COMBINED EFFECT OF TEMPERATURE AND FOOD CONCENTRATION ON THE
FILTRATION AND CLARIFICATION RATES AND ASSIMILATION EFFICIENCY OF ATRINA TUBERCULOSA SOWERBY, 1835 (MOLLUSCA: BIVALVIA) UNDER LABORATORY CONDITIONS

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Abstract - In Mexico, Atrina tuberculosa and other bivalve mollusks of commercial importance are intensively exploited, resulting in a drastic decline in their natural populations. This makes the ecophysiological studies of our native mollusks very important. The filtration and clarification rates, and the assimilation efficiency of Atrina tuberculosa at three temperatures (17, 22.5 and 28°C) and three microalgae concentrations (20,000, 40,000 and 60,000 cell·mL⁻¹) were evaluated under laboratory conditions. The data of each physiological variable were fit to a second-degree polynomial model by the method of square minimums to generate surface response graphs. The temperature and food concentration had a direct and synergic effect on the filtration and clarification rates of the species. The food concentration significantly affected the assimilation efficiency, which was observed to be the highest at the lowest food concentration. The filtration and clarification rates were lower at 17°C as compared to 28°C. No optimal values of assimilation efficiency were observed, which suggests that a wider range of temperatures and food concentration must be evaluated.

Key words: Atrina tuberculosa, filtration variables, temperature, microalgae concentration.

INTRODUCTION

In the approximately 10,760 km of the Mexican coast line (7,939 on the Pacific, and 2,821 in the Gulf of Mexico), as well as in the 932,365 ha of coastal lagoons and estuaries, at least 60 species of commercially important mollusks are found; 29 of them are intensively exploited, affecting their natural populations, some of which have diminished drastically, particularly those of the genus Pinna and Atrina (Baqueiro 1984, Moreno et al., 2005). In the bays and estuaries of the Gulf of California, the most commercially important species are Argopecten ventricosus (“catarina clam”), Pecten vogdesi (“flying clam”), Spondylus calcifer (“rock scallop”), Nodizepecten subnodosus (“lion hand”), Pinna rugosa (“long axe callus”) and Atrina tuberculosa (“Chinese axe callus”).
The distribution of *Atrina maura* (Sowerby, 1835), *Atrina tuberculosa* (Sowerby, 1835), and *Pinna rugosa* (Sowerby, 1835), three of the most abundant species (Brusca, 1980), extends from the southern part of Baja California, Mexico to South Panama and Clipperton Island (Keen, 1971). The scarce scientific literature related to these species indicates that *Atrina maura* has been more studied than *Atrina tuberculosa*. These studies are related to the egg-laying cycles of the wild populations (Aguilar, 1964, Holguín, 1975; Ángel-Pérez, 2000), and re-stocking, culture, and seed collection in exploded zones (Cendejas et al., 1985, Cardoza-Velasco, 1998). Basurto (2006) and Basurto and Coleman (2010) described the biological and ecological mechanisms supporting the fisheries of the Seri callo de hacha, including *A. tuberculosa*. The effect of diverse parameters on the filtration rate has been widely studied in many bivalves of the world (Jørgensen, 1975, Winter, 1978, Peña-Messina, 2009, Enriquez-Ocaña et al., 2012); however, more scientific research about our native species is required to evaluate their optimal management in the laboratory or in the natural environment.

In the present study, the rates of filtration and clarification, as well as the assimilation efficiency of *Atrina tuberculosa* at three levels of temperature and three food (microalgae) concentrations were evaluated to contribute on the knowledge of the physiological behavior of this species under laboratory conditions.

**MATERIALS AND METHODS**

A sample of 30-35 individuals of *A. tuberculosa* was collected three times at different seasons of the year in a coastal area located at 28° 51’ to 29° 03’ N, and 113° 27’ to 113° 36’ W. They were transported to the laboratory and put in a tank with 150 l of aerated seawater (salinity 34±1 PSU). After eliminating epibionts, organisms with a shell-length size from 17.5 to 20.5 cm were selected and placed in 40 l aquaria (5 organisms per aquarium). The water temperature was controlled with a thermoregulatory BLUE M model PCC-24A-3 and the organisms were acclimated during fifteen days while being fed with *Isochrysis galbana* cultivated with the f/2 medium of Guillard and Ryther (1962).

