Comparative analysis of oxygen consumption rates between cupped oyster spat of *Crassostrea gigas* of French, Japanese, Spanish and Taiwanese origins

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Abstract — Respiration rates of various geographical strains of *Crassostrea gigas* were compared to assess the respiratory expenditure as a physiological indicator of catabolism (cost of maintenance). Parental oysters, sampled in France (Marennes-Oléron), Japan (Hiroshima), Taiwan (Tunkang) and Spain (Cadix), were differentiated by both their geographic origin and by mitochondrial DNA markers, allowing the distinction between the two closely related taxa *Crassostrea gigas* and *Crassostrea angulata*. After reproduction of these parental oysters, respiration rates of each strain, reared under common controlled conditions, were individually estimated at 20 °C by using a volumetric microrespirometer. Our results demonstrated that physiological variability existed among the *Crassostrea gigas* strains and is likely to be related to physiological differences between geographical regions and/or genetic adaptations. The French strain showed the highest rates (0.71 μL·h⁻¹), while the Japanese, Taiwanese and the hybrid between Spanish and French strains consumed less, 0.53, 0.43 and 0.40 μL·h⁻¹, respectively. Our results confirmed the discrepancies previously suggested between *Crassostrea gigas* and *Crassostrea angulata*. Comprehensive physiological assessments should be carried out over a wide temperature range to confirm our results and to further evaluate growth potential. Appropriate decision making based on these studies will help future shellfish management in shellfish rearing areas such as the overstocked Marennes-Oléron Bay. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Respiration rates / metabolism / *Crassostrea gigas* / *Crassostrea angulata* / hybrids / oyster strains / NE Atlantic / Pacific Ocean

Résumé — Analyse comparée des taux de respiration de naissains de différentes souches d’huîtres creuses japonaises *Crassostrea gigas* originaire de France, Espagne et Taiwan. Les taux de respiration de différentes souches géographiques de l’huître creuse japonaise *Crassostrea gigas* ont été comparés afin d’évaluer, en tant qu’indicateur physiologique, le coût énergétique de maintenance résultant du catabolisme (pertes énergétiques). Les géniteurs échantillonnés en France (Marennes-Oléron), au Japon (Hiroshima), à Taiwan (Tunkang) et en Espagne (Cadix) ont été différenciés à la fois par leur origine géographique et par l’utilisation de marqueurs ADN mitochondriaux, permettant ainsi la distinction entre deux taxa très proches *Crassostrea gigas* et *Crassostrea angulata*. Après reproduction de ces géniteurs, les taux de respiration du naissain de chaque souche, élevé dans des conditions contrôlées similaires, ont été mesurés individuellement à 20 °C au moyen d’un microrespiromètre volumétrique. Nos résultats démontrent qu’il existe une variabilité physiologique entre les différentes souches, due probablement à une adaptation physiologique locale et/ou à des adaptations génétiques. Les huîtres de souche française ont montré les taux de respiration les plus élevés (0.71 μL·h⁻¹), comparés aux taux de consommation de 0.53, 0.43 et 0.40 μL·h⁻¹ respectivement pour les souches japonaise, taiwanaise et les hybrides entre la souche espagnole et française. Ces résultats confirment les différences déjà suggérées entre les souches *Crassostrea angulata* et *Crassostrea gigas*. Des évaluations complètes des fonctions physiologiques doivent être réalisées en fonction d’une gamme de température pour confirmer ces résultats et estimer le potentiel de croissance des différentes souches. Ces études doivent faciliter les futures décisions en matière d’aménagement des cultures marines dans les bassins de production français, en particulier dans des zones d’élevage surchargées comme le bassin de Marennes-Oléron. © 1999 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Taux de respiration / métabolisme / *Crassostrea gigas* / *Crassostrea angulata* / hybrides / souches génétiques / Atlantique NE / océan Pacifique

