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Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster *Crassostrea virginica*

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SUMMARY

We tested two hypotheses in this study: first, that intraspecific growth variations in a marine bivalve are correlated with physiological (basal metabolic rate and scope for growth) and biochemical (membrane lipids) characteristics, and, second, that this bivalve shows intraspecific variations in physiological and biochemical adaptations to temperature. To test these hypotheses, five genetically distinct groups of juvenile oysters *Crassostrea virginica* that showed differences in their growth rates were maintained in the laboratory (1) for further measurements of growth and standard metabolic rates and (2) subjected to acclimation at 4°C, 12°C and 20°C and further examined for scope for growth and determination of membrane lipid composition. Our results show that a lower basal metabolic rate and lower unsaturation index of membrane lipids coincides with higher growth rates and a higher scope for growth in oysters. We provide evidence that intraspecific differences in basal metabolic rate in oysters are related to membrane unsaturation as predicted by Hulbert's theory of membranes as metabolic pacemakers. Furthermore, our results suggest that the theory of membranes as metabolic pacemakers is related to intraspecific differences in growth. A perfect negative relationship was observed between the acclimation temperature and the unsaturation index of membrane lipids in oysters, as predicted by the homeoviscous adaptation theory. However, changes in the unsaturation index in response to temperature were mainly due to variations in the eicosapentaenoic (20:5n-3) fatty acid in fast-growing oysters, whereas slow-growing animals changed both docosahexaenoic acid (22:6n-3) and 20:5n-3. Thus, the pattern of biochemical compensation in response to temperature in this species shows intraspecific variation.

Key words: genetically based intraspecific variation, growth, temperature adaptation, scope for growth, lipid remodelling, homeoviscous adaptation, mollusc, aquaculture.

INTRODUCTION

The physiological components regulating intraspecific growth differences among individuals living in the same environment may be affected by differences in energy acquisition (food consumption and assimilation), differences in the allocation of energy among maintenance, growth, reproduction and other consuming activities, and differences in the metabolic cost of growth (Bayne, 1999). The energy budget or 'scope for growth' provides a means of integrating the basic physiological processes into an index of energy available for growth and reproduction. In bivalves, the scope for growth has proved to be an accurate predictor of total production, which includes growth rate and gamete production (for a review, see Bayne et al., 1985) (see also Toro et al., 1996; Labarta et al., 1997; Smaal and Vonck, 1997; Bayne, 1999; Bayne et al., 1999b; Pouvreau et al., 2000; Rueda and Smaal, 2004).

The standard metabolic rate, defined as the minimum energy requirement for the maintenance of all essential functions within an inactive animal, is an important component of total production (Bayne et al., 1985). Interestingly, the unsaturation of membrane phospholipids (the number of double bonds per 100 fatty acid chains) is positively correlated with the standard metabolic rate in allometric comparisons of mammals and birds (Couture and Hulbert, 1995; Hulbert et al., 2002b; Hulbert et al., 2002a). Membrane bilayers in metabolically active systems are more polyunsaturated and less monounsaturated than those in systems that are metabolically less active (Hulbert and Else, 1999; Hulbert and Else, 2000). Such polyunsaturated membranes have been proposed to result in an increased molecular activity of membrane proteins; in this manner, the amount of membrane and its composition can act as a pacemaker for metabolism. We have shown that intra- and interspecific differences in basal metabolic rates in bivalves relate to membrane unsaturation as predicted by Hulbert's theory of membranes as metabolic pacemakers (Pernet et al., 2006; Pernet et al., 2007b).

The eastern oyster *Crassostrea virginica* is a eurythermal suspension-feeding bivalve distributed around the east coast of North America from the Gulf of St Lawrence to the Gulf of Mexico (Galtsoff, 1964). In the Gulf of St Lawrence, *C. virginica* is restricted to the warm shallow bays and estuaries in the southwestern part. In these areas, water temperature increases from below zero during the winter to *ca* 22–25°C during the summer. *Crassostrea virginica* is of primary interest for cold-water aquaculture because of its high commercial value. However, variability in growth rates among individuals and sporadic juvenile overwintering mortalities complicate the commercial exploitation of this species (Lavoie, 1995). Therefore, there is some interest in establishing a selective breeding programme for cold-water performance in *C. virginica*, considering that bioenergetic parameters (Hawkins et al., 1989; Tremblay et al., 1998; Pernet et al., 2006), survival and growth [see

Dégremont et al. (Dégremont et al., 2007) and references therein] are genetically correlated in many bivalve species. However, little is known about the physiological and biochemical responses of *C. virginica* to temperature.

We first hypothesized that physiological (metabolic rate and scope for growth) and biochemical (membrane lipid) traits would correlate with intraspecific growth differences in *C. virginica*. We predicted that fast-growing oysters allocate a smaller proportion of their aerobic capacity to maintenance requirements than do slow-growing animals. We also predicted that differences in metabolic rate among oyster groups correlate with the unsaturation index of their membrane lipids. Finally, we predicted that the scope for growth of these oysters correlates with their growth rate as the fast- and slow-growing animals differ in their capacity to exploit food.

Our second hypothesis was that genetically distinct groups of oysters exhibit different degrees of adaptation to temperature. The underlying premise was that physiological rates in *C. virginica* would show a marked degree of temperature dependence even after a period of acclimation (Newell et al., 1977; Shumway and Koehn, 1982). Furthermore, we predicted that juvenile *C. virginica* would counteract the thermal effects on membrane fluidity by changing their membrane fatty acids as predicted by the theory of homeoviscous adaptation. Briefly, the theory of homeoviscous adaptation states that the 'viscosity' of the lipid bilayer is adjusted to offset changes imposed by temperature (Sinensky, 1974; Hazel, 1995; Hayward et al., 2007). An important component of the cold response is the increase in membrane lipid unsaturation, and this has been linked to an enhanced resistance to cold (Hayward et al., 2007). Given that membrane adaptation is now viewed as a central contributor to low temperature survival of all organisms (Hayward et al., 2007), this trait may be of particular interest for the cold-water aquaculture of marine invertebrates.

