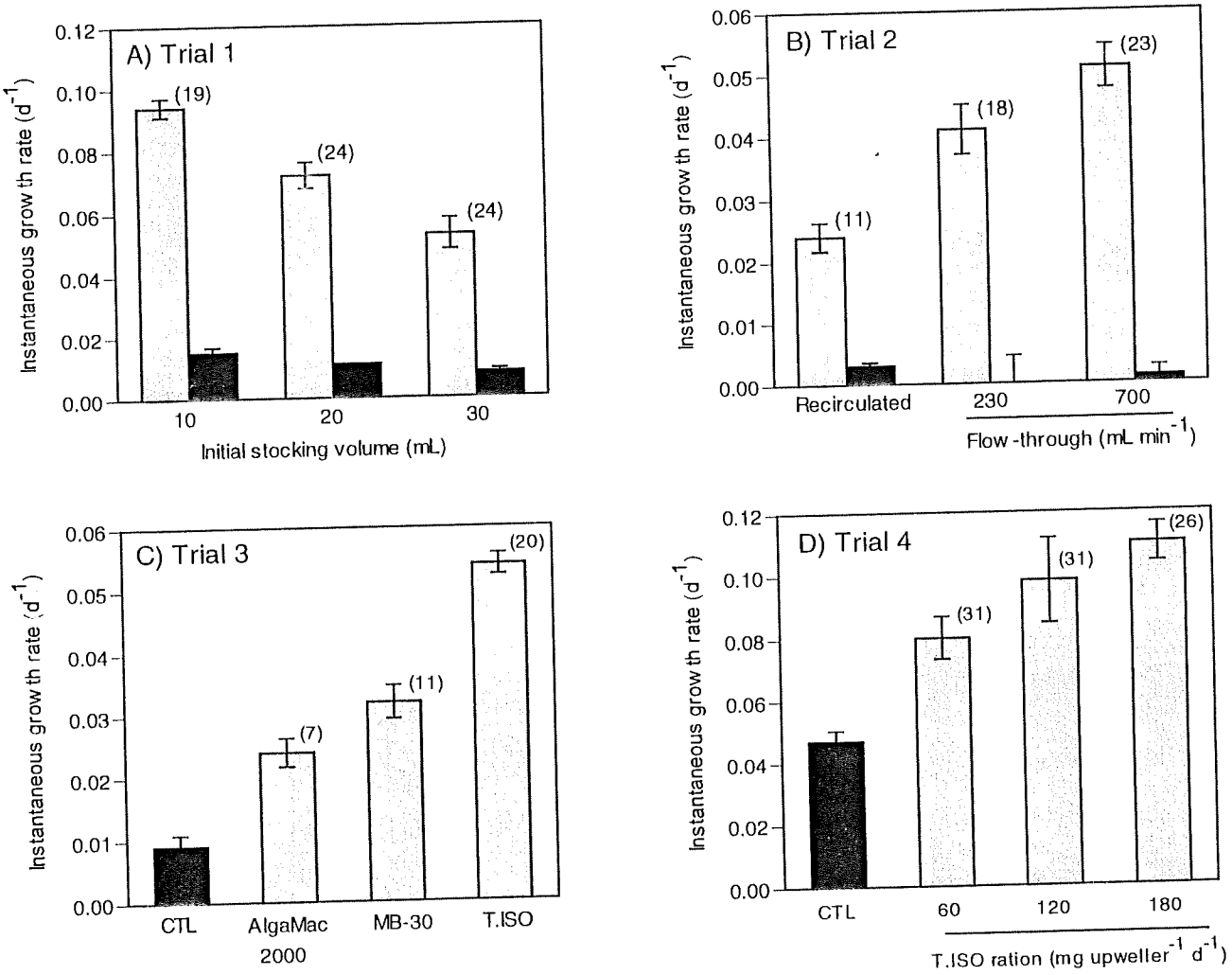
A) Trial 1
Instantaneous grow th rate (d-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
(19)
(24)
(24)
10
20
30
Initial stocking volume (mL)
B) Trial 2
Instantaneous grow th rate (d-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
(11)
(18)
(23)
Recirculated
230
700
Flow -through (mL min-1)
C) Trial 3
Instantaneous grow th rate (d-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
(7)
(11)
(20)
CTL
AlgaMac 2000
MB-30
T.ISO
D) Trial 4
Instantaneous grow th rate (d-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
(31)
(31)
(26)
CTL
60
120
180
T.ISO ration (mg upweller-1 d-1)