The experimental system consisted of 27 aquaria (40.5 x 20.5 x 26.5 cm) installed in a tank with water at controlled temperature. Each aquarium received a continuous water flux of 50 ml·min⁻¹, (in preliminary experiments the minimum filtration rate was 45 ml·min⁻¹). The water level in each aquarium was set with a vertical sliding tube to maintain a constant volume of 15 l. In order to know the effect of temperature and food concentration on the filtration and clarification rates, as well as on the assimilation efficiency of *A. tuberculosa*, a 3 x 3 factorial experimental design was performed three times during the year, after each of the mollusk collections. In each experiment nine treatments consisted of the combination of three temperatures (17, 22.5 and 28°C), and three microalgae (*Isochrysis galbana*) concentrations (20,000, 40,000 and 60,000 cell·ml⁻¹) were evaluated in triplicate (before the experiment, the absence of pseudo feces was verified with selected cell densities). Before the beginning of each experiment, the mollusks were starved during 24 h to drain the digestive tract, avoiding interferences in later measurements. Between each diet evaluation, the organisms were passed to the acclimation aquaria and fed during five days with the experimental diet.

The filtration rate (FR) was measured in a continuous flux system according to the procedure described by Hildreth and Crisp (1976):

\[
FR = F \left( \frac{C_1 - C_2}{C_0} \right) \ C_0^{-1}
\]

where F is the flow rate through the experimental aquarium, \(C_1\) is the concentration of microalgae in the entrance, \(C_2\) is the concentration in the outlet and \(C_0\) is the food concentration around the organisms.

Each filtration experiment lasted 12 h, and samplings were done every two hours. The \(C_0\), \(C_1\) and \(C_2\) were measured with a Coulter Counter Electrozone Model 112LSD/SSP with a sensor whose orifice was
48 µm in diameter, connected to a 200 µl (0.2 mL) volumetric section.

The clarification rate (CR) was evaluated according to the procedure proposed by Winter (1978) that consists of multiplying the FR by the food concentration in the entrance of the experimental aquarium (C<sub>i</sub> in mg·L<sup>-1</sup>); the units derived from this operation are in mg·h<sup>-1</sup>.

The assimilation efficiency (AE) was measured by the method of Conover (1966), which does not require a quantitative recovery of all the feces, and is expressed as:

\[
AE = \frac{(F-E)}{(1-E)\cdot F} \cdot 100
\]

where F is the weight of the organic matter of the food divided by the food dry weight and E is the weight of the organic matter of the feces divided by the feces dry weight. To calculate the assimilation efficiency (AE), samples were filtered through 4.5 cm-GFC fiberglass filters, previously incinerated at 450°C during 4 h. To measure E, every three hours during the 12 h the feces were collected. To measure F, every two hours 50 ml from the food were obtained. In both cases, the dry weight and the organic matter were evaluated according to the procedure described by Sorokin (1973).

The data were analyzed for homoscedasticity and normality to decide if a parametric or nonparametric statistical analysis should be applied (Zar, 1999; Sokal and Rholf, 2000). In addition, the same organisms (blocks) were exposed to different food concentrations at each one of the experimental temperatures. The data of each physiological variable were fitted to a second-degree polynomial model by the method of square minimums to generate the surface responses (Snedecor and Cochran, 1980), in the form of:

\[
f(T, A) = a + bT + bA + cT^2 + dA^2 + eT\cdot A
\]

where \(f(T, A)\) represents the response variable of FR, CR or AE; \(a, b, c, d,\) and \(e\) are the coefficients of the regression; \(T, T^2\) represents the linear and quadratic effect of the temperature; \(A, A^2\) the linear and quadratic effect of the food concentration and \(T\cdot A\) the interactive effect of the temperature and the food concentration.

**RESULTS**

All the mollusks survived transport to the laboratory. The dry weight of one cell of *Isochrysis galbana* was estimated in 0.0303±0.0011 ng, which implies that the dry weight of the food in each microalgae dose was 0.579, 1.195 and 1.741 mg·L<sup>-1</sup>, which corresponds to 19,109; 39,439 and 57,459 cell·mL<sup>-1</sup>, respectively.