MATERIALS AND METHODS

Animals

Oysters *Crassostrea virginica* (Gmelin 1791) were collected in the field or produced in the hatchery. Wild juvenile oysters were initially collected between 21st July and 12th August 2004 on material suspended in the water column in Baie de Miramichi, Néguac, NB, Canada (47°N, 65°W). They were immediately transferred to an aquaculture grow-out site located in Baie de Saint Simon (47°42′N; 64°45′W, lease MS-1076), Gulf of St Lawrence, NB, Canada. Oysters were cultured following usual local practices: they were separated from collectors during the fall, placed in mesh bags attached to bottom tables that were raised slightly off the substrate for overwintering under the ice-cover and then placed in floating bags at the sea surface the following spring (Lavoie, 1995).

Hatchery oysters obtained from the Coastal Zone Research Institute (CZRI, Shippagan, NB, Canada) were reared following a standard procedure (Dégremont, 2003). Briefly, 100 adults collected in February 2005 at Bouctouche (NB, Canada) were conditioned in the CZRI hatchery. The seawater temperature was gradually increased from –1°C to 20°C over a 15 day period and maintained for a 35 day conditioning period. A cultured phytoplankton diet of *Isochrysis galbana*, *Chaetoceros gracilis* and *Pavlova lutheri* was added to the seawater at a total concentration of ~100 cells μl^{-1}. After the conditioning period, five males and 20 females were randomly selected for the production of juveniles. Spermatozoids or oocytes were collected by stripping the gonad. Sperm from each male was combined with the oocytes from four different females to produce 20 full-sib families on 7th April 2005. Three-million oocytes per female were fertilized at a ratio of 200 spermatozoids per oocyte. Additionally, 650×10^3 oocytes from each female were mixed together and fertilized with a mixture of spermatozoids from each male to produce pooled offspring (Dégremont, 2003). Larvae were reared in 115 l tanks at 21°C in 0.5 μm-filtered seawater and fed daily with the diet used for broodstock at ~40 cells μl^{-1}. Water and food were renewed three times per week. Oyster larvae attained the pediveliger stage 22 days post-hatching and settled on collectors that were suspended in the larval tanks for 10 days. When hatchery-reared oysters reached at least 8 mm in shell length (9th August), they were placed in floating bags and transferred to the aquaculture grow-out site alongside the wild juvenile oysters. Oysters originating from the field, the pooled group, and three full-sib families, designated F_{24}, F_{26} and F_{35}, were harvested on 12th October 2005 and transferred to the Station Aquicole de Pointe-au-Père (Institut des Sciences de la mer, ISMER, QC, Canada). We chose these five groups of oysters for their differences in growth rate, as calculated from settlement until collection.

Oyster characterization

Growth and standard metabolic rates

Upon arrival at ISMER, ~80 oysters from each group were numbered with bee tags and placed into two 150 l aquaria (~40 oysters per group per aquarium, salinity was 28 and temperature was 20±0.8°C). Temperature, salinity and oxygen were monitored daily with multiparameter probes YSI 85 (Yellow Springs Incorporated, OH, USA). Oysters were fed continuously with a mixed suspension of *I. galbana*, *P. lutheri* and *Nannochloropsis* sp. at 10 cells μl^{-1}. The shell length of 68–74 individual oysters from each group was recorded monthly for 9 months.

In the meantime, 50 oysters from each group were numbered with bee tags and placed into a 150 l aquarium. After 21 days of acclimation to laboratory conditions, the minimum oxygen consumption ($\dot{V}_{O_2min}$ or standard metabolic rate) was measured after having starved the oysters for 10 days. Oysters were kept individually in 50 ml metabolic chambers for 60 min before starting the measurement. Empty shells were used as a control. Six chambers were used simultaneously, which allowed us to measure five oysters and one control at a time. Animals that remained closed in the chamber were excluded from physiological analysis. Oxygen consumption for an individual oyster was determined by sealing the chamber and measuring the reduction in $\%O_2$ with a Strathkelvin 928 6-channel dissolved oxygen system using a microcathode 1302 electrode (Strathkelvin Instruments, Glasgow, Scotland, UK). Seawater was well mixed with a magnetic stirrer. The output signal was monitored continuously on a computer until a decrease in O_2 of at least 20% was reached. After the oxygen uptake measurement, each set of oysters was frozen at –80°C for later determination of dry mass, which was measured after drying at 70°C for 72 h. Respiration rate is expressed as the rate expected for a standard oyster in $ml^{-1}\ g^{-1}$ dry mass h^{-1} by application of allometric correction (Widdows and Johnson, 1988).

Genetics

Upon arrival at ISMER, 50 oysters from each group were stored at –80°C until genetic analyses. DNA extraction was carried out using DNeasy tissue kits (Qiagen, Mississauga, ON, Canada). Genetic variation was determined at the polymorphic microsatellite loci *Cvi*7, *Cvi*8, *Cvi* 9, *Cvi*12, *Cvi*13, *Cvi*14 and *Cvi*23 (Brown et al., 2000; Reece et al., 2004). Amplified fragments were separated by capillary electrophoresis using an ABI PRISM 310 automated genetic analyser (Applied Biosystems, Foster City, CA, USA) and analysed

with the Genescan and Genotyper software packages (Applied Biosystems).

