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Physiological responses of female and male black-lip pearl oysters *(Pinctada margaritifera)* to different temperatures and concentrations of food

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Abstract – This study was designed to measure responses of four-year-old black-lip pearl oysters (*Pinctada margaritifera*) to different temperatures and food concentrations and to identify the energy requirements of each sex. Oysters were fed a monospecific microalgal diet of *Isochrysis* affinis *galbana* (T-iso). Measurements of oxygen consumption and ingestion rates were carried out at 24 °C and 27 °C and at two algal (T-iso) concentrations: 5000 and 30 000 cell ml^{-1}. Glycogen content in adductor muscle, absorption efficiency and scope for growth were also estimated. Females and males responded differently to environmental factors, with food level being the most influential parameter. Oxygen consumption and absorption efficiency were significantly higher in females than in males, but males had significantly higher glycogen content than females. At high food concentration, glycogen content, ingestion rate, oxygen consumption, and scope for growth were significantly higher than at the low food concentration. Only absorption efficiency was significantly higher at the low food concentration. Oxygen consumption was significantly higher at 27 °C than at 24 °C. These results indicate that females and males have different bioenergetic functioning and that energy demands for reproduction are higher in females.

Keywords: Food supply / Ingestion rate / Glycogen / Oxygen consumption / Scope for growth / Pearl oyster

1 Introduction

The black-lip pearl oyster *Pinctada margaritifera* (Pteriidae) is an economically important species because it is the basis of the pearl industry in the islands and atoll lagoons of French Polynesia. Approximately 7000 persons work on 800 farms (10 000 ha), producing ~10 t per year, worth €88 million in 2007 (Cochennec-Laureau et al. 2010). Almost all cultured pearl oysters are initially collected from the wild, but production of spat in hatcheries is intended to supply oysters with desirable traits, such as pearl colour and fast growth (Le Moullac et al. 2003; Hui et al. 2011).

P. margaritifera is a protandric species. Populations growing in both natural and cultivated conditions have low numbers of females, and females are mainly among the older oysters (Chávez-Villalba et al. 2011). Gender is determined by genotype or environmental factors or their interaction (Valenzuela et al. 2003). Since *P. margaritifera* occupies areas with relatively homogenous temperature and food, the effect of these parameters on the expression of gender has not been well established. Available information suggests that temperature influences sex ratios in this oyster because more females have been found in the northern populations of French Polynesia, where temperature is higher and the range is narrower (Chávez-Villalba et al. 2011). Furthermore, pearl oysters exposed to plenty of food may accelerate the change from male to female (Tranter 1958; Pouvreau et al. 1999).

In bivalves, the general theory is that more energy is required to produce eggs than sperm, resulting in different metabolic requirements of the sexes (Wright 1988). This theory was investigated by Baghurst and Mitchell (2002) in Pacific oyster (*Crassostrea gigas*), showing that females are better able to use a food-rich environment, grow faster, and display superior condition. Other studies show that this pattern is not consistent among bivalves. In Pectinidae, Vahl and Sundet (1985) found that *Chlamys islandica* metabolic rates of mature males increase at a faster rate with body size than mature females. Tran et al. (2008) found that oxygen consumption in male *C. gigas* is twice that of females. These studies suggested that the high cost of sperm production, involving protein synthesis, cannot be supported by stored glycogen reserves, restricting spermatogenesis to periods of more abundant food. Studies of *Crassostrea gigas* and Sydney rock oysters (*Saccostrea glomerata,* Ostreidae) show no differences in oxygen consumption between males and females at different stages of their reproductive cycles (Soletchnik et al. 1997; Honkoop 2003). It is important to consider whether metabolic rates of bivalves, as measured by oxygen consumption, are

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influenced by a number of variables, such as temperature, salinity, food concentration, oxygen tension, body size, reproductive status, activity level, and physiological condition (MacDonald et al. 2006).

Acquisition of energy in bivalves can be described by different parameters, such as the clearance rate (CR, in L h^{-1}), filtration rate and ingestion rate (IR, in cell h^{-1}). CR is the amount of water pumped by the individual, using the concentration of microalgae as a marker (Yukihira et al. 1998; Petersen et al. 2004). IR directly describes the rate at which microalgae is captured. For *P. margaritifera*, IR is a saturating function of microalgae concentration modelled with an adapted Michaelis-Menten function (Le Moullac et al. 2013). When IR is measured, this is a direct descriptor of energy acquisition. Assimilation efficiency (AE) can then be derived by considering the residual organic matter content in the faeces and pseudofaeces. Assimilation of organic matter by a bivalve varies according to the quantity and quality of suspended particulate matter (Saraiva et al. 2011). Energy losses involve oxygen consumption and excretion (Pouvreau et al. 2000). The scope for growth (SFG), resulting in energy gained, is the difference between the energy acquired by feeding and that lost by respiration and excretion (Pouvreau et al. 2000).