At the temperature of 17°C, the filtration rate (FR) was independent of the food concentration (Table 1), with a minimum mean value of 0.281 L·h<sup>-1</sup>·g<sup>-1</sup> when the microalgae concentration was 1.195 mg·L<sup>-1</sup>. At 22.5°C, the FR increased significantly from 0.449 L·h<sup>-1</sup>·g<sup>-1</sup> at the lowest microalgae concentration to 1.394 L·h<sup>-1</sup>·g<sup>-1</sup> at a concentration of 1.195 mg·L<sup>-1</sup>, diminishing to 0.443 L·h<sup>-1</sup>·g<sup>-1</sup> at 1.741 mg·L<sup>-1</sup>. At 28°C the FR increased significantly as did the microalgae concentration (1.177 L·h<sup>-1</sup>·g<sup>-1</sup> at 0.579 mg·L<sup>-1</sup> to 3.962 L·h<sup>-1</sup>·g<sup>-1</sup> at 1.741 mg·L<sup>-1</sup>). In all microalgae concentrations the FR increased with temperature. The second-degree polynomial model presents 87.9% of the total variance of FR (Table 2), where the quadratic effect of temperature (\(T^2\)) presents 58.79%, followed by the interactive effect \(T\cdot A\) with 13.2%, while the linear effects of the food concentration \(A\) and temperature \(T\) presents only 8.91% and 5.74%, respectively; the values of \(R^2\) for \(A\) and \(T\) are much lower than those registered for \(T^2\), whereas the quadratic effect of the food concentration was not significant. The isopleths obtained at intervals of 0.25 L·h<sup>-1</sup>·g<sup>-1</sup> indicates an increasing tendency of FR at temperatures over 20°C (Fig. 1a); the orientation in a diagonal direction with respect to the temperature and food concentration represents the interactive effect of both factors (\(T\cdot A\)).

The clarification rate (CR) of *Atrina tuberculosa* increased significantly with the food concentration at 17 and 28°C (Table 2). The lowest mean values (0.194 and 0.759 mg·h<sup>-1</sup>·g<sup>-1</sup>) were found at the microalgae concentrations.
concentration of 0.579 mg·l$^{-1}$, and the highest (0.617 and 6.730 mg·h$^{-1}$·g$^{-1}$) at 1.741 mg·l$^{-1}$. For the temperature of 22.5°C, the greatest CR was 1.709 mg·h$^{-1}$·g$^{-1}$ at a microalgae concentration of 1.195 mg·l$^{-1}$. All the mean values of the CR were significantly different in the nine combinations of temperature and food concentration.

In Table 2, the polynomial adjustment of CR based on the temperature and food concentration is described. Once again, the quadratic effect of the temperature $T^2$ presents 87.83% of the total variance. The interaction of the linear and quadratic effects of the temperature and the food concentration was highly significant, but the quadratic effect of the food concentration was not significant. In Fig. 1b, isopleths drawn up at intervals of 0.25 mg·h$^{-1}$·g$^{-1}$ showed an increase in the CR for temperatures greater than 19°C; the orientation in a diagonal direction to the projection plane is associated with the interactive effect between the temperature and the food concentration.

The lowest mean value of the assimilation efficiency (AE) was 42.38% and was obtained by the treatment at 17°C and at 1.71 mg·L$^{-1}$ of microalgae concentration. The highest mean was 74.93% in the combination of 28°C and 0.579 mg·L$^{-1}$. The test of multiple comparisons did not detect differences between the AE in the different food concentration levels at 22.5°C, neither among temperatures at microalgae concentration of 1.741 mg·L$^{-1}$. In general, the AE diminished as the microalgae concentration increased.