Temperature experiment

Experimental design

Upon arrival at ISMER, 243 animals from each group were equally distributed into nine 37 l aquaria (6-Pack Arctic model, Aquabiotech, Coaticook, QC, Canada). Each aquarium had its own filtration, aeration and water temperature control unit. Oysters were acclimated to laboratory conditions for 5 days prior to starting the experiment. The salinity was 28, the natural photoperiod was followed, and the temperature was maintained at 12°C (Fig. 1). The five groups of oysters were maintained together in these aquaria over the entire experiment. Animals were fed a diet of *I. galbana*, *P. lutheri* and *Nannochloropsis* sp. at a total concentration of ~10 cells μl^{-1} twice a day. Temperature was monitored every day in each tank throughout the experiment.

On 17th October 2005, the temperature of three aquaria was gradually decreased (~1.5°C day^{-1}) to 4°C while the temperature of three other aquaria was gradually increased (~1.5°C day^{-1}) to 20°C. The temperature of the remaining aquaria was maintained at 12°C as a control (Fig. 1). All animals reached the desired temperature by 24th October after which these temperatures were maintained for 6 weeks. Physiological rates and lipid composition of oysters were measured on 24th October after attaining the desired temperature (acute response) and again on 5th December (long-term acclimation; Fig. 1).

Physiological rates

A pool of five oysters per aquarium was used for physiological measurements in 500 ml chambers before the oysters were killed for dry mass determination to calculate mass-standardized physiological rates by allometric equation.

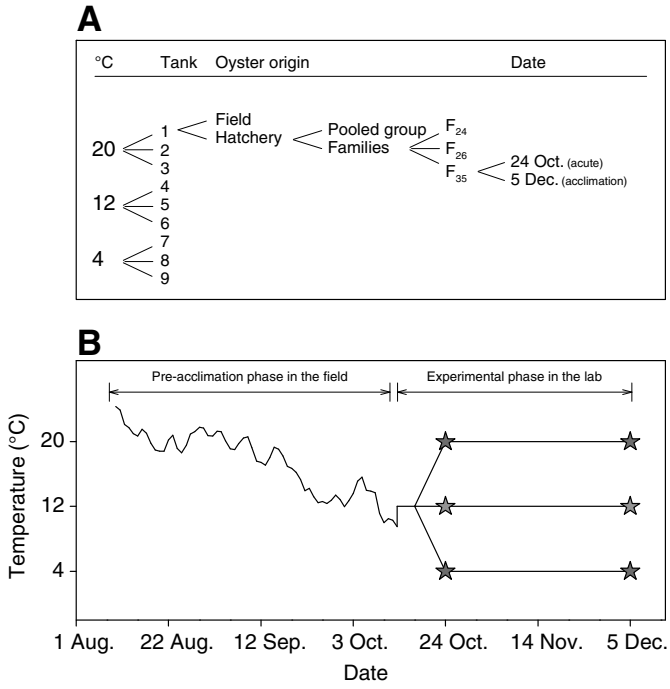

Fig. 1. (A) Schematic diagram of the split–split plot experimental design. (B) Experimental protocol for the temperature experiment. Stars indicate dates of physiological measurements and lipid sampling.

Clearance rate, defined as the volume of water cleared of suspended particles per unit time and biomass, was quantified using a static system (Pernet et al., 2007b). Briefly, animals were provided with *I. galbana* at an initial concentration of 10 cells μl^{-1} and food particles were counted every 15 min for 60 min using an electronic particle counter (Beckman Coulter Counter Z2, Mississauga, ON, Canada). The clearance rate (l h^{-1}) was then used in conjunction with the algal biomass (mg ml^{-1}) to estimate the amount of ingested energy, assuming that the energy content of the diet was 23.5 J mg^{-1} (Widdows and Johnson, 1988).

Oxygen consumption ($\dot{V}_{O_2}$) for an individual animal was determined by sealing the chamber and measuring the reduction in $\%O_2$ as described in the previous section. Respiration was then expressed as ml O_2 g^{-1} tissue dry mass h^{-1} and then converted into energy equivalents using the conversion factor 1 ml O_2=20.33 J (Widdows and Johnson, 1988).

Assimilation, defined as the product of ingested energy and absorption efficiency (Widdows and Johnson, 1988), was estimated using the Conover ratio [see Conover, 1966 in Widdows and Johnson (Widdows and Johnson, 1988)]. Food and faecal samples were filtered onto pre-combusted pre-weighed 47 mm GFC filters that were rinsed with isotonic ammonium formate (3.2%), dried at 80°C for 48 h, cooled to room temperature in a desiccator, and re-weighed. Afterwards, they were combusted overnight at 450°C, cooled to room temperature in a desiccator, and finally weighed again. This procedure provided estimates of the organic and inorganic fractions contained in the food and faeces.

The scope for growth was estimated by subtracting the energy lost through respiration and excretion from the energy obtained by food assimilation (Widdows and Johnson, 1988). As excretion represents <5% of the energy budget in most bivalves, it was ignored.

Lipid analysis

Five oysters from each group were randomly sampled in each aquarium on 24th October and 5th December in each temperature treatment for the determination of lipid composition. Tissues of different oysters from the same aquarium were pooled together to obtain *ca* 100 mg wet mass and stored in lipid-free amber glass vials with Teflon-lined caps under nitrogen in 1 ml dichloromethane at –80°C. Lipids were extracted (Folch et al., 1957), and classes determined as previously described (Pernet et al., 2007b). Lipids were separated into neutral lipids (including triglycerides and sterols) and polar lipids (including mainly phospholipids) using column chromatography on silica gel hydrated with 6% water (Pernet et al., 2007b). Fatty acid methyl esters (FAME) from polar lipids were prepared using 2% H_2SO_4 in methanol (Lepage and Roy, 1984). Gas chromatograph parameters and the procedure for FAME identification and analysis have been described previously (Pernet et al., 2007b).