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1. INTRODUCTION

The Marennes-Oléron bay, France, is the highest yielding area for Pacific oyster *Crassostrea gigas* production in Europe. Between 40 000 and 60 000 metric tons are marketed annually. However, historical trends in French oyster production have shown large fluctuations over the century, the latest being the massive introduction of *Crassostrea gigas* during the 1970s to replace the ailing *Crassostrea angulata* affected by a viral disease [14, 18, 20]. This successful introduction led to an industry rebound and annual natural spatfall has occurred regularly over the subsequent 25 years, although it was poor for 2 years. In Marennes-Oléron Bay, overstocking became a major issue in 1985 when the relationship between the ecosystem carrying capacity and the overall stocking biomass was established [22], therefore demonstrating the limits of the bay carrying capacity. Unbalanced stocks of various size classes (mainly spat and 1 year old) induced decreasing growth rates and increased mortality rates. The overall impact has been a significant shift from an 18 month to a 3 year rearing cycle time to reach a marketable size [22]. To address this matter, and improve the economic yield, several issues were discussed. The obvious one was a systematic oyster stock decrease by new regulations and their enforcement (e.g. overall authorized stocking biomass is presently 6 000 oysters bags or 72 metric tonnes per ha). However, discrepancies between optimized biological and economic yields should be taken into account to establish thresholds and regulations, therefore leading to new difficulties [1]. A simple new density regulation would be unlikely to be successful in limiting the stocking biomass, and a more specific economic analysis is required to assess the impact on the various shellfish farming trades (i.e. spat producers—growers and growers—marketers). Therefore, due to the overall complex organization, the shellfish farming industry was reluctant to push for such regulations. Instead new management practices were developed and enforced to reverse these trends: (1) limiting proliferation of trophic competitive species, including the exotic gastropod *Crepidula fornicate* by annual dredging [24, 25]; (2) destruction of natural beds of *Crassostrea gigas*, classified as sanctuaries since its introduction; (3) increasing the cleaning and restructuring of the leasing grounds to limit wild oyster populations, estimated at 20 000 metric tons [37].

Moreover, new rearing techniques are currently under evaluation using long lines and deep water oyster cultures located off shore which, combined with a decrease in intertidal leasing grounds, will limit and improve oyster stock spatial distribution (overall density decrease) [17, 19].

Besides the changes in overall shellfish management, oyster mortality rates, which have increased over 25 years, are also likely to have been affected by global environmental changes. A 1.5 °C seawater temperature increase has been recorded in the bay of Marennes-Oléron since 1978 [39].

At a constant stocking biomass, this environmental change has induced increased physiological activity in oysters, and consequently a 4% decrease in the bay carrying capacity [39]. The oyster broodstock and spat which were introduced during the 1970s came from British Columbia (Canada) and Sendai-Miyagi (Japan) [33]. However, oysters in both cases originated from the latter as demonstrated by the lack of genetic differentiation between the French and the Miyagi populations [35]. In the Japanese environment, seawater temperatures peak at 25 °C and salinity is below 33 in summer [32, 33]. Higher seawater temperatures are now regularly observed in summertime in the Marennes-Oléron bay. Moreover, mortality rates were correlated with *Crassostrea gigas* strains since oysters descended from Hiroshima and Matsushima stocks showed various mortality rates in similar environmental conditions [19, 28, 31].

Based on this information, scientific research programmes have focused on ecosystem modelling to assess the main constraints affecting the primary productivity and to improve knowledge on overall ecosystem functioning [24]. At the population level, genetic and ecophysiological studies have been carried out on *Crassostrea gigas* to assess strains characterized by optimized assimilation rates as well as by genetic characteristics [6, 7, 10, 13]. A comprehensive research programme has been developed to study wild and cultured *Crassostrea gigas* populations on a worldwide basis to define the genetic structure and stock variability for these populations. In the near future, controlled introductions of shellfish populations may be of interest to the aquaculture industry by providing desirable traits and improved productivity through selective breeding. The main short-term objective is the search for original genetic characteristics capable of improving the overall oyster culture yield in France, and in the long-term to specify a policy for a genetic management strategy for *Crassostrea gigas* [6, 7].