The objective of this study was to test the hypothesis that the energy needs of female and male pearl oysters *P. margaritifera* of the same age and originating from the same location are different. An experimental plan was designed to simultaneously test the effect of temperature and amount of food in females and males to measure the metabolic rates and calculate the scope for growth, according to sex. A low level of algae was chosen to support the mitotic process of the germinal stem cells in order to achieve full gametogenesis (Le Moullac et al. 2013). We did not measure excretion because it only represents a small part of the energy balance (less than 5%) in *P. margaritifera* (Yukihira et al. 1998). These eco-physiological experiments were performed as a first step to identifying the energy requirements of each gender so that this information can be used to modify gender expression in future experiments.

2 Materials and methods

The pearl oysters used in this study came from a cultured population located in Vairao lagoon in Tahiti, French Polynesia. Here, oysters are maintained in traditional systems consisting of suspended long-lines immersed at 10 m depth, with oysters "ear-hanging" in pairs on down-lines by groups of twenty.

A batch of 450 four-year old oysters (135 ± 13 mm average shell height) was taken from the cultivation system and examined to determine gender. Each animal was partly opened and the mantle and gills were temporarily displaced to gain access to the gonad to collect a sample with a 1 ml syringe. Gonad smears were placed on slides and observed under a microscope to identify the type of gametes. An identifying plastic tag was attached to each oyster. Since the sex identification procedure is stressful, tagged female (84) and male (290) oysters were re-placed separately on the long lines and left for at least two weeks to recuperate between sexing and experimentation.

2.1 Experimental design

Oysters were maintained in an acclimation system containing four 500-L open-flow tanks, with a water replacement rate of 100 L h^{-1}. The experimental oysters were continuously supplied with a monospecific microalgal diet (*Isochrysis* aff. *galbana*) to obtain the desired food concentrations around them. A raceway system in each tank produced constant mixing of water and generated a homogenous concentration of microalgae and a well-oxygenated environment. During the acclimation period, waste (faeces and pseudofaeces) from the oysters in each tank were collected to measure assimilation efficiency of organic matter by analysing microalgae and waste products (Conover 1966). The oysters were laid out in a collector, where their waste was retained on a 10 μm sieve. The waste was analysed according to Le Moullac et al. (2012).

The four conditioning experimental treatments consisted of combinations of two temperatures and two food concentrations: (1) 24 °C and 5000 cell ml^{-1}, (2) 24 °C and 30 000 cell ml^{-1}, (3) 27 °C and 5000 cell ml^{-1}, and (4) 27 °C and 30 000 cell ml^{-1}. The smaller concentration of algae was designated as low food level (LFL); the higher concentration was designated as high food level (HFL).

Four series of experiments were performed sequentially. One experimental series consisted of the four combinations of temperature and food concentration, each tested on four oysters (2 females and 2 males). Each series was composed of two phases: (1) two weeks in acclimation and (2) 48 h in an ecophysiological measurement system (EMS) to monitor clearance and oxygen consumption (Fig. 1).

2.2 Ecophysiological measurement system

After two weeks of acclimation, each oyster in each treatment was placed in the ecophysiological measurement system (EMS) to monitor clearance rate and oxygen consumption. The EMS consists of five open-flow chambers. For each treatment, each of the four oysters was placed, successively, in one of the chambers and the fifth chamber remained empty to be used as a control (Fig. 1). Experimental conditions during acclimation were replicated in the EMS during measurements. For this, the chambers contained water at the same temperature and concentration of algae as in the treatments tanks. Flow rates in the chambers were constant at 10 L h^{-1}.

Each chamber was equipped with a two-way electromagnetic valve activated by an automaton. When the valve of one measuring chamber was opened, the released water was analysed for 3 min using a fluorometer (AU100, Turner Designs, Sunnyvale, CA) to measure fluorescence, then an oximeter (OXI 358, WTW, Weilheim, Germany) to measure dissolved oxygen (data stored on a computer). Each cycle was completed within 3 min and another cycle started in the control chamber for 3 min (sequence: chamber 1, control, chamber 2, control, chamber 3, etc.). Specimens in each treatment remained in the chambers for at least 48 h; measurements of each variable were taken every 24 min until 120 measurements of clearance and oxygen consumption had been recorded. A total of 64 oysters were individually monitored in the EMS (eight females and eight males per treatment).

