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Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea gigas* (Thunberg)]

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Abstract

Physiological measurements were made on Pacific oysters from controlled crosses between inbred lines. Hybrid individuals were expected to perform better than inbred oysters, for a variety of traits related to feeding behaviour. The oysters were offered a diet simulating natural suspended particulate matter. By quantifying the organic and inorganic fractions of food, faeces and pseudofaeces, various aspects of feeding were elucidated. The results agreed with expectation; on average, hybrid oysters had higher rates and efficiencies of feeding and growth than inbreds. In one experiment there were significant differences between hybrids and inbreds for seventeen out of twenty cases; in another experiment hybrids performed better than inbreds for eight out of sixteen cases. In both experiments, we find significant differences between the reciprocal hybrids, though heterosis for growth is evident for all hybrids. Our experiments therefore confirmed heterosis for growth and hybrid superiority for physiological traits, independent of ration level; emphasised the complexity of these relationships amongst genotypes; and demonstrated the segregation of physiological traits in the F_2 generation.

Keywords: Growth; Feeding; Heterosis; Pacific oysters

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1. Introduction

The physiological basis for hybrid vigour or heterosis (Griffing, 1990) is poorly understood (Hedgecock et al., 1996). For some species of bivalve molluscs, a weak but suggestive correlation has been established between multilocus heterozygosity (MLH), as measured by protein electrophoresis, and rate of growth (Mitton and Grant, 1984; Zouros and Foltz, 1987; Britten, 1996). Other studies have in turn linked this growth/heterozygosity relationship to various physiological components of growth, such as whole-animal metabolic rate (Koehn and Shumway, 1982; Toro et al., 1996), the intensity of protein turnover (Hawkins et al., 1986) and the efficiency of protein deposition (Bayne and Hawkins, 1997). Such studies have posed the hypothesis that heterosis of growth may be explained, at least in part, by differences in metabolic efficiency between individuals. However, the term 'metabolic efficiency' requires definition, and a coherent theory of the physiological basis for the heterosis of growth in these animals remains elusive.

Hedgecock et al. (1995) describe an experimental approach to understanding hybrid vigour in the Pacific oyster [*Crassostrea gigas* (Thunberg)], based on controlled crosses amongst inbred lines (see also Hedgecock et al., 1996; Vavra et al., in press). By this approach genomic heterozygosity is experimentally manipulated, and the reliance on ex post facto correlations of physiological traits and allozyme heterozygosity within natural populations, which has been a feature of much previous work with bivalves (Britten, 1996), is avoided. Hedgecock et al. (1995, 1996) recorded strong evidence of heterosis of growth in oyster larvae and juveniles, including an example of negative heterosis. They concluded that the evidence implicated epistasis as an explanation for experimental observations to date. Vavra et al. (in press) studied the larvae from such controlled matings and concluded that ''... while no single physiological mechanism accounted for all cases of hybrid vigor... the results do favor metabolic efficiency as an explanation of growth heterosis in larval oysters''.

In this paper we record results of physiological measurements made on the oysters from some of these inbred and hybrid families. Our aims were threefold: (1) to explore the hypothesis that hybrid individuals express both higher physiological rates and higher efficiencies when compared with inbred oysters; (2) to re-examine our earlier finding (Hedgecock et al., 1995) that significant differences between reciprocal hybrid crosses are common; (3) to challenge the further hypothesis that the variability in physiological traits that correlate with growth and genotype in the F_1 generation will persist into the F_2 generation and so help to explain observed differences in rates of growth amongst those individuals.

2. Materials and methods

Inbred lines from a naturalised population of *Crassostrea gigas* (Thunberg) in Washington, USA, were used to establish experimental crosses at the Bodega Marine Laboratory. The experimental design for these crosses, and the hatchery and subsequent methods used to rear the larvae and juveniles, are described by Hedgecock et al. (1995).

Electrophoresis of polymorphic enzymes was done to confirm brood stock pedigrees and theoretical expectations for genetic diversity in the inbred lines, as described by McGoldrick and Hedgecock (1997).

Juvenile oysters from three experimental crosses were used during 1994, 1995 and 1996. In 1994, the oysters were from a factorial cross of first generation individuals from lines 89-6 and 89-7. This cross is described fully by Hedgecock et al. (1995) as well as by Vavra et al. (in press) (their cross 2); three progeny groups are designated here as *6×6, 6×7* and *7×6*. *6×6* identifies the inbred genotype for Line 6; *6×7* is the hybrid with male parent from Line 6 and female parent 7; and *7×6* is the hybrid for male 7 and female 6. *6×7* and *7×6* are referred to as reciprocal hybrids. Only one individual of inbred line 7 survived to Day 340 (see Table 5 of Hedgecock et al., 1995), so physiological analysis of this parental line was not possible. During the larval stage, parent inbred line 7 had higher mortality and slower growth than parent inbred line 6.