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The polynomial of second-degree adjustment that describes the relation of the temperature and the food concentration on the AE, presents 35.29% of the total variance and only the linear effect of the microalgae concentration (10.43%) and quadratic effect of the temperature (23.79) had a high level of significance. The direction of the isolines with respect to the axis that represents the microalgae concentration indicates that the interaction is not significant, confirming that AE decrease as the food concentration increases (Fig. 1c).
Most of the ecophysiological studies on mollusks have evaluated variables individually. However, this is not what happens in natural environments where some different variables change independently from others (Norkko and Thrush, 2006). In this context, the studies that involve simultaneously more than one variable are much more useful (Widdows, 1978).

In the present study, the filtration rate of *Atrina tuberculosa* reflected three different patterns related to temperature level when the food concentration was varied. At 17°C, the filtration rate remained independent of the food concentration, probably because the gills ciliary activity diminishes at this temperature and organisms are not capable to filtrate more, although the concentration of microalgae increases. Thompson and Bayne (1974) obtained similar results for *Mytilus edulis* at 15°C in a microalgae concentration rank from 500 to 25,000 cell·mL⁻¹. At 22°C, the filtration rate increased with microalgae concentration up to a maximum in the intermediate concentration; af-

![Table 1. Means ± se of the of Filtration Rate (FR L·h⁻¹·g⁻¹), Clarification Rate (CR in mg·h⁻¹·g⁻¹), Assimilation Efficiency (AE %) of *Atrina tuberculosa* fed *Isochrysis galbana* at nine combinations of temperature (T in °C) and food concentration (A in mg·L⁻¹).](image)

<table>
<thead>
<tr>
<th>T</th>
<th>FR</th>
<th>CR</th>
<th>AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.579</td>
<td>0.37₄⁻ 0.75₀⁻ 1.177⁻ 0.194⁻ 0.44₉⁻ 0.75₉⁻ 74.₄₄⁻ 55.₇₆⁻ 7₄.₉₃⁻</td>
<td>±0.₀₃₄  ±0.₀₂₅  ±0.₀₃₁  ±0.₀₁₈  ±0.₀₀₈  ±0.₀₂₄  ±2.₈₇  ±₅.₆₇  ±₄.₉₀</td>
<td>±0.₂₈₁  ±1.₃₉₄⁻  ±₂.₃₂₃⁻  ±0.₃₁₄⁻  ±1.₇₀₉⁻  ±₂.₈₅₄⁻  ±₇₃.₂₄⁻  ±₄₃.₁₆⁻  ±₅₃.₆₅⁻</td>
</tr>
<tr>
<td>1.₁₉₅</td>
<td>±0.₀₁₈  ±0.₀₂₃  ±0.₀₃₂  ±0.₀₂₂  ±0.₀₁₆  ±0.₀₂₇  ±₂.₃₀  ±₃.₃₅  ±₂.₇₈</td>
<td>±0.₃₈₉⁻  ±₀.₄₄₃⁻  ±₃.₉₆₂⁻  ±₀.₆₁₇⁻  ±₀.₇₄₉⁻  ±₆.₇₃₀⁻  ±₄₂.₃₈⁻  ±₄₈.₂₉⁻  ±₄₉.₉₀⁻</td>
<td>±1.₁₉₅⁻  ±0.₂₈₁⁻  ±₃.₉₆₂⁻  ±₀.₆₁₇⁻  ±₀.₇₄₉⁻  ±₆.₇₃₀⁻  ±₄₂.₃₈⁻  ±₄₈.₂₉⁻  ±₄₉.₉₀⁻</td>
</tr>
<tr>
<td>1.₇₄₁</td>
<td>±0.₀₁₉  ±0.₀₃₀  ±0.₀₂₂  ±0.₀₂₃  ±0.₀₁₇  ±0.₀₃₁  ±₅.₉₈  ±₅.₇₄  ±₅.₄₃</td>
<td>±0.₃₈₉⁻  ±₀.₄₄₃⁻  ±₃.₉₆₂⁻  ±₀.₆₁₇⁻  ±₀.₇₄₉⁻  ±₆.₇₃₀⁻  ±₄₂.₃₈⁻  ±₄₈.₂₉⁻  ±₄₉.₉₀⁻</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Multiple regressions by steps corresponding to Filtration Rate (FR in l·h⁻¹·g⁻¹), Clarification Rate (CR in mg·h⁻¹·g⁻¹), and Assimilation Efficiency (AE %) of *Atrina tuberculosa* fed *Isochrysis galbana*, at nine combinations of temperature (T) and food concentration (A).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>FR (%)</th>
<th>p</th>
<th>CR (%)</th>
<th>p</th>
<th>AE (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>59.₇⁹</td>
<td>0.₀₀₀</td>
<td>5₈.₂₅</td>
<td>0.₀₀₀</td>
<td>₂₃.₇₉</td>
<td>0.₀₀₀</td>
</tr>
<tr>
<td>T*A</td>
<td>₁₃.₂₀</td>
<td>0.₀₀₀</td>
<td>₂₀.₈₀</td>
<td>0.₀₀₀</td>
<td>₀₇.₅₇</td>
<td>0.₃₇₇</td>
</tr>
<tr>
<td>A</td>
<td>₈.⁹₁</td>
<td>0.₀₀₀</td>
<td>₃.₇₂</td>
<td>₀.₀₀₀</td>
<td>₁₀.₄₃</td>
<td>₀.₀₀₁</td>
</tr>
<tr>
<td>T</td>
<td>₅.₇₄</td>
<td>₀.₀₀₀</td>
<td>₅.₀₆</td>
<td>₀.₀₀₀</td>
<td>₃.₁₉</td>
<td>₀.₅₄₄</td>
</tr>
<tr>
<td>A²</td>
<td>₀.₂₆</td>
<td>₀.₃₁₁</td>
<td>₀.₀₀</td>
<td>₀.₈₇₆</td>
<td>₀.₀₁</td>
<td>₀.₉₃₀</td>
</tr>
<tr>
<td>T²</td>
<td>₈₇.₉₀</td>
<td>₈₇.₈₃</td>
<td>₃₅.₂₉</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Regression equations:

FR = 10.₂₁₉⁻7.₈₈₄₈₇T⁻₃.₂₈₀₂₄A₀.₀₁₈₆T²⁻₀.₃₇₃₄₄A²⁻⁺0.₂₁₆₁T*A
CR = ₁₉.₈₇₅₈⁻₁.₆₄₃₇₇T⁻₈.₀₇₉₄₄A₀.₀₃₁₅₇T²⁻⁺₀.₁₀₂₉₄₄A²⁻⁺₀.₄₃₃₅T*A
AE = ₂₉₇.₉₉₄⁻₁₉.₂₈₇₃₃T⁻₂₉.₂₁₃ₐ₄A₀.₄₀₈₅₅T²⁻⁺₀.₈₄₈₅₄₄A²⁻⁺₀.₄₇₄₉₉T*A

**DISCUSSION**

Most of the ecophysiological studies on mollusks have evaluated variables individually. However, this is not what happens in natural environments where some different variables change independently from others (Norkko and Thrush, 2006). In this context, the studies that involve simultaneously more than one variable are much more useful (Widdows, 1978).

In the present study, the filtration rate of *Atrina tuberculosa* reflected three different patterns related to temperature level when the food concentration was varied. At 17°C, the filtration rate remained independent of the food concentration, probably because the gills ciliary activity diminishes at this temperature and organisms are not capable to filtrate more, although the concentration of microalgae increases. Thompson and Bayne (1974) obtained similar results for *Mytilus edulis* at 15°C in a microalgae concentration rank from 500 to 25,000 cell·mL⁻¹. At 22°C, the filtration rate increased with microalgae concentration up to a maximum in the intermediate concentration; af-
terwards, it decreased significantly at the highest concentration. This tendency agrees with the generalized relation between the filtration rate and the food concentration. Nam Han (2008) found that *Ruditapes philippinarum*, thriving in a temperature range from 5 to 25°C, had a higher filtration rate at 20°C. Similarly, Sylvester et al. (2005) documented that *Limnoperna fortunei* presented a greater clarification rate at 25°C, as compared to lower temperatures. Winter (1978) found that the mollusk filtration rate increased remarkably as the microalgae concentration increased up to a maximum value, but if the concentration continued increasing, the filtration rate decreased. At 28°C, the filtration rate always increased with the increase of the food concentration and an optimal value was never reached for the concentrations evaluated.