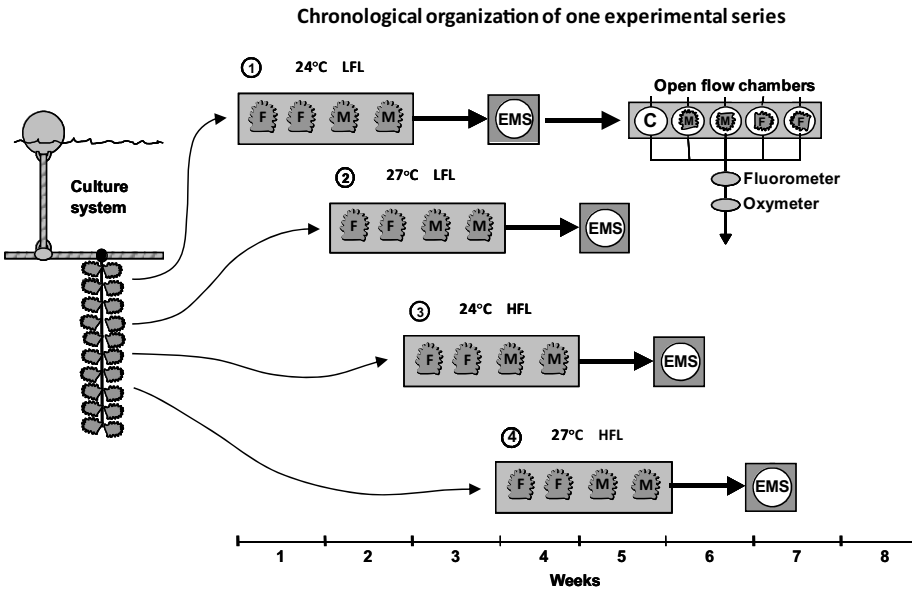

Fig. 1. Experimental design and ecophysiological measurement system (EMS). (1) 24 °C, 5000 cell ml^{-1}; (2) 24 °C, 30 000 cell ml^{-1}; (3) 27 °C, 5000 cell ml^{-1}; (4) 27 °C, 30 000 cell ml^{-1}; F: Female, M: Male, C: Control chamber (empty).

2.3 Ecophysiological measurements

Ingestion rate, an indicator of feeding activity, is defined as the quantity of microalgae cleared per unit of time. Ingestion rate (*IR*) was estimated using fluorescence measurements and calculated as:

$$IR = V(C_1 - C_2),$$

where C_1 is the fluorescence level of the control chamber, C_2 is the fluorescence of the experimental chamber containing one oyster, and V is the constant water flow rate (10 L h^{-1}).

Oxygen consumption rate was measured (mg O_2 h^{-1}). Differences in oxygen concentration (*OC*) between the control and experimental chambers were used to calculate oxygen consumption rates:

$$OC = V(O_1 - O_2),$$

where O_1 is the oxygen level in the control chamber, O_2 is the oxygen level in the experimental chamber, and V is the water flow rate.

Ingestion and oxygen consumption rates were estimated and an average calculated for each oyster, taking into account only values recorded when the oysters were actively filtering or consuming oxygen. To compare ingestion and oxygen consumption rates, it was necessary to correct for differences in specimen weight. Values of the ecophysiological activities were converted to a standard animal basis (1 g, dry weight), using the formula:

$$Ys = (Ws/We)^{b} \times Ye,$$

where *Ys* is the physiological activity of a standard oyster, *Ws* is the dry weight of a standard oyster (1 g), *We* is the dry weight of the specimen, *Ye* is the measured physiological activity, and *b* is the allometric coefficient of a given activity. The average *b* allometric coefficients were 0.66 for ingestion rate and 0.75 for oxygen consumption rate (Savina and Pouvreau 2004).

Assimilation efficiency of organic matter was assessed by analysing microalgae, faeces and pseudofaeces according to the method of Conover (1966). The oysters were laid out in a collector, in which the deposits were collected on a 10-μm sieve. Faeces and pseudofaeces were centrifuged for 15 min at 3400 × g. The supernatant was removed and the pellet was washed twice with 37% ammonium formate in distilled water. The pellet was placed in an aluminium cup (tare weight) and dried at 60 °C for 48 h, before being burnt at 450 °C for 4 h. Microalgae organic matter was obtained by centrifugation of 5 L of the microalgae and followed by treatment of the pellet according to the same procedure as for organic waste. Assimilation efficiency (AE) was calculated (Conover 1966) as:

$$AE = (OM_m - OM_w)/[(1 - OM_w) \times (OM_m)],$$

where *AE* is the assimilation efficiency, OM_m is the microalga organic matter (0.87 for *Isochrysis* aff. *galbana*) and OM_w is the waste organic matter.

