Scope for Growth of the Oyster *Crassostrea corteziensis* (Hertlein, 1951) in Effluents from an Intensive White Shrimp *Litopenaeus vannamei* Farm

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(Received 31.7.12, Accepted 18.10.12)

Key words: effluent, filtration, scope for growth, *Crassostrea corteziensis*

Abstract

An *in situ* study was carried out in the effluent of an intensive shrimp farm in southern Sinaloa, México, to analyze the scope for growth of the pleasure oyster, *Crassostrea corteziensis*. Oysters were collected from a wild population, placed in Nestier trays, and submerged in the farm effluent. The oysters were sampled on days 41, 54, 70, and 91 of the 104-day shrimp culture cycle. The average temperatures during the samplings varied 26-34°C, and salinity ranged 15-22 psu. The seston concentration was 51-71 mg/l with a high proportion of organic matter (51-66%). The average clearance rates of the *C. corteziensis* were 0.58-1.31 l/h/g and average filtration rates were 26-76 mg/h/g. Assimilation efficiency varied 0.4-0.79. Under the experimental conditions, the scope for growth of *C. corteziensis* was positive and ranged 25-340 J/h/g. On this basis, we conclude that this bivalve should be studied for use in integrated aquaculture systems, with farm effluents as a feed source.

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Introduction

Intensive coastal aquaculture involves the discharge of waste water enriched with high concentrations of dissolved and particulate materials that could impact the environment. However, the high concentration of organic components of these materials represents a source of energy that can be made available to filter-feeding organisms in integrated aquaculture systems (Jones et al., 2001; Páez-Osuna, 2001; Ramos et al., 2008; Martínez-Córdova et al., 2009).

Several species of macroalgae, bivalve mollusks, and other invertebrates are capable of using the discharged materials from shrimp and fish farms and represent a remedial solution to the possibly negative environmental impacts of aquaculture activities (Jones et al., 2001; Ramos et al., 2008; Martínez-Córdova et al., 2009). According to in situ and controlled laboratory studies, the filtering activity of some bivalves can remove large volumes of particulate matter from farm effluent water, indicating a potential to integrate the bivalve into aquaculture systems (Lefebvre et al., 2000; Jones et al., 2001; Palmer and Rutherford, 2005; Miranda et al., 2009; Nieves-Soto et al., 2011).

In general, in situ studies performed directly on farm effluents do not include analysis of the energy budget needed to estimate the scope for growth. The scope for growth provides a rapid and quantitative assessment of the energy available for growth and reproduction of bivalves grown in an aquaculture system. By definition, scope for growth is an integrative physiological indicator reflecting the net energy gain of an organism and is an accurate predictor of yield (Bayne et al., 1999; Yukihiro et al., 2000; Filgueira et al., 2011).

The pleasure oyster *Crassostrea corteziensis* is naturally distributed in lagoons and estuarine environments from the Gulf of California to Panama (Keen, 1971). This euryhaline eurythermal oyster is highly-valued for human consumption and, therefore, the focus of interest for local fisheries and low-technology cultures (Stuardo and Martínez, 1975; Chávez-Villalba et al., 2005). *Crassostrea corteziensis* could be a worthwhile option for integrated aquaculture if their ability to exploit farm effluents is confirmed. In this research, the filtering response and scope for growth of *C. corteziensis* were analyzed in situ in the effluent from an intensive white shrimp, *Litopenaeus vannamei*, farm in the subtropical region of southern Sinaloa, México.

Materials and Methods

*Experimental design and sampling.* The experiment was conducted during an intensive production cycle of white shrimp (*Litopenaeus vannamei*) in southern Sinaloa, México (23°42’N; 106°48’W). The farm uses water from a pumping station on the Piaxtla River mixed with ocean water from the Pacific in varying proportions, depending on rainfall. Oysters (*Crassostrea corteziensis*) were collected from a nearby wild population, planted in Nestier trays, and placed in the effluent on day 20 of the shrimp culture. The experimental system consisted of a continuous water flow through closed chambers that functioned as respirometers. The chambers were 1.2-L transparent plastic cylinders with screw caps and a water inlet and outlet made of plastics connectors.