Statistical analyses

The mean number of alleles per locus, and observed and expected heterozygosities were calculated using the software Genetix 4.05 (Belkhir et al., 1998). The software FSTAT (Goudet, 2001) was used to calculate the fixation index F_{IS} and F_{ST}. The F_{IS} values were tested (1000 randomizations) for significant differences from zero to assess the compliance with the Hardy–Weinberg equilibrium. F_{ST} values were used as an index of genetic differentiation among oyster groups. All probability values were adjusted for multiple comparison tests using sequential Bonferroni adjustments (Rice, 1989).

Three-way split–split plot ANOVAs were conducted to determine differences in the physiological rates and the fatty acid composition

Table 1. Summary of the split–split plot three-way ANOVAs on the effect of temperature, oyster origin and sampling time on physiological rates and lipid composition in juvenile oysters *Crassostrea virginica*

Source of variation	d.f.
Main plot analysis	
Temperature	2
Error A	6
Subplot analysis	
Origin	4
Field *vs* hatchery	1
Pooled group *vs* families	1
F_{35} *vs* F_{24}, F_{26}	1
F_{24} *vs* F_{26}	1
Temperature × origin	8
Error B	24
Sub-subplot analysis	
Time	1
Time × temperature	2
Time × origin	4
Time × temperature × origin	8
Error C	30

Physiological rates: scope for growth, clearance rate and oxygen consumption. Lipid composition: unsaturation index, total polyunsaturated fatty acid (PUFA) and major individual PUFA of polar lipids.
Error A: tank (temperature).
Error B: origin × tank (temperature).
Error C: time × tank (temperature) + origin × time × tank (temperature).
Independent variables were temperature (4°C, 12°C and 20°C), oyster origin (field or hatchery; in the hatchery: pooled group or families F_{24}, F_{26} and F_{35}) and time (acute and acclimation response).

of the polar lipids in oysters, i.e. the unsaturation index and the major polyunsaturated fatty acids (PUFA), namely docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3), as a function of overwintering temperature, oyster origin and sampling time (Table 1). The unit of replication was the aquarium in which the temperature was applied. The main plots were temperature levels, subplots were oyster origin, and sub-subplots were sampling time. Here we used a mixed linear model, which models not only the means of our data but also their variances and covariances. The repeated option was applied to the term 'time' to take into account temporal dependence (SAS 9.1.3 Help and documentation; SAS Institute Inc., Cary, NC, USA).

Where differences were detected, least-square means multiple comparison tests were used to determine which means were significantly different. When differences among groups of oysters were detected without interaction with other factors (main effect), planned contrasts between group means were used (Table 1). Residuals were screened for normality using the expected normal probability plot and further tested using the Shapiro–Wilk test. Data on scope for growth, $\dot{V}_{O_2}$ and 20:5n-3 were log+1 transformed to achieve normality of residuals and homogeneity of variances. Homogeneity of variance–covariance matrices was graphically assessed. Analyses were carried out using SAS 9.1.3.

RESULTS

Oyster characteristics

Although oysters originating from the field were bigger than those produced in the hatchery at the time of collection, they showed a reduced growth rate compared with that of hatchery-reared animals (Table 2). The growth rate of oysters collected in the field from settlement to 12th October 2005 (72.6 μm day^{-1}) was almost half

Table 2. Characteristics of juvenile oysters *Crassostrea virginica*

		Hatchery (age 0+)			
			Families		
Variable	Field (age 1+)	Pooled group	F_{24}	F_{26}	F_{35}
General characteristics at the onset of the experiment					
Mating scheme	ND	$f_{20–39}\times m_{5–9}$	$f_{24}\times m_8$	$f_{26}\times m_9$	$f_{35}\times m_7$
Shell length (mm)	31.3±2.0	22.1±1.5	24.3±2.6	26.1±2.6	23.4±1.9
Growth rate (μm day^{-1})					
Before experiment	72.6±4.6	116.7±7.8	128.5±14.0	137.9±13.7	123.7±9.8
After experiment	32.2±7.4	38.2±4.3	46.2±3.3	47.5±4.8	36.7±4.4
Basal metabolic rate (ml O_2 g^{-1} tissue dry mass h^{-1})					
$\dot{V}_{O_2min}$	0.34±0.09	0.27±0.08	0.19±0.04	0.18±0.07	0.27±0.10
Genetic characteristics (average at 7 loci using DNA microsatellite markers)					
N	51	42	25	28	27
Mean no. alleles per locus	13.9	7.6	2.9	4.9	6.4
H_o	0.60	0.44	0.49	0.57	0.47
H_e	0.73	0.65	0.44	0.62	0.66
F_{IS}	**0.19**	**0.32**	–0.10	0.08	**0.29**

Oysters were collected in the field or produced in the hatchery. Five males (m) and 20 females (f) were mated to produce animals in the pooled group whereas F_x represents full-sib families.
Shell length was measured on 100 animals upon arrival at ISMER on 12th October, at the onset of the temperature experiment.
Growth rates were calculated from settlement to arrival at ISMER, at the onset of the temperature experiment (Before experiment), and in the laboratory at 20°C from the end of the experiment (November 2004) to July 2005 (After experiment, *N*=68–73 animals).
Basal metabolic rate was calculated for individual juvenile oysters maintained at 20°C for 6 weeks (*N*=23–59 animals).
N, sample size; H_o, observed proportion of heterozygotes; H_e, Hardy–Weinberg proportions of heterozygotes; F_{IS}, deviation from Hardy–Weinberg equilibrium. Loci that show significant deviation from Hardy–Weinberg equilibrium are in bold.
Data are means (±s.d. where appropriate).