Ecophysiology data were converted into energy values to define the scope for growth (SFG) for each male and female oyster:

$$SFG = (IR \times AE) - OC,$$

where *IR* is the ingestion rate, *AE* is the assimilation efficiency, and *OC* is oxygen consumption. We used 20.3 J for 1 mg of particulate organic matter (Bayne et al. 1987) and 14.1 J for 1 mg O_2 (Bayne and Newell 1983; Gnaiger 1983).

2.4 Dry weight and glycogen measurements

After the oysters were removed from the EMS, they were dissected and sexed. Tissues were detached from the shells and separated into three portions (adductor muscle, gonad, and mantle-gills). These were placed in Petri dishes and

Table 1. Statistical analysis of flesh, visceral mass, muscle, and mantle-gills dry weight, and gonadosomatic index (GSI) of *Pinctada margaritifera* at the end of the experiments, according to treatments based on combinations of temperature, concentration of food (LFL: low food level, HFL: high food level, F: female, M: male). *ANOVA performed on *Asin*-transformed GSI.

Temperature (°C)	Food level (cell ml^{-1})	Sex	*N*	Flesh (g)	Visceral mass (g)	Muscle (g)	Mantle-gills (g)	GSI
24	LFL	F	7	5.35 ± 0.97^{a}	1.62 ± 0.38^{b}	1.71 ± 0.32^{c}	2.06 ± 0.44^{d}	0.30 ± 0.04^{d}
		M	9	5.64 ± 1.20^{a}	1.88 ± 0.62^{b}	1.89 ± 0.41^{c}	1.87 ± 0.36^{c}	0.33 ± 0.06^{d}
	HFL	F	8	5.39 ± 1.51^{a}	1.66 ± 0.39^{b}	1.82 ± 0.66^{c}	1.91 ± 0.56^{c}	0.32 ± 0.05^{d}
		M	8	5.77 ± 1.31^{a}	2.04 ± 0.56^{b}	1.92 ± 0.51^{c}	1.82 ± 0.35^{c}	0.35 ± 0.03^{d}
27	LFL	F	8	5.50 ± 1.34^{a}	1.80 ± 0.60^{b}	1.83 ± 0.56^{c}	1.87 ± 0.35^{c}	0.33 ± 0.08^{d}
		M	7	4.97 ± 0.85^{a}	1.41 ± 0.30^{b}	1.70 ± 0.31^{c}	1.86 ± 0.36^{c}	0.29 ± 0.04^{d}
	HFL	F	7	5.53 ± 1.20^{a}	1.92 ± 0.62^{b}	1.79 ± 0.53^{c}	1.82 ± 0.40^{c}	0.35 ± 0.08^{d}
		M	7	5.38 ± 1.41^{a}	1.91 ± 0.64^{b}	1.75 ± 0.59^{c}	1.73 ± 0.40^{c}	0.36 ± 0.07^{d}
	p			0.964	0.384	0.985	0.910	0.227**

stored at −20 °C. Later, all samples were freeze-dried for 72 h. Then, each sample was weighed to the nearest 0.1 g and frozen until further analysis. Freeze-dried muscle was used to measure glycogen content. Muscles were powdered in a mortar, and a 10 mg sample was homogenized in 1 ml de-ionized water. Changes in the glycogen composition of muscle were measured by the phenol-sulphuric acid method (Dubois et al. 1956).

2.5 Statistical analyses

Normality of data distribution and homogeneity of variance were tested, using the Shapiro-Wilk test and the F-test, respectively (Wessa 2012). Normality and homogeneity of variance were determined for dry weight data. Gonadosomatic index (GSI) data were transformed to arcsine values for statistical analyses. Means of total flesh, muscle, visceral mass, muscle dry weight, and GSI were compared using one-way ANOVA for each combination of sex, temperature, and food concentration.

Since the assumptions of normality and homogeneity of variance were not met for the data used to determine metabolic rates (*AE, IR, OC,* and *SFG*) and glycogen, we used the non-parametric Kruskall-Wallis test to simultaneously compare means of each combination of sex, temperature, and food concentration. The Mann-Whitney U test was used to compare means separately, according to temperature, food level, and sex on metabolic rates and glycogen content. Results were considered significant at $p < 0.05$. We performed a 3-way ANOVA on all transformed variables.

3 Results

3.1 Biometric analysis

One-way ANOVA indicated that the pearl oysters in this experiment were homogeneous, since final dry weights of muscle, visceral mass, mantle-gills and gonadosomatic index (GSI) were not significantly different (Table 1).