The oysters were sampled on days 41, 55, 70, and 91 of the 104-day shrimp culturing cycle to estimate the physiological variables needed to determine scope for growth. All samplings were performed in the shade and at the same time of day. For each sampling, two organisms (54±5 mm) were removed from the Nestier trays and placed in each of 10 experimental chambers. The empty shells of two oysters of similar size were placed in each of three additional chambers and used as controls. The chambers were closed and the water flow started as described below.

A submersible 1/6 HP pump was placed at a depth of one meter in the middle of the effluent channel to pump water into a 60-L tank at the top of the experimental system (Fig. 1). The water inlet of the pump was covered with a plastic net (2-mm mesh) to prevent the entry of larger particles. The water was distributed from the upper tank to the respirometers through a manifold with vinyl feeder hoses connected to the water inlet of each chamber. The flow of pumped water from the effluent and the water column in the upper tank was constant, maintaining constant pressure in the chambers. The
Scope for growth of oyster raised in effluent from a shrimp farm

Continuous flow was adjusted manually to 3.6 l/h in each chamber by measuring the outflow rate and, if necessary, the main valve of the manifold was adjusted and tightened by clamping the feeder hoses. The outflow of each chamber was continually monitored to prevent changes. Overflow from the upper tank prevented overheating of the water.

Fig. 1. The experimental system in which scope for growth of pleasure oysters was determined.

Once filtering activity and production of feces were initiated by the oysters, sampling was begun. Each sampling lasted approximately 3 h to prevent the loss of biodeposits, as had occurred in pre-experiment observations. Temperature and salinity were recorded, and water samples were taken from the feeder tank every 30 min to determine the chlorophyll a concentration according to Clesceri et al. (1998). Samples of the water outflow from the experimental and control chambers were collected to estimate concentrations of oxygen with a fiber optic oxygen minisensor connected to an oxygen transmitter (PreSens-Microx TX2), and ammonium by the phenol-hypochlorite method (Solorzano, 1969). At the same time, water samples were extracted from the outflow of the control chambers to determine the concentration of seston in terms of total particulate matter (TPM), particulate organic matter (POM), and particulate inorganic matter (PIM). After sampling, the chambers were opened and biodeposits (feces and pseudofeces) were collected. When it was impossible to separate biodeposits from other sediments, both were collected, and quantification of the biodeposits was determined by subtracting the sedimented materials in the control chambers (Iglesias et al., 1998).

To estimate TPM, POM, and PIM, the biodeposits and water samples were immediately filtered in previously washed, incinerated, and weighed Whatman GF/C filters, rinsed with 4% ammonium formate to remove salts, and dried at 60°C until a constant weight was obtained. Thereafter, the filters were incinerated at 450°C for 6 h and again weighed to calculate ash (inorganic material content) and the organic content of the samples was obtained by calculating the difference between the total dry weight and the weight of the ash. The organisms were shucked and their soft tissues were dried at 60°C to obtain a constant weight. The dry weights of the soft tissues were used to standardize all physiological rates to reflect one gram of dry weight from the organisms.

**Physiological variables.** Clearance (l/h) and filtration (mg/h) rates were calculated using the method for biodeposits (Hawkins et al., 1996; Iglesias et al., 1998): clearance rate = (IM\textsubscript{b}/t)/PIM and filtration rate = clearance rate × TPM, where IM\textsubscript{b} = inorganic
matter contained in the biodeposits, \( t \) = duration of the sampling, and TPM and PIM are the concentrations of total and inorganic particulate matter of the seston, filtered by the oysters as food, and obtained from the outflow of the control chambers.

Assimilation efficiency was based on feces samples that were processed similarly to the biodeposits to quantify their organic and inorganic contents and calculated as \( (F - E)/[(1-E)F] \), where \( F \) is the organic fraction of the food and \( E \) is the organic fraction of the feces (Conover, 1966). The respiration rate (mg \( O_2/h \)) was calculated by multiplying oxygen consumption (the difference between dissolved oxygen concentrations at the outflows of the control and experimental chambers) by the water flow. The ammonia excretion rate (mg \( NH_4-N/h \)) was calculated by multiplying ammonia excretion (the difference between ammonium concentrations at the outflows of the experimental and control chambers) by the water flow.