The filtration rate in the three microalgae concentration evaluated increased as the temperature did, which was an expected result due to the effect of temperature in the metabolic activity of the organisms. Particularly in the present study, the filtration rate of *A. tuberculosa* never reached the critical limit in the food concentrations evaluated. These results are partially similar to those obtained by Ali (1970) for *Hiatella arctica* fed the microalgae *Phaeodactylum tricornutum* at temperatures ranging from 1.5 to 25°C, where he found that the filtration rate increased almost exponentially up to a maximum value at 15 to 17°C, but diminished to almost zero at 25°C. Similar results were documented by Theede (1963), Schulte (1975) and Widdows (1978) for *M. edulis*. Enríquez-Ocaña et al. (2012) reported that *Crassostrea corteziensis* has higher filtration and clarification rates at moderately high temperatures (29°C) as compared to lower (23°C) or higher (32°C) temperatures. Contrarily, Miranda-Baeza et al. (2006) reported that *Anadara grandis* showed no thermal preference in the experimental thermal range of 22 to 31°C.

During the 12 h of the experiment, pseudofeces were not detected, therefore the clarification rate must be considered equivalent to the ingestion rate, which is a function of body size, temperature, and food concentration (Winter, 1978).

At 17°C, the clarification rate of *A. tuberculosa* was independent of the microalgae concentration. Contrarily, at 22.5 and 28°C the CR was strongly affected by the microalgae concentration and the filtration rate. These tendencies agree with the model proposed by Winter (1978) according to which the ingestion rate of the offered food shows an almost linear increase in the clarification rate, correlating with an increase in the filtration rate until the stable phase. This phase corresponds to the threshold that initiates the decay of the filtration rate that was totally identified in this study, although at 22.5°C, a maximum value in the clarification and filtration rates was observed. Probably experimenting around the intermediate food concentration would be possible to detect the stable phase of the clarification rate of *A. tuberculosa*. The increase in the clarification and filtration rates as the temperature increases, observed in the present study, agrees with the results obtained by Sylvester et al. (2005) for *Limnoperna fortunei* at temperatures ranging from 15 to 25°C with a microalgae concentration of 7000 to 10,000 cell·mL⁻¹ of *Chlorella vulgaris*.

The assimilation efficiency of bivalves varies widely (Foster-Smith, 1975), depending mainly on the quantity and quality of feed, especially microalgae species (Ren et al., 2006), the physiological state of the organism and the environmental conditions (Bayne, 1985, Iglesias et al., 1996, Navarro and Widdows, 1997). In the present study, despite the high variability of the data, it is possible to detect similar tendencies to those observed in other bivalves. At 17 and 28°C, the assimilation efficiency diminished with the increase in the food ration. These results agree with those obtained by Thompson and Bayne (1972) and Foster-Smith (1975). Nevertheless, in this study, at 22.5°C the situation was different, because the assimilation efficiency had a slight fluctuation; it is very probable that this was because of the amount...
of accumulated food in the digestive tract of *A. tuberculosa* and that the digestive gland was unable to digest all the ingested food. The previous assumption is reinforced clearly with the minimum value of the assimilation efficiency found when a high value in the clarification rate was detected. This is congruent with the results obtained by Winter (1973) who found that for *Mytilus edulis* periods of low filtration activity coincided with periods of maximum digestive activity.

The fluctuations on the AE when the temperature increased could suggest that this physiological variable is independent of temperature. Nevertheless, Winter (1970) found for *Modiolus modiolus* and *Arctica islandica* augmented digestive activity with temperature increase. On the other hand, Buxton et al. (1981) did not find significant differences in the assimilation efficiency of *Ostrea edulis* at temperatures of 5, 10, 15, 20 and 25°C, and at a concentration of 20,000 cells·ml⁻¹ of *Tetraselmis suecica*. Viegut et al. (2012) reported that the assimilation rate of *Corbicula fluminea* is affected by environmental temperature.

At the temperatures and microalgae concentrations evaluated in this study, optimal values of assimilation efficiency were never observed, which suggests that a wide range of these parameters must be evaluated.

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