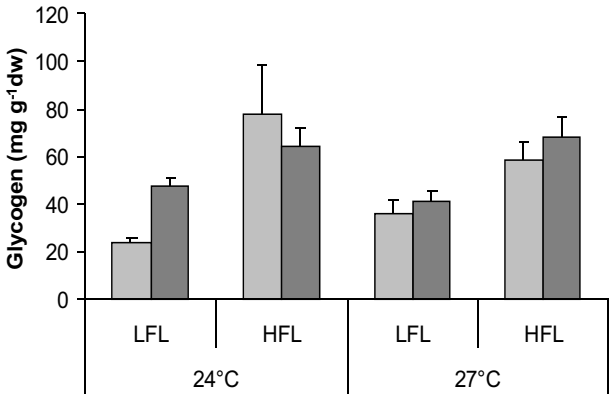

Fig. 2. Effect of high (HFL) and low (LFL) food concentration and temperature (24 °C and 27 °C) on glycogen content in adductor muscle in females (grey) and males (black) of the black-lip pearl oyster *Pinctada margaritifera*. Means are presented with standard error ($5 < n < 10$).

3.2 Energy management: glycogen content of the adductor muscle

The Mann-Whitney test showed that the difference in glycogen content in adductor muscle between treatments at 24 °C and 27 °C was not significantly different ($U = 427$, $p = 0.739$). Glycogen content of oysters fed HFL diet was significantly higher than oysters fed LFL diet ($U = 163$, $p < 0.0001$). Muscle glycogen content was also significantly higher in males than in females ($U = 294$, $p = 0.021$) (Fig. 2, Table 2).

3.3 Digestion: assimilation efficiency (AE)

Nonparametric analysis with the Mann-Whitney test indicated that temperature did not significantly influence assimilation efficiency ($U = 416$, $p = 0.625$), but that food concentration and gender did induce significant changes. Assimilation efficiency was significantly higher: (i) in oysters fed LFL than in those fed HFL ($U = 141$, $p < 0.0001$), and (ii) in females than in males ($U = 313$, $p = 0.043$) (Fig. 3, Table 2).

Table 2. Significance level of Mann-Whitney non-parametric test of ingestion rate (IR), absorption efficiency (AE), oxygen consumption (OC), scope for growth (SFG), and glycogen content in adductor muscle of *Pinctada margaritifera.* LFL: low food level, HFL: high food level, F: female, M: male.

		Metabolic rate			Energy acquisition	Energy storage
Factors	Abbrev.	IR	AE	OC	SFG	Glycogen
Temperature (°C)	24/27	ns	ns	0.004	ns	ns
Food level (cell ml^{-1})	LFL/HFL	0.0002	<0.0001	<0.0001	0.004	<0.0001
Sex	M/F	ns	0.044	0.027	ns	0.021

ns: no significant difference.

3.4 Metabolic rates: ingestion and oxygen consumption

Analysis of ingestion rates with the Mann-Whitney test showed no significant differences between the two temperature treatments (U = 400, p = 0.464) or between males and females (U = 421.5, p = 0.673). Ingestion rates of oysters fed HFL diet were significantly higher than oysters fed LFL diet (U = 198, p = 0.0002) (Fig. 4a, Table 2).

Oxygen consumption rate (OC) was sensitive to all factors tested: it was significantly higher at 27 °C, than at 24 °C (U = 253, p = 0.0037), significantly higher than oysters fed LFL diet than in those fed HFL diet (U = 184, p < 0.0001), and significantly higher in females than in males (U = 300, p = 0.0266) (Fig. 4b, Table 2, see the 3-way ANOVA, Table 3).

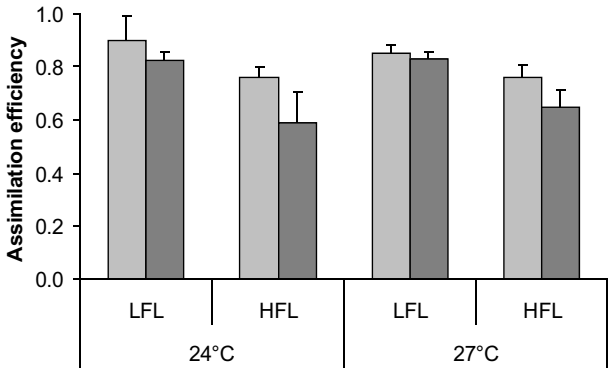

Fig. 3. Effect of high (HFL) and low (LFL) food concentration and temperature (24 °C and 27 °C) on absorption efficiency in females (grey) and males (black) of the black-lip pearl oyster *Pinctada margaritifera*. Means are presented with standard error (5 < n < 10).

3.5 Standardized scope for growth (SFG)

Analysis of metabolic rates indicated that, on average, the mean energy balance remained positive. Only food concentration induced significant changes in SFG, which was increased in oysters fed HFL diet (U = 255, p = 0.004). Neither temperature (U = 391, p = 0.387) nor gender (U = 384, p = 0.329) significantly changed SFG (Fig. 5, Tables 2 and 3).