In 1995, oysters were from a factorial cross of second generation individuals of inbred line 89-5 and first generation individuals of inbred line 92-1, yielding the third generation of 89-5 and two reciprocal hybrid progenies (cross 4 of Vavra et al., in press). These progenies are designated here as *5×5, 5×1* and *1×5*, respectively. Again, no individuals of parent inbred line 1 were available for physiological study, none having survived to the juvenile stage. In 1996, the oysters were from an F_2 hybrid population, designated *7×6A*. This population was derived from a single pair cross involving one male and one female from the F_1 *7×6* hybrid population that was studied earlier (Hedgecock et al., 1995).

Three days (in 1994) and seven days (1995) prior to the start of experiments, the oysters were recovered from their cages in nearby Tomales Bay and held in an outdoor tank at the Bodega Marine Laboratory. Individuals were tagged by numbering directly onto the shell and suspended indoors in a large vat (850 l) of seawater in which the appropriate experimental diets (see below) were made up. During these experiments half the volume of seawater in the holding vats was changed for fresh seawater daily, and the diets checked frequently. The vats were vigorously aerated to maintain the particulate diets in suspension. The seawater temperatures were 17.8±0.8°C and 17.0±0.7°C in 1994 and 1995, respectively, and salinities were 33±0.4‰.

The design of the 1996 experiment was different to 1994 and 1995. Newly settled larvae from a cross between *7×6* siblings, which were created by hybridization of lines 89-7 and 89-6 in 1993, were collected from the laboratory cultures in June, 1995. The spat oysters were maintained in the hatchery for 30 days on a diet of 10^4 cells of *Isochrysis* sp. (Tahitian strain; T-ISO) per ml, then transplanted to Tomales Bay in trays of plastic mesh. After 203 days these oysters were all measured for shell height (greatest shell dimension) and total live weight (shell plus flesh); Fig. 1A is a histogram of the distribution of shell heights in this sample.

Eighty-eight oysters were then selected by shell height, starting at the extreme tails of this distribution (Fig. 1B). The smallest were each given a white numbered tag and the largest given a red numbered tag. These tagged oysters were mixed in equal numbers of each colour in each of four cages, and returned to the bay for 142 days prior to bringing them back into the laboratory (at 14.5±2°C; oceanic salinities; natural particulates as food) for genetic and physiological analysis. We report here the results of the