For the latter approach, a quarantine station at the Ifremer research hatchery (La Tremblade, France) has allowed experiments to be carried out on various *Crassostrea* sp. strains. The present study aims to assess respiration rate variability of several *Crassostrea gigas* strains to provide insights for the genetic management strategy. Respiration rate is considered as an appropriate physiological indicator of catabolism representing the overall bio-energetic status and respiratory expenditure (i.e. costs of maintenance) of the shellfish [38, 40]. In examining the oxygen consumption rate, we supposed that oysters allocating a smaller proportion of their aerobic capacity to maintenance requirements would be able to allocate more energy to growth [21].
Table I. Sampling sites, status and characteristics of experimental batches of the various *Crassostrea gigas* strains (wild versus culture). Oyster strains: Cgf, *Crassostrea gigas*, France; Cgi, *Crassostrea gigas*, Japan; CgCa, hybrid resulting from the cross between *Crassostrea gigas*, France and *Crassostrea angulata*, Spain; Cat, *Crassostrea angulata*, Taiwan.

<table>
<thead>
<tr>
<th>Oyster populations</th>
<th>Geographical origin 1</th>
<th>Strain</th>
<th>Total weight (mean ± SD) (mg)</th>
<th>Dry meat (mean ± SD) (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crassostrea gigas</em></td>
<td>Marennes-Oléron, France</td>
<td>Cgf</td>
<td>392.8 ± 76.8</td>
<td>14.5 ± 3.4</td>
</tr>
<tr>
<td><em>Crassostrea gigas</em></td>
<td>Hiroshima, Japan</td>
<td>Cgi</td>
<td>261.2 ± 88.9</td>
<td>12.9 ± 4.5</td>
</tr>
<tr>
<td>Hybrid <em>Crassostrea gigas</em> × <em>Crassostrea angulata</em></td>
<td>Marennes-Oléron, France</td>
<td>CgCa</td>
<td>349.2 ± 40.9</td>
<td>13.6 ± 2.1</td>
</tr>
<tr>
<td><em>Crassostrea angulata</em></td>
<td>Tunkang, Taiwan</td>
<td>Cat</td>
<td>340.6 ± 80.2</td>
<td>17.9 ± 5.4</td>
</tr>
</tbody>
</table>

SD: Standard deviation.

2. MATERIALS AND METHODS

2.1. Geographical samples

Parental oyster samples were obtained from Marennes-Oléron (France) (Cgf), Hiroshima (Japan) (Cgi), Tunkang (Taiwan) (Cat) and Cadiz (Spain) (Ca) (Table I) and held in quarantine in similar environmental conditions at the IFREMER research hatchery. Each oyster population was characterized by both their geographical origin and mitochondrial DNA markers as previously described [6, 7, 36]. These recent studies showed that *Crassostrea angulata*, considered by most authors as a subspecies of *Crassostrea gigas* [34], can be clearly distinguished from *Crassostrea gigas* using mitochondrial DNA markers and that it originates from Taiwan. Although all the tested strains must be considered as *Crassostrea gigas*, the oysters originating from Tunkang (Taiwan) and Cadiz (Spain) are named ‘*Crassostrea angulata*’ rather than ‘*Crassostrea gigas ssp. angulata*’ as this name is in common use in aquaculture [6, 15, 25, 36]. Breeding was achieved by mass spawning of 100 oysters (strains, Cgf and Cat) or by stripping of the gametes of mature oysters (Cgf: 21 females × 10 males, hybrid strain CgCa: 21 females Cg × 15 males Ca). Larval rearing, settlement and spat rearing were performed according to usual hatchery methods. Five-month-old spat of each strain were then randomly sampled for oxygen consumption measurements.