4 Discussion

Bioenergetics are related to the overall genetic makeup of individual bivalves (Bayne 2000). It is known that a high level of heterozygosity in marine bivalves is the basis of a wide variability in individual phenotypic responses (Harrang et al. 2013). This variability makes it possible to improve growth and stress resistance by genetic selection, which modifies physiological responses such as reproduction (Huvet et al. 2010) and immunity (Delaporte et al. 2006). Phenotypic responses to selection are commonly associated with the speed of physiological fluxes via available energy (reserves and accessibility to food) (Bayne 2000) and the heterozygosity level (Tremblay et al. 1998). Reproduction is energetically expensive, leading to higher consumption of oxygen (Rueda and Smaal 2004). For example, after spawning, *C. gigas* do not feed for 36 hours, but their oxygen consumption doubles (Lambert et al. 2007).

There are numerous studies on metabolic rates of bivalves (e.g., Velasco 2007; Han et al. 2008; Yu et al. 2010;

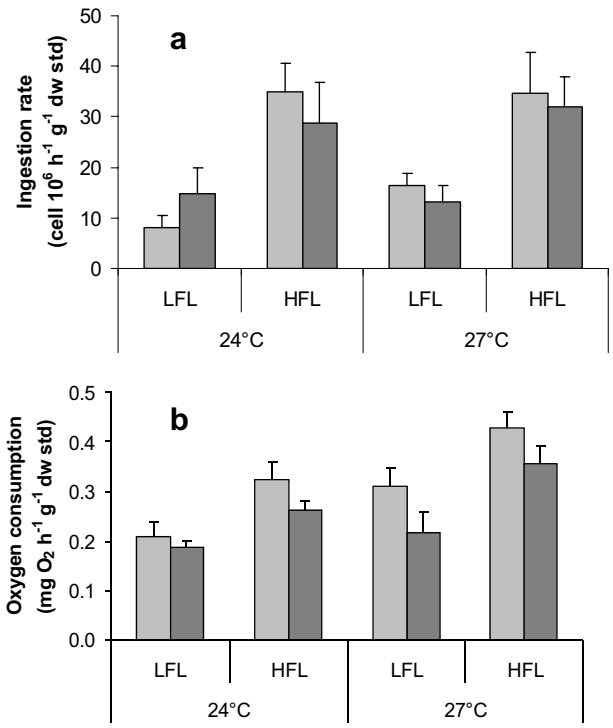

Fig. 4. Effect of high (HFL) and low (LFL) food concentration and temperature (24 °C and 27 °C) on ingestion rates (a), and oxygen consumption (b) in females (grey) and males (black) of the black-lip pearl oyster *Pinctada margaritifera*. Means are presented with standard error (5 < n < 10).

Table 3. Three-way ANOVA of transformed data.

Sources of variations	BC* Glycogen		BC* Ingestion rate		Asin** Assimilation efficiency		BC* Oxygen consumption		Ln*** Scope for growth	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Temperature	0.20	0.66	0.41	0.53	0.38	0.54	10.58	***0.002***	0.61	0.44
Food level	25.20	***<0.0001***	16.89	***0.0001***	21.35	***0.0001***	26.59	***<0.0001***	6.08	***0.02***
Sex	5.19	***0.03***	0.07	0.93	6.08	***0.02***	5.25	***0.03***	0.34	0.56
Temperature × Food	0.10	0.72	0.52	0.47	0.01	0.94	0.12	0.73	0.24	0.62
Temperature × Sex	0.55	0.46	0.30	0.59	0.05	0.83	1.41	0.24	0.11	0.92
Food × Sex	2.41	0.13	0.25	0.62	0.27	0.61	0.02	0.89	0.83	0.37
Temperature × Food × Sex	2.05	0.16	1.14	0.29	0.41	0.53	0.17	0.67	1.52	0.22

*Box-Cox transformation (Box and Cox 1964) was used to adjust data to normality, ** Asin $\sqrt{p}$ transformation, *** Ln(y+20) transformation.

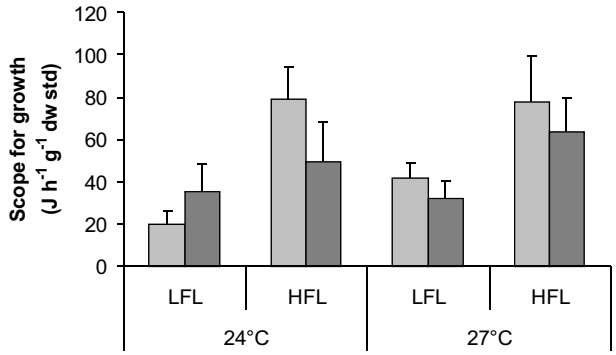

Fig. 5. Effect of high (HFL) and low (LFL) food concentration and temperature (24 °C and 27 °C) on standardized scope for growth (SFG) in females (grey) and males (black) of the black-lip pearl oyster *Pinctada margaritifera*. Means are presented with standard error ($5 < n < 10$).