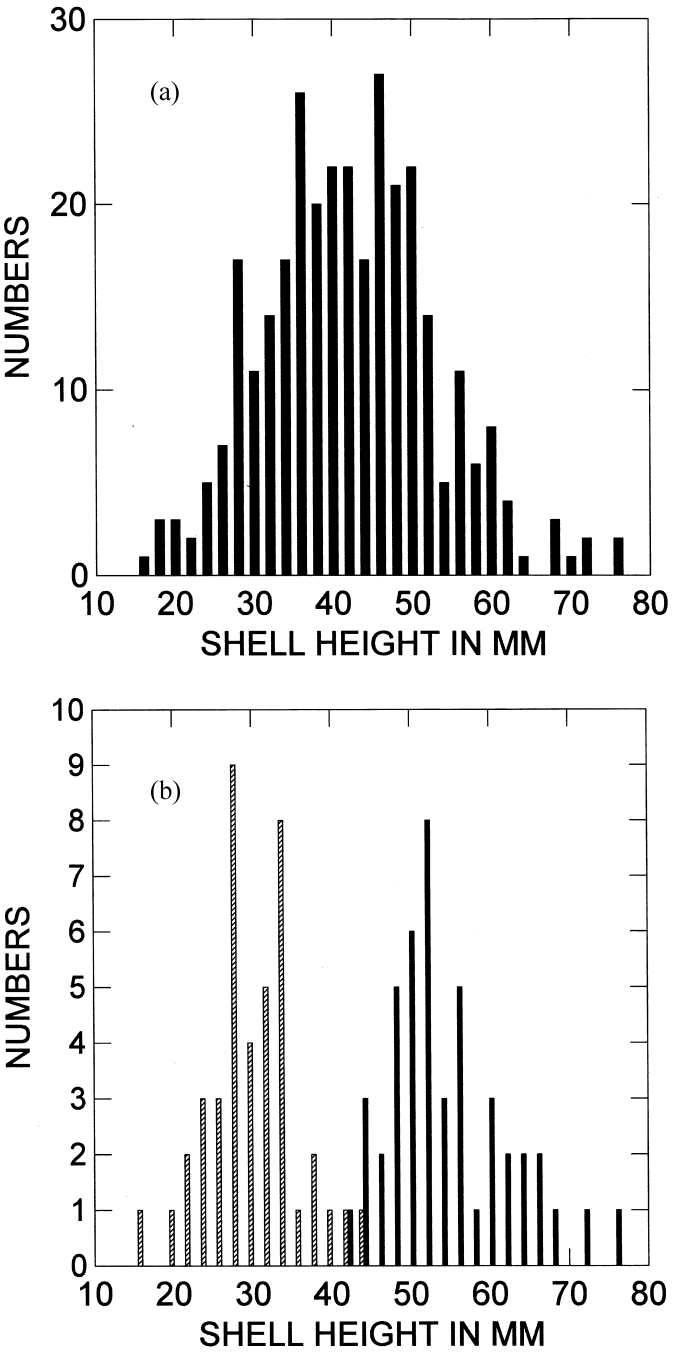

Fig. 1. (A) The initial size distribution of oysters for the 1996 experiment ($n=314$; mean shell height in mm: 41.1±SD 10.8). (B) Oysters selected for the 1996 experiment, showing the 'slow' ($n=42$; mean shell height 31.3 mm) and 'fast' ($n=42$; mean shell height 53.3 mm) growth categories.

physiological measurements only; a genetic analysis of growth is given by McGoldrick (1997). Small oysters (white tags) are designated 'slow growing', the larger (red tags) are 'fast growing'.

In 1994 and 1995, the experimental diets consisted of mixtures of cells of the alga T-ISO and natural silt. The alga was grown in 200 l batch cultures and harvested daily towards the end of the log phase of growth. The silt consisted of surface sediment (2–3 mm depth) that was scraped from the intertidal flats of Tomales Bay and held in the laboratory at 4°C. Each day, an appropriate volume of this silt was sieved through nylon mesh, first of 150 μm then 10 μm, left to stand for 60 min, then decanted for use. The composition of the diets was checked by size-frequency analysis with a Coulter Counter, in addition to measures of total mass and organic content as described below.

In the experiments during 1994 and 1995, water was pumped at 150 ml min^{-1} from the holding vats to eight shallow trays, six of which were used, with oysters, for measurements of feeding behaviour, and two as blanks, without oysters. Frequent sampling of the water in the trays for the concentration of suspended particulates and for temperature confirmed equivalence of conditions between the measurement trays and the vats. At the start of a period of measurement, individual oysters were placed in the trays and left undisturbed until they were seen to be discharging both pseudofaeces and true faeces. Samples of these biodeposits were collected over 60 min, together with water samples from the control trays. The trays were then cleared of all deposited material and the oysters again left undisturbed until a second period of sampling. The two measurements were averaged for subsequent analysis.

In 1996, oysters were selected directly from the holding trough in the laboratory for incubation with ^{15}N-labelled *Isochrysis* cells, as described below.

At the end of each series of measurements, each oyster was measured for total live weight (= shell + live flesh) and this measurement converted to an equivalent dry flesh weight using a relationship established at the BML:

$$\text{Dry Weight (g)} = 0.103 + [0.016 \times \text{Live Weight(g)}];\ r^2 = 0.95 \text{ for } n = 106.$$

The method used in 1994 and 1995 to quantify aspects of the feeding behaviour of the oysters is the so-called 'biodeposition' method, as recently reviewed by Iglesias et al. (1998). Both pseudofaeces (material filtered by the oyster from suspension, but then rejected from the pallial cavity without being ingested) and true faeces (filtered material which is then ingested and passed through the digestive tract) were separately collected quantitatively from the measurement trays and filtered onto ashed and pre-weighed GF/C filters. Samples of the available food, from the water within the control trays, were treated similarly. The filters were dried overnight at 60°C, weighed, then combusted at 450°C for 4 h before cooling in a desiccator and final weighing. Chlorophyll contents were measured on filtered material by extraction in acetone and spectrophotometric estimation. Table 1 lists the measurements made and the derivations of components of feeding behaviour (rates and efficiencies).

There are two important assumptions in this method (Iglesias et al., 1998). Firstly, the proportion of organic to inorganic matter is assumed to be similar for both the material in suspension (i.e. the available food) and the material filtered by the animals. In the