Scope for growth was determined by the equation of Winberg (1960): absorbed energy - (respired energy + excreted energy). The respiration and ammonia excretion rates were transformed into energy units (J/h) using 14.06 J/mg for oxygen consumed (Crisp, 1971) and 24.8 J/mg for excreted \( NH_4-N \) (Elliott and Davison, 1975).

The net organic absorption rate was determined as (filtration rate \( \times \) organic fraction of the food) - (biodeposit production rate \( \times \) organic fraction of the biodeposits). Absorbed energy was calculated by converting the net organic absorption rate to energy units using the caloric content of the effluent seston. The caloric content (J/mg) was calculated from lyophilized seston samples obtained by filtering at least 40 l effluent water at 1 micron with a Parr 1425 semi-micro calorimeter.

Data analysis. Physiological variables were tested for normality and homoscedasticity to determine whether a parametric or nonparametric analysis should be used. One-way ANOVA, Kruskal-Wallis, Tukey, and Dunn multiple comparison tests were used to compare the physiological variables. Pearson correlation coefficients were calculated between physiological rates, and between physiological rates and environmental variables (Zar, 1999). The statistical procedures were performed using SigmaStat software, 2006.

Results
Temperature and salinity tended to decrease as the experiment progressed (Table 1). Total particulate and particulate organic matter were lowest in the first sampling, and characterized by a relatively low proportion of organic matter. The average calorific value of the organic fraction of the seston of the four samplings was 17 J/mg. The concentrations of total particulate and particulate organic matter highly and positively correlated with the chlorophyll \( a \) concentration (\( r = 0.92 \) and 0.91, respectively, \( p<0.0000 \)). The mean clearance and filtration rates negatively correlated with salinity of the effluent; the correlation was low for clearance rate (\( r = -0.33, p<0.05 \)) and moderate for filtration rate (\( r = -0.46, p<0.01 \)). There was virtually no production of pseudofeces. Assimilation efficiency significantly differed between treatments and significantly correlated with environmental variables: the correlation with temperature and salinity was moderately negative (\( r = -0.54 \) and -0.64, respectively, \( p<0.001 \)) but high and positive with total and particulate organic matter concentrations and chlorophyll \( a \) (\( r = 0.92, 0.89 \) and 0.90, respectively, \( p<0.001 \)). There were no significant correlations between mean respiration rate and any environmental variable or physiological rate but the trend was similar as for the filtration and clearance rates. There was a moderate positive correlation between the ammonia excretion rate and temperature (\( r = 0.47, p<0.01 \)), and moderately negative correlations between this rate and total and organic particulate matter and chlorophyll \( a \) concentrations (\( r = -0.64, 0.59, \) and 0.71, respectively, \( p<0.001 \)).

In general, absorbed energy highly and positively correlated with the filtration and clearance rates (\( r = 0.91 \) and 0.84, respectively, \( p<0.0000 \)), and had a low but positive correlation with assimilation efficiency (\( r = 0.34, p<0.05 \)). Potential energy for growth was always positive and its lowest value was observed in the first sampling in which the energy spent on respiration and excretion represented higher proportions of the total...
energy than in other samplings. The scope for growth moderately and negatively correlated with salinity and temperature \((r = -0.39, p<0.05\) and \(-0.57, p<0.001\), respectively), and moderately but positively correlated with variables related to food concentration \((r = 0.44, p<0.01\). The scope for growth showed high positive correlation with the filtration and clearance rates, and absorbed energy \((0.87, 0.8, \text{ and } 0.98, \text{ respectively; } p<0.05)\).

Table 1. Environmental variables of effluents from an intensive white shrimp Litopenaeus vannamei farm (means±SD), and physiological variables of oyster Crassostrea corteziensis grown in the effluent (means±SE).