2.2. Respiration rates

Respiration rates were determined by using a volumetric micro-respirometer [41]. The measurements were carried out individually on 12 oysters per strain using a system of micro-chambers connected to capillaries and kept under constant pressure (Figure 1). One oyster was placed per micro-chamber filled with seawater where a KOH solution was settled as a suspending drop. The latter maintained a constant CO2 concentration inside the micro-chamber, so that changes in gas volume were caused only by oxygen consumption. One micro-chamber with no oyster was used simultaneously as a control functioning similarly to a thermal barometer. All measurements were made at 20 °C ± 0.1 °C using an ultra thermostat to maintain constant seawater temperature. We chose to examine the oxygen consumption at such a high temperature (20 °C) to maximize metabolism, and therefore accentuate the difference in maintenance efficiency [9, 29]. Respiration rates were measured every 2 min over a 40 min experimental time, leading to 20 values per oyster. Following the measurements, oysters were shocked and the wet meat frozen for 24 h. Then, individual dry meat weight was obtained by freeze drying for 36 h, and weighing using a 1/100 mg precise scale. Experiments were conducted with *Crassostrea gigas* of 5.4–24.7 mg dry meat weight.

2.3. Data analysis

To take into account allometric differences, which were mainly due to growth variability among oyster batches, respiration rate was calculated per unit dry meat weight (mg) using the formula [3, 4]:

\[
R_s = \frac{(W_s/W_e)^b \times R_e}{R_s}
\]

where \(R_s\) is the respiration rate of the standard animal in (LO2-h-1), \(W_s\) is the weight of the standard animal (1 mg), \(W_e\) is the measured dry meat weight of the animal, \(b\) is the allometric coefficient and \(R_e\) is the uncorrected respiration rate in (LO2-h-1-ind-1). The allometric coefficient of 0.77 was used for all batches [16] rather than 0.8, obtained previously [9] on a larger oyster size range.

All the statistical treatments, including normality tests, ANOVA and tests were performed using the software Statgraphics® V7. The data were analysed using the following model:

\[
Y_{ijk} = \mu + s_i + j_{ij} + e_{ij}
\]

where \(Y_{ij}\) is the measurement of the \(k\)th individual of the \(i\)th strain, \(\mu\) is the overall mean, \(s_i\) is the population effect, \(j_{ij}\) is the individual within the strain effect and \(e_{ij}\) is the residual.
3. RESULTS

Characteristics of oyster strains are presented in Table I. Average total weight ranged from 261 mg (Cgj) to 393 mg (Cgf), while average dry meat weight varied from 13 to 18 mg for the Japanese and Taiwanese strains, respectively.

Oxygen consumption rates of the different strains were compared using an ANOVA statistical test, and showed a significant origin effect (Table II). Figure 2. More than 20% of the total variance was explained by this strain effect.

The French strain (Cgf) showed the highest mean respiration rate (0.72 L·h⁻¹), while the Japanese (Cgj), Taiwanese (Cat) and the hybrid oysters (CgCa) consumed 0.53, 0.43 and 0.41 L·h⁻¹, respectively (Table III).

Based on their respective respiration rates, the four strains can be divided into two groups, incorporating CgCa–Cat–Cgj and Cgf–Cgj (Table III). Pair comparisons of respiration rates by using the least square method showed that only two pairs of means differed significantly, these were the strain Cgf versus the hybrid CgCa, and the local Cg versus the Taiwanese strain Cat.