Fig. 1

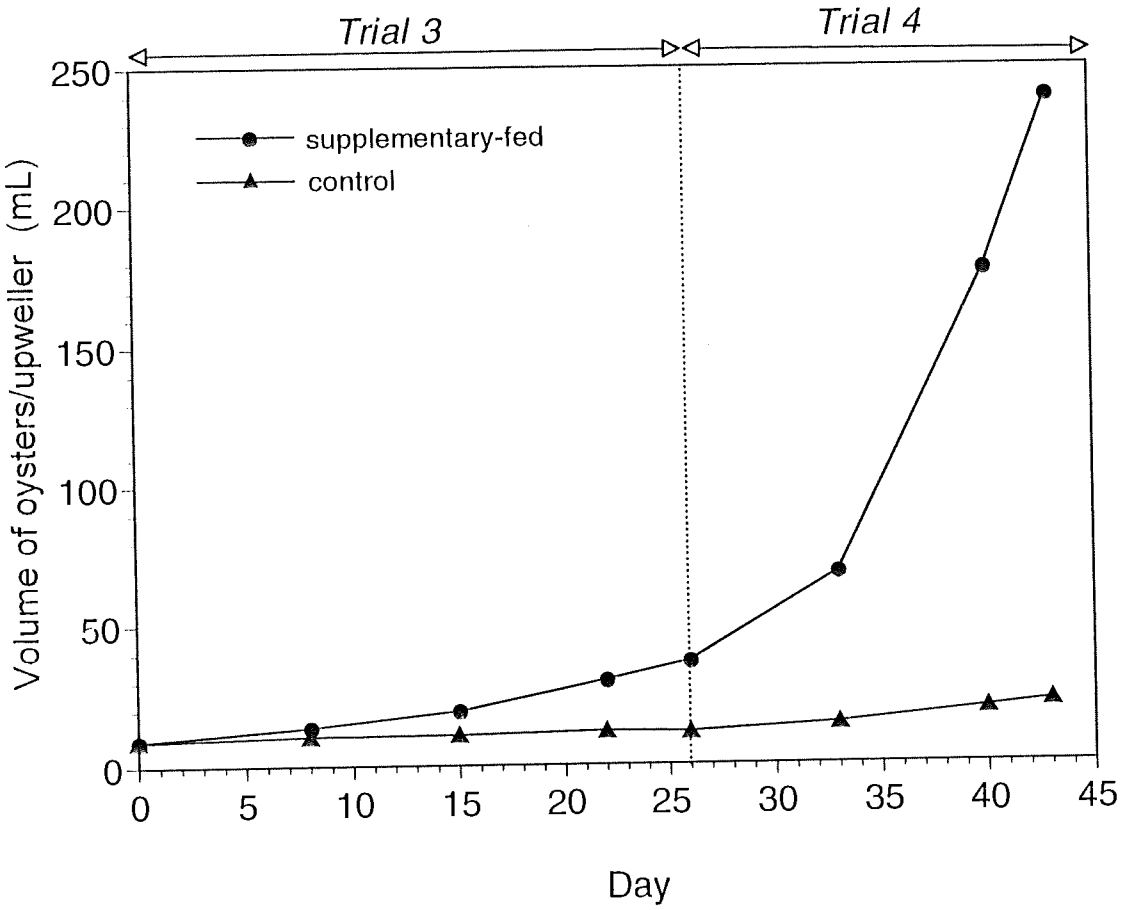
Trial 3
Trial 4
supplementary-fed
control
Volume of oysters/upweller (mL)
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
Day

Fig. 2

Table 1. Average concentrations of chlorophyll a, total particulate matter (TPM) and particulate organic matter (POM) in seawater at Pipe Clay Lagoon, and 1600-1800 µm juvenile Pacific oyster (C. gigas) growth rates over 5 productions seasons. k_{ow} = instantaneous growth rates, based on measurement of organic weight. n.a. = data not available for this season.