<table>
<thead>
<tr>
<th>Sampling (day)</th>
<th>41</th>
<th>55</th>
<th>70</th>
<th>91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>34.1±0.06</td>
<td>32.4±0.11</td>
<td>32.9±0.06</td>
<td>26.4±0.1</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>22</td>
<td>17</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Chlorophyll a (μg/l)</td>
<td>24.86±2.65</td>
<td>128.76±23.98</td>
<td>70.0±0.62</td>
<td>150.6±8.94</td>
</tr>
<tr>
<td>Total particulate matter (mg/l)</td>
<td>50.78±2.88</td>
<td>71.08±2.43</td>
<td>61.94±2.6</td>
<td>64.01±4.37</td>
</tr>
<tr>
<td>Particulate organic matter (mg/l)</td>
<td>25.88±2.33</td>
<td>46.89±3.68</td>
<td>37.6±2.52</td>
<td>39.7±4.5</td>
</tr>
<tr>
<td>Particulate organic matter (%)</td>
<td>50.97</td>
<td>65.96</td>
<td>60.74</td>
<td>61.95</td>
</tr>
<tr>
<td>Caloric content of seston (J/mg)</td>
<td>9.75±0.33</td>
<td>10.72±0.35</td>
<td>10.16±0.18</td>
<td>10.31±0.42</td>
</tr>
<tr>
<td>Caloric content of organic seston (J/mg)</td>
<td>19.13</td>
<td>16.25</td>
<td>16.73</td>
<td>16.62</td>
</tr>
<tr>
<td>Clearance rate (l/h/g)</td>
<td>0.58±0.08</td>
<td>0.56±0.05</td>
<td>1.31±0.21</td>
<td>0.94±0.18</td>
</tr>
<tr>
<td>Filtration rate (mg/h/g)</td>
<td>26.70±3.71</td>
<td>38.90±3.18</td>
<td>76.01±12.24</td>
<td>60.57±11.82</td>
</tr>
<tr>
<td>Assimilation efficiency</td>
<td>0.40±0.02</td>
<td>0.79±0.01</td>
<td>0.52±0.02</td>
<td>0.73±0.03</td>
</tr>
<tr>
<td>Respiration rate (mg O2/h/g)</td>
<td>3.60±0.77</td>
<td>5.19±0.67</td>
<td>6.42±0.73</td>
<td>3.77±0.79</td>
</tr>
<tr>
<td>Ammonia excretion rate (mgNH3-N/h/g)*</td>
<td>0.96±0.17</td>
<td>0.09±0.02</td>
<td>1.13±0.14</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td>Absorbed energy (J/h/g)*</td>
<td>98.90±15.58</td>
<td>307.35±25.83</td>
<td>411.61±59.37</td>
<td>398.39±86.14</td>
</tr>
<tr>
<td>Respired energy (J/h/g)</td>
<td>50.64±10.81</td>
<td>72.90±9.38</td>
<td>90.26±10.33</td>
<td>52.95±11.1</td>
</tr>
<tr>
<td>Excreted energy (J/h/g)*</td>
<td>23.70±4.33</td>
<td>2.31±0.57</td>
<td>28.02±3.52</td>
<td>5.31±1.19</td>
</tr>
<tr>
<td>Scope for growth (J/h/g)*</td>
<td>24.56±13.88</td>
<td>232.14±23.38</td>
<td>293.34±60.76</td>
<td>340.13±91.79</td>
</tr>
</tbody>
</table>

Different superscripts in a row indicate significant differences between samplings \(p<0.05\).

*Nonparametric test.

Discussion

Crassostrea corteziensis maintained its filtering activity associated with positive values for scope for growth in all samplings, in spite of different environmental conditions. The filtering activity was comparable to that observed in other bivalve species that have potential for bioremediation (Miranda et al., 2009; Peña-Messina et al., 2009; Nieves-Soto et al., 2011). Although this study was limited by uncontrolled conditions, several possible relationships were detected. The lower filtration and clearance rates in the first sampling could be related to the high temperature of the effluent water, which reached 34°C. According to findings for other bivalves, filtration and clearance rates increase as the temperature increases to an optimal value, then gradually or suddenly decrease (Winter, 1978; Ran et al., 2000; Yukihira et al., 2000; Guzmán-Agüero et al., 2013). In the last three samplings, the temperatures were lower and the filtration and clearance rates were higher.