Hansen et al. 2011), but few of them examine gender as a source of variation. Studies of females and males have been made on Icelandic scallop *Chlamys islandica* (Vahl and Sundet 1985), freshwater eastern floater mussel *Pyganodon cataracta* (Tankersley and Dimock 1993), Sydney rock oyster *Saccostrea glomerata* (Honkoop 2003), and Pacific oyster *Crassostrea gigas* (Soletchnik et al. 1997; Tran et al. 2008). In our study, apart from a wide range of factors acting on metabolic functioning of *P. margaritifera* (Yukihira et al. 1998, 2000; Pouvreau et al. 1999, 2000; Le Moullac et al. 2012), we focused on specific metabolic responses of females and males. This provided the first information concerning specific metabolic behaviours related to gender in this species. We showed that metabolic needs in females were greater than those in males. Since the oysters in our experiments were adults, it can be concluded that the differences noted in energy consumption are associated with reproduction, rather than growth.

4.1 Effect of temperature

Our results showed that ingestion rates in *P. margaritifera* were independent of temperature in the range 24 °C–27 °C. Such feeding behaviour was previously reported for *P. margaritifera* and *P. maxima* between 23 °C and 32 °C, but an effect of temperature was detected between 19 °C and 23 °C (Yukihira et al. 2000). In oysters, metabolism is dependent on temperature; usually, an increase in temperature causes an increase in oxygen consumption, and experimental approaches have confirmed this relationship in many bivalves (Aldridge et al. 1995; Bougrier et al. 1995; Marsden and Weatherhead 1998; Hicks and McMahon 2002). The same relationship is also found in tropical bivalves, such as the Calafia pearl oyster *Pinctada mazatlanica* (Saucedo et al. 2004). Shumway (1982) pointed out that respiration rate usually increases with rising temperature, up to a maximum or optimum limit beyond which it rapidly decreases. Sugiyama and Tomori (1988) reported that the respiratory rate shifts at 33 °C (tested range 15–35 °C). However, as far as we know, this highest temperature is not reached within the geographic distribution of *P. margaritifera*.

4.2 Effect of food

The only parameter that significantly affected ingestion at 24 °C and 27 °C was food level, as ingestion rate was found to increase with food concentration. Ingestion rate in *P. margaritifera* is a saturating function of seston concentration as modelled with an adapted Michaelis-Menten function: ingestion rate increases with food density at a decelerating rate until a maximum is reached above which ingestion rate remains constant (Le Moullac et al. 2013). Filtration rate of *P. margaritifera* decreases in a correlated manner because the increase in algal concentration means that less effort is required to obtain food (Yukihira et al. 1998). This behaviour is common to most bivalves, including Atlantic bay scallop *Argopecten irradians concentricus*, Eastern oyster *Crassostrea virginica* (Palmer 1980), and king scallop *Pecten maximus* (Laing 2004). Filtration promotes growth in bivalves only up to an optimum food concentration, beyond which growth rates decline (Winter 1978).

Feeding is closely associated with respiration because the gills capture the particles present in the circulating water; therefore, differences in respiration rates are a likely response to nutrition (Bierbaum and Shumway 1988). Oxygen consumption increases with algal density in various bivalves, including Japanese pearl oyster *P. fucata*, blue mussel *Mytilus edulis* (Tomaru et al. 2002; Bayne et al. 1975) and juvenile

Sydney rock oyster *Saccostrea glomerata* (Kesarcodi-Watson et al. 2001). In our study, *P. margaritifera* also followed this pattern: when availability of food increased, oysters continued to simultaneously consume oxygen and feed, suggesting that oyster activity was optimal under these experimental conditions, as in *C. gigas* (Haure et al. 2003).

Assimilation is the last step in the feeding process. Our study confirmed that AE is highest at low food concentration and declines at high food concentration, a result also described in *P. margaritifera* by Le Moullac et al. (2013) who studied the link between food concentration and ingestion rate. Iglesias et al. (1992) showed that assimilation of the organic matter in food increased with concentration of particles, and Hawkins et al. (1998) showed that assimilation was correlated with food quality (lipid and protein content). The diet used was composed of a single species (*I. galbana*); therefore, our results cannot be attributed to variation in the microalgal quality. Because *P. margaritifera* lives in conditions of continuously limited food availability (Pouvreau et al. 1999), a possible response to food depletion is an increase in assimilation efficiency to maintain physiological processes unaltered. Our results also indicate that reserves of glycogen increase with the availability of food. In *C. gigas*, only part of the mobilization of lipid and glycogen reserves is attributed to gonad maturation (Soletchnik et al. 1997). In atoll lagoons of French Polynesia, wind regime impacts food availability and spatiotemporal variability of plankton concentration, and consequently reproduction dynamics of *P. margaritifera* (Fournier et al. 2012).