Production season	Chorophyll a ($\mu g\ L^{-1}$)	TPM ($mg\ L^{-1}$)	POM ($mg\ L^{-1}$)	Oyster k_{ow} (d^{-1})
Dec '92 – May '93	1.0 ± 0.4	5.5 ± 0.7	n.a.	0.020 ± 0.011
Dec '93 – June '94	1.1 ± 0.5	6.9 ± 4.4	1.2 ± 0.8	0.010 ± 0.018
Jan '95 – Sep '95	1.5 ± 1.1	3.2 ± 5.0	0.82 ± 0.93	0.019 ± 0.015
Jan '96 – Nov '96	0.86 ± 0.40	1.8 ± 1.0	0.60 ± 0.27	0.014 ± 0.019
Jan '97 – June '97	0.43 ± 0.17	1.1 ± 0.4	0.57 ± 0.19	0.003 ± 0.023

Table 2. Average daily temperatures and concentrations of chlorophyll a, total particulate matter (TPM) and particulate organic matter (POM) (± s.d; n ≥ 3) in seawater at Pipe Clay Lagoon and Little Swanport, compared to oyster instantaneous growth rate (k_{VOL}; based on volume) of non-supplementary fed oysters (± range/2; n = 2).

	Min-Max T (°C)	Chlorophyll a ($\mu g\ L^{-1}$)	TPM ($mg\ L^{-1}$)	POM ($mg\ L^{-1}$)	Oyster $k_{VOL}(d^{-1})$
Trial A (Feb 1997)					
Pipe Clay Lagoon	19–20	0.31 ± 0.10	1.3 ± 0.3	0.40 ± 0.05	0.005 ± 0.001
Little Swanport	20–24	2.5 ± 1.1	4.2 ± 4.0	1.2 ± 0.8	0.14 ± 0.002
Trial B (April 1997)					
Pipe Clay Lagoon	12*	0.57 ± 0.04	1.0 ± 0.4	0.36 ± 0.03	0.004 ± 0.002
Little Swanport	13*	2.0 ± 0.1	1.4 ± 0.1	0.67 ± 0.08	0.056 ± 0.001

* Min-Max temperature data not available. Data is average daily temperature.

Table 3. Experimental details and properties of inflowing seawater during four supplementary-feeding trials undertaken with juvenile Pacific oysters at Pipe Clay Lagoon (November 1996 to March 1997). Values of seawater include average temperature range and concentrations of chlorophyll a, total particulate matter (TPM), particulate organic matter (POM) and an estimate of phytoplankton concentration (± s.d.; n ≥ 3). T.ISO = Isochrysis sp. (T.ISO)

	Trial 1	Trial 2	Trial 3	Trial 4
Initial trial conditions				
Stocking volume (mL)	10, 20, 30	20	9	12
Oyster size range (μm)	500-700	500-700	400-500	700-1300
Diet	T.ISO	T.ISO – fed by different methods	T.ISO, commercial diets	T.ISO
Ration (mg upweller^{-1} d^{-1})	220	200	96*	60, 120, 180
Ration/mL oyster (mg d^{-1}) $	22, 11, 5.5	10	11	5, 10, 15
Trial duration	16	16	26	17
Background water parameters during trial				
Min-Max T (°C)	19-21	16-19	14-17	16-19
Chlorophyll a (μg L^{-1})	0.31 ± 0.10	0.35 ± 0.08	0.55 ± 0.39	0.71 ± 0.28
TPM (mg L^{-1})	1.3 ± 0.3	1.7 ± 0.7	1.3 ± 0.4	1.9 ± 0.8
POM (mg L^{-1})	0.40 ± 0.05	0.46 ± 0.14	0.40 ± 0.14	0.61 ± 0.27
Phytoplankton DW (μg L^{-1})#	31 ± 10	35 ± 8	55 ± 39	71 ± 28
Phytoplankton DW through upweller d^{-1} (mg)	30 ± 10	34 ± 8	53 ± 38	69 ± 27

* Ration was 135 mg upweller^{-1} weekday^{-1}; oysters did not receive supplementary feed on weekends.
$ To compare rations fed with those of other studies (eg. Epifanio et al., 1979), 1 mL ≈ 1.25 g wet weight of oysters.
# Estimated based on chlorophyll a representing 1.0% of algal dry weight (Brown, 1991; Brown and Jeffrey, 1992).