Table 1
A definition of terms used to describe components of feeding behaviour

Measured variable	Derived variable	Description
Total suspended particulate matter		TPM (mg l^{-1}): Suspended matter dried overnight at 60°C
Particulate organic matter		POM (mg l^{-1}): TPM ashed at 450°C
	Particulate inorganic matter	PIM (mg l^{-1}): TPM-POM
	Particulate organic content	OC (fraction): POM/TPM.
Total faeces		Faeces $prod^n$ (mg h^{-1}): Faeces dried at 60°C
Faeces organic matter		FOM (mg h^{-1}): Faeces ashed at 450°C
	Faeces inorganic matter	FIM (mg h^{-1}): Faeces $prod^n$-FOM
	Faeces organic content	FOC (fraction): FOM/Faeces $prod^n$
Total pseudofaeces		Rejection rate, RR (mg h^{-1}): Pseudofaeces dried at 60°C
Pseudofaeces organic matter		PsOM (mg h^{-1}): Pseudofaeces ashed at 450°C
	Pseudofaeces inorganic matter	PsIM (mg h^{-1}): RR-PsOM
	Pseudofaeces organic content	PsOC (fraction): PsOM/RR
	Filtration rate	FR (mg h^{-1}): (FIM + PsIM) × (TPM/PIM)
	Selection efficiency	SE (fraction): 1 − (PsOC/OC)
	Ingestion rate (organics)	IR (mg organics h^{-1}): (FRxOC)-PsOM
	Absorption rate (organics)	AR (mg organics h^{-1}): IR-FOM
	Absorption efficiency	AR/IR (fraction)

Variables measured directly are distinguished from the derived variables, and methods of calculation are shown in the final column. Suspended particulates and biodeposits were dried overnight at 60°C and then ashed at 450°C for 4 h. An estimate of clearance rate (CR: l h^{-1}) may also be derived as (FIM + PsIM)/PIM, but this is to be distinguished from direct measurement of clearance rates as described in the text.

present experiments >90% by volume of the food particles (*Isochrysis* + silt) were between 4.5 and 9.0 μm diameter (as established by Coulter Counter), and therefore within the size-range of maximal retention efficiency of the Pacific oyster (Barrillé et al., 1993). The first assumption is therefore met. A second assumption is that both the pseudofaeces (which are produced within seconds of filtration) and the true faeces (produced after a gut passage time that may be many minutes long) are the products of the animal filtering the same suspended particles. In the present experiments the

concentration and the composition of the food presented to the oysters were similar throughout both the holding and the measurement period, meeting the requirements of the second assumption.

Other details of experimental conditions and physiological measurements are described below.

2.1. The 1994 experiment

Six oysters from each of the three crosses were held in the vats at each of two diets, which were designated as Low Quality and High Quality, as follows (values are means ± 1 S.D., for $n = 12$ determinations).

Low Quality Diet: Total Particulate Material (TPM) = 8.48 ± 1.54 mg l^{-1};

Organic Content (OC) = $21 \pm 4\%$; Chlorophyll *a* (Chla) = 5.0 ± 3.3 μg l^{-1}.

High Quality Diet: TPM = 7.61 ± 2.33 mg l^{-1};

OC = $35 \pm 8\%$; Chla = 21.5 ± 5.3 μg l^{-1}.

Measurements of feeding behaviour were made after 4 *and* 10 *days exposure to these diets* (*subsequently averaged for analysis*) *on six oysters from each cross.*

2.2. The 1995 experiment

Ten oysters from each of the three crosses were held for seven days at one diet condition as follows ($n = 7$): TPM = 3.84 ± 0.89 mg l^{-1}; OC = $33 \pm 10\%$; Chla = 11.9 ± 4.2 μg l^{-1}. Over the following 10 days, oysters were selected at random for analysis of feeding behaviour. In addition, clearance rates (volume of water cleared of suspended material per h) and respiration rates (ml oxygen consumed per h) were measured as follows.

2.2.1. Clearance rates

The oysters were placed in 3 l of seawater + food and left undisturbed for 15 min. A small volume of *Isochrysis* culture was then added, to return the particle concentration to a value similar to the starting value, and samples of 5 ml taken immediately and at 10-min intervals up to 1 h, to determine by Coulter Counter the concentration of particles in suspension (in the diameter range 4–50 μm). The particle counts were graphed over time to establish linearity in the decline in concentration, and clearance rate calculated according to Coughlan (1969).

2.2.2. Oxygen consumption rates

The oysters were placed individually within air-tight chambers of filtered seawater immersed in a temperature-controlled water bath. Samples of 2 ml volume were taken at intervals and rates of oxygen consumption determined as described by Bayne and Hawkins (1997).

2.2.3. Growth rates

On July 10, 1995, and whilst they were being held in trays in Tomales Bay, all oysters from the three crosses were individually tagged and measured for shell height and total live weight. 107 days later these oysters were transferred to an outdoor tank at the Bodega Marine Laboratory, and on November 6 (120 days from the initial measurement) the individuals were measured again. These data were then used to calculate a rate of growth in dry flesh weight, converted from total live weight measurements, for individuals over this period.

2.3. The 1996 experiment

A total of 44 oysters, 22 from each of the fast- and slow-growing groups, were randomly selected from the holding bags for measurements of their rates of protein synthesis, and clearance rates. Subsequently, two individuals died, and data from a further two were rejected due to the failure of the oysters to filter algal cells from suspension during experimental incubations. In the analysis that follows, therefore, we present data for $n=21$ individuals of the fast-growing category and $n=19$ of the slow-growing category.