Table 3. Matrix of index of genetic differentiation (F_{ST}) among the five groups of juvenile oysters *Crassostrea virginica*

	Pooled group	F_{24}	F_{26}	F_{35}
Field	0.100	0.252	0.143	0.089
Pooled group	–	0.195	0.151	0.136
F_{24}		–	0.304	0.261
F_{26}			–	0.192

All pairwise comparisons were significant (P<0.05). Oysters were collected in the field or produced in the hatchery. Five males and 20 females were mated to produce animals in the pooled group whereas F_x represents full-sib families.

that of animals produced in the hatchery, which averaged 126.6 μm day^{-1}. Furthermore, the average growth rate of oysters collected in the field and then exposed to laboratory conditions for 9 months at 20°C was only 32.2 μm day^{-1} compared with 42.2 μm day^{-1} for animals produced in the hatchery. Oysters from families F_{24} and F_{26} showed higher growth rates than other hatchery-reared groups for the two time periods.

The minimum oxygen consumption or standard metabolic rate ($\dot{V}_{O_2min}$) of oysters collected in the field (0.34 ml O_2 g^{-1} h^{-1}) showed an increase of 49.5% compared with that of hatchery-reared animals, where $\dot{V}_{O_2min}$ for the four groups averaged 0.23 ml O_2 g^{-1} h^{-1} (Table 2). It is noteworthy that the fast-growing oysters from families F_{24} and F_{26} showed lower $\dot{V}_{O_2min}$ than the other group of oysters. Interestingly, the $\dot{V}_{O_2min}$ of oysters exposed to laboratory conditions decreased linearly with increasing growth rate ($\dot{V}_{O_2min}$=–0.01×growth rate+0.65; r^2=0.998, N=5, P=0.001; Table 2).

The average number of alleles was much higher in oysters from the field compared with those from the pooled group or the families (Table 2). Interestingly, oysters from F_{24} and F_{26} showed no deviation from Hardy–Weinberg equilibrium whereas the overall F_{IS} values for oysters from other groups indicated significant heterozygote deficiencies. The F_{ST} values among the five groups of oysters varied from 0.09 to 0.30, and all pairwise comparisons were significant (P<0.05), thus indicating a high level of genetic differentiation (Table 3).

Temperature experiment

Physiological rates

The overall scope for growth varied as a function of oyster origin (Fig. 2A). Fast-growing oysters produced in the hatchery showed an average scope for growth of 46.6 J h^{-1} g^{-1} compared with only 18.7 J h^{-1} g^{-1} in animals from the field (contrast: field *vs* hatchery, P<0.001). Although oysters originating from the hatchery showed a similar scope for growth, it is noteworthy that fast-growing animals from F_{26} showed the highest scope for growth. More broadly, the scope for growth of oysters increased linearly with increasing growth rate measured under laboratory conditions at 20°C for 9 months (y=2.75×growth rate–68.5; r^2=0.956, N=5, P=0.004).

Differences in scope for growth among groups of oysters were mainly due to clearance rate (Fig. 2A and B, inset). Overall, clearance rate increased with temperature (main effect, P=0.025), although it also varied as a function of temperature × origin (Fig. 2B). While clearance rate was similar among groups of oysters at 4°C and 12°C (P=0.785), it increased by 2.7 times for fast-growing oysters from F_{26} with the temperature increase from 12°C to 20°C (P<0.001). Although oysters from other groups showed a similar trend for increasing clearance rate with increasing temperature from 12°C to 20°C, the means were not significantly different

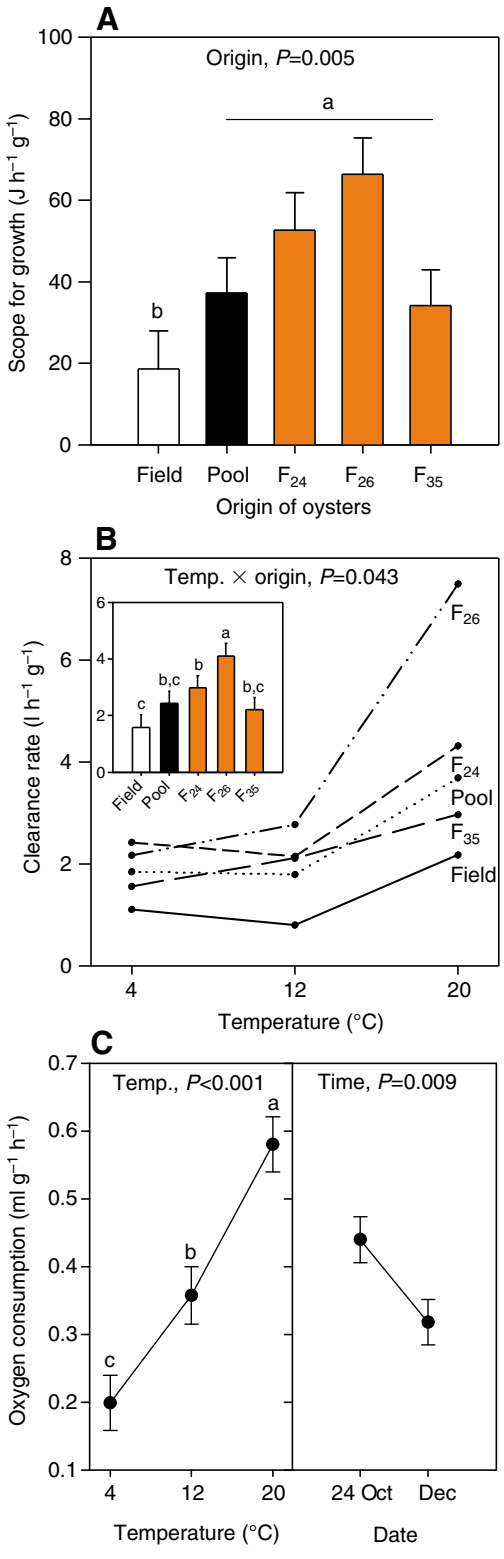

Fig. 2. (A) Scope for growth as a function of oyster origin. (B) Clearance rate of oysters as a function of temperature × origin. The inset shows clearance rate as a function of oyster origin only (main effect). (C) Oxygen consumption rate of oysters as a function of temperature (left) and date (right). Oysters were collected in the field or produced in the hatchery. Five males and 20 females were mated to produce animals in the pooled group (Pool) whereas F_x represents full-sib families. Data are means ± s.e.m., n=3 aquaria. Data from different temperatures and times were pooled together when these effects were not significant. Different letters indicate significant differences.