4. DISCUSSION

Based on our results, this preliminary study of oxygen consumption rates in juvenile oysters demonstrated that physiological variability exists among Crassostrea gigas strains and is probably related to geographical, physiological and/or genetic adaptations. The method appeared efficient for comparing oyster strains and detecting those characterized by low metabolic rates; it might, therefore, be of interest for aquaculture purposes. Moreover, the constraints concerning species transfers and introductions specified by the ICES Code of Practice [27] make a survey at such an early stage of particular interest from a practical point of view. These constraints explain the limited number of physiological comparisons avail-

Table II. Variance analysis (ANOVA) table for standardized respiration rates (µL O₂·h⁻¹) of Crassostrea gigas strains.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>F-Ratio</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between strains</td>
<td>0.69592</td>
<td>3</td>
<td>0.231972</td>
<td>3.49</td>
<td>0.0233**</td>
</tr>
<tr>
<td>Within strains</td>
<td>2.92259</td>
<td>44</td>
<td>0.066423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (corr.)</td>
<td>3.61851</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant difference; ** highly significant; NS, not significant.
Table III. Multiple range tests for standardized respiration rates of *Crassostrea gigas* strains using the least square deviation (LSD) method at 95 %, and statistically significant difference between means.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Count</th>
<th>Mean oxygen consumption rates (μL O₂·mg⁻¹ dry body weight·h⁻¹)</th>
<th>Homogeneous group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CgCa</td>
<td>12</td>
<td>0.40957</td>
<td>A</td>
</tr>
<tr>
<td>Cat</td>
<td>12</td>
<td>0.43563</td>
<td>A</td>
</tr>
<tr>
<td>Cgj</td>
<td>12</td>
<td>0.53008</td>
<td>A, B</td>
</tr>
<tr>
<td>Cgf</td>
<td>12</td>
<td>0.7165</td>
<td>B</td>
</tr>
</tbody>
</table>

Contrast | Difference | +/- limits |
----------|------------|------------|
Cgf–CgCa  | *0.3069    | 0.21205    |
Cgf–Cgat  | *0.2809    | 0.21205    |

able in the literature in contrast to genetic studies [2, 6, 7, 11, 12, 35].

Although not statistically significant, the Hiroshima strain showed an intermediary position in terms of cost of maintenance between the French *Crassostrea gigas* strain and both Taiwanese and *Crassostrea gigas−C. angulata* hybrid strains. This trend could be related to the discrepancies reported among Japanese strains [28, 31]. The low cost maintenance observed with the hybrid *C. gigas−angulata* and the Taiwanese strain tends to support the similarity between Taiwanese and European *C. angulata* populations, both from a genetical and physiological point of view [6]. However, the various costs of maintenance may induce overall growth differences between these strains and the European *Crassostrea gigas* as already suggested with *Crassostrea virginica* [30]. Meanwhile, decreased growth has been reported for hybrids, 8 and 49 % for *C. gigas−angulata* and *C. angulata−gigas*, respectively [8]. Although lacking a complete scope for growth assessment, a growth difference between the European *Crassostrea gigas* strain and *Crassostrea angulata* was found to be correlated with a greater assimilation rate (by a factor of 1.7) for *Crassostrea gigas* [23]. Similar oxygen consumption rates and various feeding activities were reported to explain growth differences between hybrid and inbred *Crassostrea gigas* oysters [5]. Therefore, it appears critical to further evaluate growth potential and explain the relationships with physiological measurements by a comprehensive scope for growth assessment. Meanwhile, new assessments should be carried out over the annual temperature range to improve comparisons. *Crassostrea gigas* physiological activity has been measured over a wide range of temperature [9], and it was demonstrated that *Crassostrea angulata* has a reduced filtration activity below 10 °C, in contrast to *Crassostrea gigas* which was still active at 5 °C [26].

For a similar growth potential or scope for growth (SFG), the environmental impact of a species might be significantly different depending on whether energy is allocated mainly to maintenance or to production. In the case of the overstocked Marennes-Oléron bay, an optimal management strategy would be to culture a genetic strain which maximizes the use of energy input by the association of high assimilation rate, limited food retention and low energy maintenance cost. The *Crassostrea gigas* strain currently used in European waters showed the highest energy maintenance cost in this study, which might result in lower yields, increased mortality rates and higher pressure on the carrying capacity.

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