Rates of protein synthesis were measured using the ^{15}N technique described by Hawkins (1985) and as summarised by Bayne and Hawkins (1997). Clearance rates were measured as described above for the 1995 experiments. The growth of these oysters over the preceding 142 days in Tomales Bay was measured as changes in total live weight, which was then converted to growth in dry flesh weight.

2.4. Weight-standardisation of the physiological rates

As a result of differences between families with respect to rates of growth, individual oysters were of very different size at the time of physiological measurement (Table 2). Our interest is in the variance in physiological traits amongst individuals that is not weight-dependent but might be due to different genotypes. We therefore selected to remove weight-dependent effects from the physiological measurements (Bayne and

Table 2
The flesh dry weights of the oysters used in the three experiments

Year	Cross/growth category	Dry flesh wt (g)
1994	*6×6*	0.150±0.040
	6×7	0.302±0.046
	7×6	0.346±0.074
1995	*5×5*	0.093±0.039
	5×1	0.197±0.050
	1×5	0.208±0.059
1996	Fast growers	0.258±0.091
	Slow growers	0.088±0.058

These weights were estimated from measured live weights (shell plus flesh) using a regression equation as discussed in the text.

Newell, 1983) using weight-exponents (from the normal allometric relationship between each physiological rate and body weight) in the following equation:

$$\text{Standardised rate} = \text{Observed rate} \times (\text{Standard weight}/\text{Observed weight})^{\beta},$$

where β is the appropriate weight-exponent.

From Table 2, standard weights (i.e. estimated dry flesh weight in g) were established as: 1994, 0.300 g; 1995, 0.150 g; 1996, 0.190 g. Weight-exponents were taken from a comprehensive study by Bougrier et al. (1995); $\beta = 0.439$ for all feeding rate determinations (clearance, filtration, ingestion and absorption rates) and $\beta = 0.800$ for rates of oxygen consumption. We confirmed the validity of these exponents from within our own, more limited, data set, where β for clearance rates was 0.594 ± 0.238 and β for respiration rates was 0.743 ± 0.097.

The weight-exponent for growth rate was derived by plotting growth as a function of individual body size and fitting the power function by regression; for 1995, $\beta = 1.11$; for 1996, $\beta = 0.83$. For the 1996 experiment, we established an exponent for rates of protein synthesis as $\beta = 0.475$.

For the 1996 experiment, in which oysters of different size (=different rates of growth) were actively selected for comparison, we also chose to treat body size as a covariate in analyses of covariance. Conclusions drawn from this analysis were no different to conclusions reached following weight-standardization of the data as described above.

2.5. Statistical analysis

Results were analysed by Analysis of Variance and Analysis of Covariance using SYSTAT 6.0 for Windows (Wilkinson, 1992). Post hoc pairwise comparisons between crosses were made using Fisher's Least Significant Difference test. Other statistical procedures are described in the text.

3. Results

3.1. 1994 experiment

The results are presented in Table 3, as physiological determinations on oysters held for 10 days on either the low quality or the high quality diet, and standardised to a dry flesh weight of 0.300 g. The results of the pairwise comparisons between crosses are also shown.

In 17 out of 20 comparisons, the hybrids are significantly different ($P_{LSD} < 0.05$) from the inbreds, and in all of these cases the former show faster rates and higher efficiencies than the latter. Of the three non-significant hybrid/inbred comparisons, one is a measure of selection efficiency and two are feeding rates; two occurred on the low quality diet and one on the high quality diet. Comparing the reciprocal hybrid crosses, there are significant differences in seven out of ten cases, and in six of these the 6×7 cross shows

Table 3
The results of the 1994 experiments

Trait	Cross	Low quality diet	P_{LSD}: *7×6* vs. *6×7*	P_{LSD}: *7×6* vs. *6×6*	P_{LSD}: *6×7* vs. *6×6*	High quality diet	P_{LSD}: *7×6* vs. *6×7*	P_{LSD}: *7×6* vs. *6×6*	P_{LSD}: *6×7* vs. *6×6*
Filtration rate	*7×6*	5.39±1.03	<0.001			17.98±2.14	0.007		
(mg TPM h^{-1})	*6×7*	10.25±0.92		<0.001		13.15±2.14		<0.001	
	6×6	1.48±0.84			<0.001	6.12±2.35			0.001
Selection efficiency	*7×6*	−0.50±0.36	0.028			0.32±0.08	ns		
(fraction)	*6×7*	0.09±0.32		ns		0.30±0.08		0.001	
	6×6	−0.72±0.29			0.003	0.08±0.09			0.003
Ingestion rate	*7×6*	1.03±0.18	ns			2.78±0.59	0.001		
(mg organics h^{-1})	*6×7*	1.10±0.16		<0.001		4.49±0.71		ns	
	6×6	0.11±0.15			<0.001	1.95±0.64			<0.001
Absorption efficiency	*7×6*	−0.62±0.90	ns			0.21±0.09	ns		
(fraction)	*6×7*	−0.05±0.80		ns		0.23±0.10		0.003	
	6×6	−1.81±0.73			0.007	−0.04±0.11			0.001
Absorption rate	*7×6*	−0.63±0.19	0.001			0.58±0.14	0.019		
(mg organics h^{-1})	*6×7*	−0.03±0.17		0.002		1.05±0.14		0.005	
	6×6	−0.14±0.16			ns	−0.01±0.17			<0.001