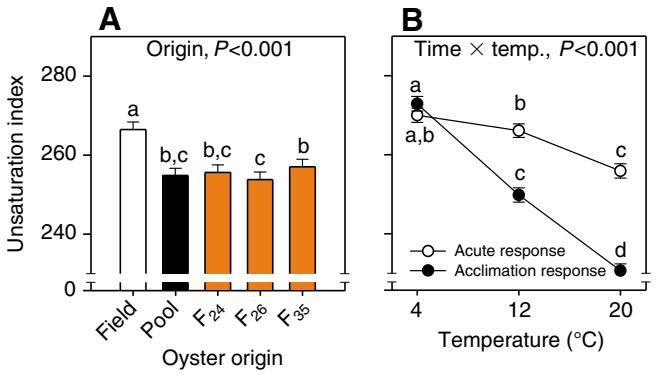

Fig. 3. Unsaturation index of polar lipids in oysters as a function of (A) their origin and (B) time × temperature. Oysters were collected in the field or produced in the hatchery. Five males and 20 females were mated to produce animals in the pooled group (Pool) whereas F_x represents full-sib families. Data are means ± s.e.m., n=3 aquaria. Different letters indicate significant differences.

(0.10<P<0.05 for F_{24} and the pooled group, P>0.4 for others). The absorption efficiency was unaffected by any of the tested factors. Oxygen consumption rate remained unaffected by oyster origin (origin effect: P=0.131; temperature × origin effect: P=0.898; time × origin effect: P=0.326; time × temperature × origin effect: P=0.935). However, oxygen consumption increased with increasing temperature and decreased during acclimation (Fig. 2C).

Fatty acid composition of polar lipids

The unsaturation index, which is the number of double bonds per 100 molecules of fatty acids, was noticeably higher in animals collected in the field (266.1) compared with that of fast-growing oysters produced in the hatchery, where it averaged only 255.0 (contrast: field *vs* hatchery, P<0.001; Fig. 3A). The unsaturation index also varied as a function of time × temperature (Fig. 3B), decreasing with increasing temperature. However, the unsaturation index of animals maintained at 4°C remained constant irrespective of sampling time whereas oysters exposed to higher temperatures showed a major decrease in their unsaturation index with acclimation.

A stepwise multiple regression model using groups of fatty acids as explanatory variables and the unsaturation index as the response variable showed that the unsaturation index was positively correlated with PUFA (y=7.0×PUFA–111.7; r^2=0.916, N=85, P<0.001). A second regression model using individual PUFA as explanatory variables showed that variations in the unsaturation index were mainly attributable to 22:6n-3 and 20:5n-3 (y=3.6×22:6n-3+38.3×20:5n-3+90.3; r^2=0.958, N=85, P<0.001). These two PUFA accounted for ~60% of the total PUFA in oyster polar lipids.

Overall, the level of 22:6n-3 in the fast-growing hatchery oysters (18.5% of total fatty acid) was 35.6% lower than that of oysters from the field, where it averaged 25.1% (Fig. 4A). The level of 22:6n-3 in animals collected in the field increased by 25% with a temperature decrease from 12°C to 4°C, whereas it remained constant (pooled and F_{26}) or increased only marginally (by 8% and 13% for F_{24} and F_{35}, respectively) in oysters produced in the hatchery. The level of 22:6n-3 varied as a function of time × temperature (Fig. 4B). Indeed, animals maintained at 4°C showed similar 22:6n-3 levels irrespective of sampling time whereas oysters exposed to higher temperatures showed a major decrease in 22:6n-3 following acclimation (Fig. 4B).

The effects of time, temperature and oyster origin interacted on 20:5n-3 (Fig. 4C). The level of 20:5n-3 in the fast-growing oysters