The negative correlation between salinity and the filtration and clearance rates may be an incorrect assessment because its estimation was influenced by the low filtration rate related to the high temperature in the first sampling. If data of the first sampling are excluded from the analysis, then the correlations become positive, although insignificant. In controlled laboratory conditions, C. corteziensis can maintain its filtering activity over a wide range of salinity and temperature (Guzmán-Agüero et al., 2013; Enríquez-Ocaña et al., 2012). However, in neither of these studies was the temperature as high as 34°C, as in the first sampling. The salinity recorded in the first sampling (22 psu) was slightly higher than the salinity (20 psu at 29°C) at which the highest filtration and clearance rates were obtained for C. corteziensis fed a high-quality microalgae diet in a laboratory
study (Guzmán-Agüero et al., 2013), supporting the assessment that low salinity is not associated with the low filtration rate. This argument can also apply to the negative correlation between salinity and all the other rates. The higher seston concentration may have reduced the filtration and clearance rates in the second sampling. The response of bivalves to food concentration is highly variable intra and inter-specifically (Pastoureaud et al., 1996; Barnes, 2006) and filter feeding can be adversely affected beyond a certain seston concentration (Ran et al., 2000; Velasco and Navarro, 2002).

The scope for growth was higher than found in similar salinity and temperature conditions (Guzmán-Agüero et al., 2013) and comparable to values considered high for other bivalves maintained under controlled experimental conditions (Yukihi et al., 2000; Velasco and Navarro, 2003; Nieves-Soto et al., 2011). The high scope for growth can be explained by the high seston concentration in the effluent and the high energy content due to the high proportion of POM. In addition, the high chlorophyll a concentration in all samplings implies a significant presence of phytoplankton in the composition of the seston. The caloric content of the seston was 70-84% of the energetic value attributed to phytoplankton species, which represent high-quality feed for filter-feeding bivalves (Thomas et al., 1984; Piña-Valdez, 2004).

Although the four samplings were performed at different stages of the intensive shrimp production cycle, which involves increased food use and waste production as the day of harvesting approaches, the seston concentration varied within a narrow range. The average concentration was high but did not reach the extreme values that can be observed in intensive aquaculture farms (Páez-Osuna, 2001). Thus, the presence of high-quality seston, in high but not extreme concentrations, may explain why the C. corteziensis did not produce pseudofeces as a selection mechanism to regulate particle ingestion. Considerable decreases in the production of pseudofeces have been observed in bivalves when the seston concentration was high and/or of high quality (Pastoureaud et al., 1996; Velasco and Navarro, 2002).

The highly positive correlations between assimilation efficiency and TPM, POM, and chlorophyll a concentrations indicates that the assimilation efficiency was related mainly to the concentration and quality of the food, as observed in other bivalve species (Hawkins et al., 1996; Velasco and Navarro, 2003). Similar assimilation efficiencies were previously obtained for this species (Guzmán-Agüero et al., 2013). The lowest assimilation efficiency and large proportion of energy spent on respiration and excretion obtained in the first sampling could mean that the C. corteziensis were in metabolic stress generated by the high temperature. Nevertheless, the energy had a net positive effect on the scope for growth.

In conclusion, the filter feeding activity and positive scope for growth of C. corteziensis in this in situ study suggests the possibility of growing this organism in aquaculture farm effluents. However, its incorporation into integrated aquaculture systems must be assessed by comprehensive studies of a broad environmental framework, covering all possible conditions of effluents and including analysis of growth and survival of juvenile and adult organisms. For these purposes, our study can serve as a starting point.

**Acknowledgements**

This work was supported by Programa de Fomento y Apoyo a Proyectos de Investigación de la Universidad Autónoma de Sinaloa (PROFAPI 2009/121) and Programa para el Mejoramiento del Profesorado (PROMEP 2008-2011) “Caracterización de organismos acuáticos con potencial para la bioremediación de efluentes acuícolas”. The first author wants to thank CONACyT for the scholarship (Reg. 51881) received to participate as a student in the Programa de Doctorado en Biotecnología de la Universidad Autónoma de Sinaloa. We thank Ing. Fernando Osuna, CEO of Acuícola Boca del Piaxtla S.A. de C.V., for his support to this research.
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