Rates and efficiencies are presented for each cross as means±S.D. for $n=6$ determinations, for the low and high quality diets. The statistical significance of differences in pairwise comparisons between crosses are shown as P-values following Fisher's Least Significant Difference test; ns, $P>0.05$.

higher rates or efficiencies than the *7×6* cross; the exception is filtration rate on the high quality diet. The overall result with respect to physiological performance, where high performance equates with fast feeding rates and/or high efficiencies, is a rank order: $6\times7 > 7\times6 \gg 6\times6$.

Comparing feeding behaviours between diets, in all cases physiological performance is superior on the high quality diet. Filtration rates increase with increase in diet quality, as does selection efficiency, with the result that the ingestion rates for organic matter are higher by an average factor of 8.2, which compares with an actual difference in dietary organic content of a factor of 1.7. Absorption efficiencies are also higher on the high quality diet, with consequent increase in absorption rates. There is no evidence, therefore, that the scale of difference between hybrids and inbreds differed between diet qualities.

3.2. 1995 experiment

The results are presented in Table 4, standardised to a dry flesh weight of 0.150 g. Oysters from the hybrid cross *1×5* are significantly different from the inbreds *5×5* in all but one of the eight traits measured, the exception being the rates of oxygen consumption. With respect to clearance rate, filtration rate, ingestion rate, absorption rate and rate of growth, these hybrids are clearly superior in performance to the inbreds ($P_{LSD} \leq 0.001$). Although significantly different also for selection and absorption efficiencies, the hybrid/inbred differences here are less striking ($P_{LSD} = 0.002$ and 0.027, respectively).

In contrast, hybrid oysters from the *5×1* cross are only distinguishable from the inbreds in their rates of oxygen consumption. Comparing between reciprocal hybrids, the *1×5* oysters express faster rates and higher efficiencies than the *5×1* hybrids in all but one trait, viz. absorption efficiency, where the difference is not significant. Overall, the rank order amongst crosses was $1\times5 \gg 5\times1 = 5\times5$ for feeding rates and efficiencies. However, means for the *5×1* hybrids exceed, though not significantly, those of the *5×5* inbreds for four of six measures of feeding behaviour and for growth.

The daily rates of growth shown in Table 4 are for dry flesh, averaged over 120 days, from July to November 1995 and range from 0.5% to 1.3% of body weight per day. Heterosis for growth is clearly evident in the *1×5* hybrids, though not in the reciprocal, *5×1*, cross. Nevertheless, it should be noted that the *5×1* hybrids remained larger than the *5×5* inbred oysters at the end of the experiment, owing to their greater initial dry weights (Table 2).

3.3. 1996 experiment

This experiment compares oysters in the upper and lower tails in size distribution of an F_2 hybrid population, representing fast- and slow-growing individuals, respectively. There is therefore a large difference, by design, in mean size of the oysters in the two main experimental groups (Table 2). Standardising the data for differences in flesh weight is effective in removing significant size-dependent effects for growth rate, rate of protein synthesis and clearance rate. For example, although rate of growth is clearly a

Table 4
The results of the 1995 experiment

Trait	Cross	Mean±S.D.	P_{LSD}: *1×5* vs. *5×1*	P_{LSD}: *1×5* vs. *5×5*	P_{LSD}: *5×1* vs. *5×5*
Clearance rate	*1×5*	3.89±0.45	<0.001		
($l\ h^{-1}$)	*5×1*	2.31±0.49		<0.001	
	5×5	1.99±0.65			ns
Filtration rate	*1×5*	9.71±3.72	<0.001		
(mg TPM h^{-1})	*5×1*	4.76±2.54		<0.001	
	5×5	5.35±2.15			ns
Selection efficiency	*1×5*	0.40±0.34	0.013		
(fraction)	*5×1*	0.09±0.57		0.002	
	5×5	0.01±0.65			ns
Ingestion rate	*1×5*	2.74±1.37	<0.001		
(mg organics h^{-1})	*5×1*	1.19±0.83		<0.001	
	5×5	1.35±0.83			ns
Absorption efficiency	*1×5*	0.31±0.47	ns		
(fraction)	*5×1*	0.16±0.45		0.027	
	5×5	0.09±0.26			ns
Absorption rate	*1×5*	0.93±0.47	0.004		
(mg organics h^{-1})	*5×1*	0.25±0.41		0.001	
	5×5	0.16±0.26			ns
Oxygen consumption	*1×5*	0.75±0.10	<0.001		
(ml $O_2\ h^{-1}$)	*5×1*	0.20±0.13		ns	
	5×5	0.68±0.22			<0.001
Growth rate	*1×5*	1.92±0.22	<0.001		
(mg dry flesh d^{-1})	*5×1*	1.08±0.33		<0.001	
	5×5	0.83±0.45			ns