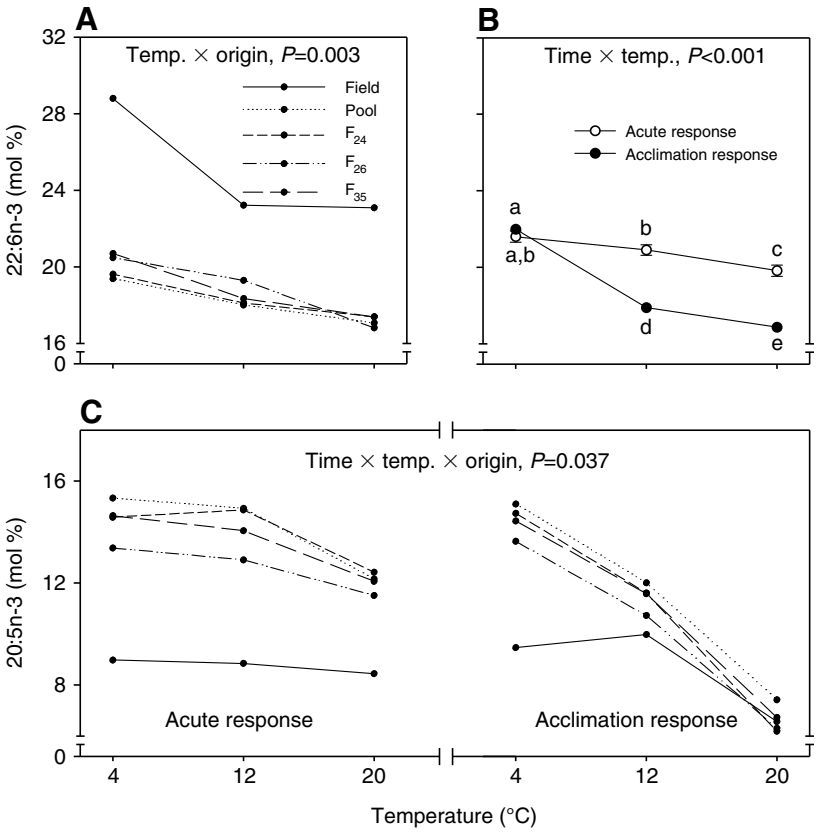

Fig. 4. Docosahexaenoic acid (22:6n-3; mol %) in polar lipids as a function of (A) temperature × oyster origin and (B) time × temperature. (C) Eicosapentaenoic acid (20:5n-3; mol %) in the polar lipids as a function of time × temperature × oyster origin. Oysters were collected in the field or produced in the hatchery. Five males and 20 females were mated to produce animals in the pooled group (Pool) whereas F_x represents full-sib families. Data are means (± s.e.m. for B but omitted for clarity in A and C), n=3 aquaria. Different letters indicate significant differences.

produced in the hatchery (13.6%) was 54.8% higher than that of oysters originating from the field, where it averaged 8.8% before acclimation (Fig. 4C, left side). The 20:5n-3 level in oysters produced in the hatchery decreased with increasing acclimation temperature from 4°C to 20°C (Fig. 4C, right side). However, 20:5n-3 in hatchery-reared oysters maintained at 4°C remained constant irrespective of sampling time, whereas those exposed to temperatures of 12°C and 20°C showed a marked decrease in 20:5n-3 following acclimation. In contrast, 20:5n-3 in oysters from the field maintained at 4°C and 12°C remained constant irrespective of sampling time.

DISCUSSION

Components of growth

For the first time, we have shown that a reduction in the standard metabolic rate ($\dot{V}_{O_2min}$) and in the unsaturation index of membrane phospholipids coincides with higher growth (as expressed by growth rate and scope for growth) in hatchery oysters. Standard $\dot{V}_{O_2}$ and membrane unsaturation were lower in fast-growing hatchery oysters compared with that of slow-growing oysters from the field. Similarly, standard $\dot{V}_{O_2}$ and phospholipid unsaturation showed a positive correlation in comparison to wild and selectively bred hard clams (Pernet et al., 2006) and in comparison to mussels and oysters (Pernet et al., 2007b). Here we provide evidence that intraspecific differences in basal metabolic rate in bivalves relate to membrane unsaturation as predicted by Hulbert's theory of membranes as metabolic pacemakers (Hulbert and Else, 1999; Hulbert and Else, 2005).

The higher unsaturation index observed in membrane lipids of oysters from the field was mainly due to higher 22:6n-3 levels compared with those of fast-growing oysters from the hatchery. The importance of 22:6n-3 in regulating an animal's metabolic rate was previously emphasized by others (Hulbert and Else, 2005). Mammalian phospholipids showed a statistically significant allometric decline in the unsaturation index with increasing body size (Hulbert et al., 2002a). This decline in the phospholipid unsaturation index was predominantly due to the fact that the 22:6n-3 content of tissue phospholipids decreased as the mammal species increased in size. These results are very similar to those reported in birds (Hulbert et al., 2002b; Turner et al., 2006). The functional significance of the elevated 22:6n-3 levels in the membranes of small mammals and birds is potentially related to their high mass-specific metabolic rate compared with larger endotherms. Because a substantial proportion of the basal metabolism is associated with membrane-linked processes [mitochondrial proton leak, Na^+ and Ca^{2+} cycling together account for approximately half of standard metabolic rate (Rolfe and Brown, 1997)], it was suggested that membrane lipids, particularly 22:6n-3, may play a role in determining the metabolic rate of different species *via* an influence on the molecular activity of membrane-bound enzymes (Hulbert and Else, 1999; Hulbert and Else, 2000).

Differences in 22:6n-3 levels between oysters from the field and those from the hatchery may reflect differences in their capacity for selective incorporation of dietary PUFA into membrane lipids. Indeed, long-chain PUFA such as 20:5n-3 and 22:6n-3 have been reported to be essential for sustaining optimal growth in several bivalve species (DeMoreno et al., 1976; Langdon and Waldock, 1981). Although the biosynthetic production of these PUFA is rather low or absent, meaning that the fatty acid composition of lipids in bivalve tissues generally reflects that of the diet, several studies have shown that the fatty acid composition in bivalve membrane lipids is regulated by selective incorporation or elimination of fatty acids (e.g. Delaunay et al., 1993). It is unlikely that differences in 22:6n-3 between groups of oysters reflect differences in their diet as these animals were kept in the same conditions for more than 2 months before the experiment.

Fast-growing oysters from the hatchery showed (1) a lower standard metabolic rate and (2) a higher scope for growth due to higher food consumption compared with slow-growing animals from the field. Similarly, Pacific oysters *Crassostrea gigas* from a fast-growing line showed a higher rate of ingestion and absorption and a lower metabolic rate at maintenance than did those from a slow-growing line (Bayne, 1999). A related field experiment with the Sydney rock oyster *Saccostrea glomerata* (formerly *S. commercialis*) showed that selection for fast-growing animals leads to faster rates of feeding across a wide range of food concentrations (Bayne et al., 1999b). Finally, the superior growth in *C. gigas* compared with that of *S. glomerata* maintained in the same conditions also coincided with a faster rate of feeding in *C. gigas* (Bayne, 1999; Bayne, 2002). Therefore, our results on the bioenergetics of juvenile *C. virginica* are in good agreement with previously published data on other oyster species.