4.3 Effect of sex

P. margaritifera are primarily males until the shell height reaches about 90 mm; females appear in the population when these oysters are two years old (Chávez-Villalba et al. 2011). The new females then grow faster than the males, but may change back to being males, probably as a result of stress (under reared rather than wild conditions) or limited food (Pouvreau et al. 1999). This suggests that female status is more demanding of energy than male status.

Oxygen consumption of mature Iceland scallop *Chlamys islandica* males and females was higher than immature scallops, and mature males had a higher metabolic rate than females (Vahl and Sundet 1985). Similarly, Tran et al. (2008) proposed that male and female *C. gigas* have different energy needs for somatic maintenance and growth. They found that oxidative metabolism is much higher in males than in females, as males had twice the oxygen consumption, 30% more arterial oxygen partial pressure, pO_2, and 8.2 times higher oxygen extraction coefficient of hemolymph than females. Our findings corroborate that *P. margaritifera* has gender-related differences in energy metabolism costs, but females here have the higher energy expenditure. This expenditure is covered by an energy gain via better assimilation efficiency. Usually, an increase in oxygen consumption is associated with the metabolic costs of reproduction (MacDonald et al. 2006). Combined with the differences we observed, this principle suggests that metabolic needs for production of gametes are different between the sexes in *P. margaritifera*, as in the freshwater eastern floater mussel *Pyganodon cataracta* (Tankersley and Dimock 1993). Our observations, together with the high AE of organic matter and elevated demand for energy for reproduction, involving transfer of reserves, might explain higher oxygen needs in *P. margaritifera* females.

Different needs and capacities of male and female *P. margaritifera* could partly explain the differences in the way glycogen is managed in their reproduction. Gender has a significant effect on glycogen content in the adductor muscle, which is higher in males than females. Glycogen reserves are mobilized in bivalves during periods of food depletion and high energy demand (Bayne 1976; Barber and Blake 1981; Berthelin et al. 2000). These reserves are usually regulated by a metabolic flux towards reproductive output, as demonstrated by Kong et al.(2007) in *C. gigas* triploids. Laboratory experiments showed that glycogen storage in *C. gigas* is related to the availability of food, and thus, reproductive effort is correlated with glycogen concentration in oysters (Delaporte et al. 2006). The energy balance for bivalves indicates that males mobilize only part of their reserves for gamete formation compared with females, which need to produce vitelline reserves to develop oocytes (Beninger and Le Pennec 1997). Although results obtained in the laboratory may differ from field studies, our findings suggest two ways in which females use energy: (1) glycogen storage is low because females use more energy directly from food, which is used for reproduction without going through a storage stage; and (2) the needs for glycogen are greater in females than in males; after a period of storage, females use glycogen for reproduction, to supplement energy provided by food.

4.4 Energy budget (SFG)

Standardized scope for growth (SFG) measures an oyster's energy status, describing the energy available for growth and reproduction. Since net energy is the result of energy obtained from feeding and digestion, and energy expenditure from metabolism and excretion (Bühringer and Danischewski 2001), we focused on describing any SFG difference between females and males. In general, we found that the mean energy balance remained positive for *P. margaritifera*. Food concentration affected significantly SFG. SFG values (20–40 J h^{-1} g^{-1}) of standardized oysters (1 gram), when fed LFL diet (5 cell μ l^{-1} of *Isochrysis galbana*) are similar (30.8–41.5 J $h^{-1}g^{-1}$) to the results of Yukihira et al. (1998, 2000) for *P. margaritifera* under comparable conditions (standardized oysters of 1 g, food: 5 cell μ l^{-1} of *I. galbana* (T-iso); oyster size: 121–180 mm; temperature of 24 °C or 28 °C). Nevertheless, we observed that SFG was about double (50–80 J $h^{-1}g^{-1}$) when the concentration of food was increased to 30 cell μ l^{-1}.

This is the first study on the black-lip pearl oyster to report differences in metabolic rates between genders. Female and male *Pinctada margaritifera* have different bioenergetic functioning, which is mainly affected by food concentration. Evidence indicates that energy demands for reproduction are higher in females than in males. This provides valuable information for finding approaches to managing gender expression in this species.

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