Rate and efficiency measurements, presented as means±S.D. for $n=10$ determinations, for each cross, except for growth rates, where $n=84$. The statistical significance of differences in pairwise comparisons between crosses are shown as *P*-values following Fisher's Least Significant Difference test; ns, $P>0.05$.

function of body size ($P<0.001$ for $n=40$), standardisation removes this dependence and growth rate 'category' (i.e. fast or slow growth) no longer explains a significant amount of the remaining individual variability in growth rate ($P=0.345$; $n=40$).

Differences between slow and fast-growing individuals for weight-standardised rates of protein synthesis are also not significant ($P=0.435$; $n=40$). In the absence of a direct measure of flesh protein content, we calculate an index of protein growth as flesh growth (mg total dry flesh d^{-1}) per mg protein synthesised. The result (Fig. 2) illustrates a significantly higher level of efficiency in the faster growing F_2 hybrids, explaining 36% of variance in this efficiency index ($P<0.001$; $n=40$).

Weight-standardised clearance rates also differ significantly between growth categories ($P<0.001$; $n=35$). The faster growing oysters have higher clearance rates (0.550±0.073 $l\ h^{-1}$ per 0.190 g body weight) than the slower growers (0.284±0.069 $l\ h^{-1}$ at the same body weight).

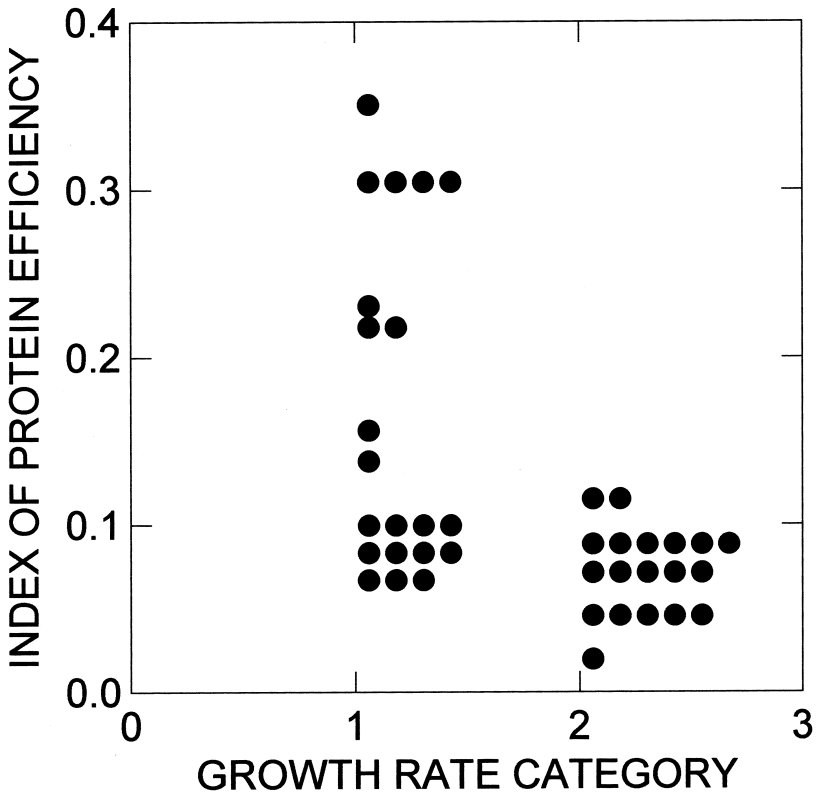

Fig. 2. An index of protein efficiency (growth in dry flesh weight/rate of protein synthesis) in the two growth rate categories, 1996: fast (=category 1) and slow (=category 2). There is a significant difference between categories ($P<0.001$; $n=40$).

4. Discussion

Variability between individual Pacific oysters is considerable with respect to most of the measurements reported here. Much of this is due simply to differences in body size. Some variability may be attributed to patchiness in the distribution of food and other environmental factors, both in the laboratory and in the field. But a significant proportion of the total variability between individuals is due to genotype, both between hybrids and the relevant inbreds and between the reciprocal hybrids. On average, the hybrid oysters out-perform the inbred oysters from the appropriate parental lines. Increased 'performance' is taken here to mean, in particular, higher rates and efficiencies of feeding (ingestion, absorption) and growth.