Finally, there was a smaller deficiency in heterozygote frequencies in fast-growing oysters from F_{24} and F_{26} compared with that of animals from other groups. Several studies have shown negative correlations between heterozygote deficiency and fitness-related traits in marine bivalves, attributed to lower metabolic requirements for heterozygous individuals (Hawkins et al., 1989; Tremblay et al., 1998; Bayne et al., 1999a). In our study, higher metabolic demands in oysters from the field, the pooled group and the F_{35} associated with a higher heterozygote deficiency probably impose a supplementary stress that results in a reduction of growth rate in these animals.

Temperature adaptation

A perfect negative relationship was observed between acclimation temperature and the unsaturation index of polar lipids in juvenile oysters, as predicted by the homeoviscous adaptation theory (Hazel, 1995). Interestingly, changes in the unsaturation index in response to temperature were mainly due to 20:5n-3 levels in fast-growing oysters from the hatchery, whereas slow-growing animals changed levels of both 22:6n-3 and 20:5n-3, as previously observed in adult *C. virginica* (Pernet et al., 2007a; Pernet et al., 2007b). This pattern may reflect differences in the proportions and thus the availability of 22:6n-3 and 20:5n-3 among groups of oysters (see previous section). In a previous study, we showed that the inverse relationship between the unsaturation index of gill phospholipids and acclimation temperature was principally due to changes in 22:6n-3 and 20:5n-3 levels, but that the magnitude of the response of these fatty acids varied between oysters and mussels (Pernet et al., 2007b). Indeed, the decrease of 20:5n-3 with a rise in temperature was much stronger in mussels than in oysters. Although speculative, the efficiency of homeoviscous adaptation in fast-growing oysters from the hatchery may be higher than that of slow-growing animals from the field, since the melting point of 20:5n-3 is 10°C lower than that of 22:6n-3.

The unsaturation index and the fatty acid composition of oysters showed only small differences among temperature treatments on 24th October compared with those observed on 5th December, after 6 weeks of acclimation. Therefore, the fatty acid remodelling in the membrane lipids of juvenile oysters may be viewed as a long-term adjustment to temperature. It is generally accepted that changes in the fatty acid composition of membrane lipids usually occur after a period of acclimation, varying in duration from 1 week

in the case of warm acclimation to several weeks during cold acclimation (Hazel and Williams, 1990). A previous study on rainbow trout showed that exposure to low temperatures led to a gradual increase in PUFA during cold acclimation (Hazel and Landrey, 1988). Likewise, hard clams exposed to a lowering of environmental temperature showed a gradual increase in the unsaturation index that was viewed as a long-term adjustment to winter temperature (Pernet et al., 2006). However, few studies showed that changes in fatty acid composition could occur in the short term. For example, in oysters, the unsaturation index of gills subjected to daily fluctuations in temperature between 12°C and 25°C for 7 days varied in a way consistent with membrane homeoviscous adaptation, mainly due to rapid changes in 22:6n-3 and 20:5n-3 levels (Pernet et al., 2007a).

There was no significant temperature effect on the scope for growth of juvenile oysters maintained at 4°C, 12°C and 20°C, reflecting the fact that both energy expenditure (respiration) and acquisition (ingestion) increased with temperature. This result contrasts with several published studies on other bivalve species (for a review, see Bayne et al., 1985) (see also MacDonald and Thompson, 1986; Beiras et al., 1995; Yukihira et al., 2000; Cusson et al., 2005). For example, the scope for growth of juvenile oysters *O. edulis* that were initially maintained at 20°C and further exposed to 14°C, 20°C or 26°C for 3 weeks increased with temperature (Beiras et al., 1995). Likewise, the scope for growth of mussels *M. edulis* acclimated at 8°C was found to be higher than that of mussels kept at –1°C (Cusson et al., 2005). Furthermore, considering that scope for growth is a predictor of total production (growth rate in juveniles), our result suggest that growth rate in oysters would be similar over temperatures ranging from 4°C to 20°C, which is very unlikely. The lack of a temperature effect on the scope for growth of oysters in our study may reflect an inability of the statistical model to detect the effect of temperature due to the low number of replicate tanks (n=3) coupled with high variation: animals acclimated at 4°C, 12°C and 20°C showed scope for growth of 24.5±15.3, 30.6±17.5 and 59.3±35.8, respectively.

For the first time, we have shown intraspecific variations in the effect of temperature on clearance rate. Overall, the clearance rate of animals exposed to 4°C was lower than that of oysters kept at 20°C (P=0.014). Although very few studies have specifically examined the effect of temperature on feeding in *C. virginica*, one paper reported that the pumping rate in this species increased steadily as temperature rose from 8°C to 28°C (Loosanoff, 1958). More recently, we showed that adult *C. virginica* acclimated to overwintering temperatures (<9°C) exhibited a reduced clearance rate compared with that of animals acclimated to spring–summer conditions at 20°C (Pernet et al., 2007b), which agreed well with the pattern observed in F_{26}.

Oxygen consumption increased with temperature irrespective of sampling time, thus suggesting that the respiration rate of these animals did not acclimate even after 6 weeks. We know from the physiological literature that thermal acclimation is not a universal feature of bivalve metabolism: the ability of bivalves to alter metabolism following a temperature change is species specific [see for example Beiras et al. (Beiras et al., 1995) and references therein]. More particularly, the oxygen consumption in oysters *C. virginica* and *Ostreas edulis* remains strongly dependent on the ambient temperature even after 3 weeks, thus suggesting that these species have a limited ability to acclimate to temperature change (Newell et al., 1977; Shumway and Koehn, 1982). Therefore, the marked thermal dependence of the respiration rates found in this work on juvenile *C. virginica* is consistent with previous studies on oyster species.

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