This study was designed to explore physiological correlates of growth heterosis expressed in the progeny from crosses between inbred lines. Hedgecock et al. (1995) reported that 11-month old juveniles from the same cross recorded in the present paper as the '1994 experiment' were 150% of the mean shell height for the 6×6 inbred

genotype, a highly significant difference. Mean initial dry flesh weights for these same families in our 1994 experiment show a difference of greater than 200% between hybrids and the *6×6* inbred (Table 2). Likewise, the *1×5* and *5×1* hybrids in our 1995 experiment were initially >200% heavier than the *5×5* inbreds of the same age (Table 2). During the interval over which growth was measured here, only the *1×5* hybrid sustained a significantly greater rate of growth than the *5×5* inbred (Table 4). However, across six experiments in which inbred line 89-5 has been used, it significantly outperformed three inbred lines and was not significantly different from two others (Hedgecock et al. 1996; unpubl. data). Our comparison of *1×5* and *5×1* hybrids is likely to have been to the better parent inbred line. Heterosis for growth is therefore a common though not exclusive observation in our experimental hybridizations to date (Hedgecock et al., 1996; Vavra et al., in press). A major inference from the present study is that hybrid physiological superiority contributes significantly to this growth heterosis.

Differences in performance between reciprocal hybrids are unexpected if growth heterosis results from heterozygosity for nuclear genes in hybrids. The progeny of $A \times B$ should have the same heterozygosity as the progeny of $B \times A$. Often both reciprocal hybrids express significant growth heterosis (e.g. $6\times7 > 7\times6 \gg 6\times6 > 7\times7$), but in some cases one hybrid may show significantly less growth heterosis (e.g. $1\times5 \gg 5\times1 = 5\times5 \approx 1\times1$). Differences between reciprocal hybrids can be explained either by maternal effects, which in turn could be either genetic or environmental in nature, or by interactions between the nuclear and mitochondrial genomes. It is difficult to believe that maternal effects would still be present in animals over 1 year of age. Indeed, in the case of the *6×7* cross, the *7×6* larvae were significantly larger on Day 7 than the *6×7* larvae (129 vs. 113 μm), the opposite of their ranking, in our experiment, by feeding rates and efficiencies and by growth rate. On the other hand nuclear-cytoplasmic interactions affecting energy metabolism are entirely feasible. Reciprocal backcrosses can be used to explore this interaction further.

Many of the physiological measurements recorded here were an attempt to explain observed heterosis in growth by exploring some of the physiological processes, particularly feeding, that support growth. As expected, differences in growth rate were reflected in differences in the feeding traits. In 1994 both hybrid groups, on average, fed more quickly and efficiently than the inbreds, again reflecting observed differences in growth (Hedgecock et al., 1995). In the 1995 experiment, *1×5* hybrid oysters had higher filtration, ingestion and absorption rates than did the *5×5* inbreds; both selection and absorption efficiencies were also higher. The rates of oxygen consumption did not differ significantly. In these circumstances a difference in the rate of growth, as observed, would be strongly indicated. Conversely, the *5×1* hybrids and *5×5* inbreds showed no differences in rates or efficiencies of feeding, and although there was a difference in their rates of metabolism (as oxygen consumption), the observed rates of growth did not differ significantly. Nevertheless, the *5×1* hybrids were twice as large as the *5×5* inbreds at the start of the experiment, indicating that a growth advantage must have existed earlier in development.

We therefore conclude that the hypothesis of heterosis for the feeding traits underlying growth in these oysters is, for the most part, supported. There is a consistency to the data on feeding rate and efficiency and the measured growth differences between hybrids and

inbreds. This consistency also extends to differences between reciprocal hybrids. And where oysters differed in their feeding rates, they differed also in the various measures of efficiency. As with growth per se, however, the physiological traits did not consistently show hybrid advantage and in both 1994 and 1995 differences between hybrids were a strong feature of our results.

For the 1996 experiment we selected slow- and fast-growing individuals to express the most extreme segregating phenotypes, from the original lines 89-6 and 89-7, within the F_2 generation of the 7×6 cross. With the effects of body size on growth removed statistically from the data, oysters in the two growth categories differed in clearance rate and in an index of the efficiency of protein deposition. This result further supports the view that differences both in feeding behaviour and in metabolic efficiency (Bayne and Hawkins, 1997; Vavra et al., in press) contribute to the observed heterosis of growth. Progress in elucidating the particular genetic basis of these physiological phenotypes will depend on a better understanding of the genetic basis for differences between reciprocal hybrids, and on the results of studies to map physiological variability upon the genetic architecture of this species of oyster (McGoldrick, 1997).

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