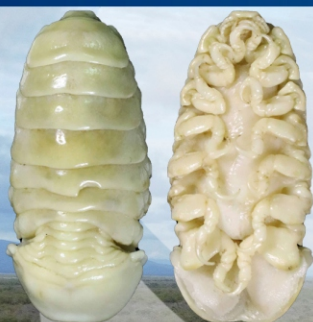




# NUEVA ESPECIE

*Cymothoa facimar*



**DRA. MAYRA GRANO-MALDONADO Y DR. JOSÉ SALGADO-BARRAGÁN**  
DEDICAN UN ISÓPODO PARÁSITO A LA FACIMAR





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## Artículo Científico

### Total Mercury content in edible tissues of *Crassostrea iridescens* (Hanley, 1854) fed with three different diets.

Contenido total de mercurio en tejidos comestibles de *Crassostrea iridescens* (Hanley, 1854) alimentados con tres dietas diferentes.



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## Total Mercury content in edible tissues of *Crassostrea iridescens* (Hanley, 1854) fed with three different diets.

Contenido total de mercurio en tejidos  
comestibles de *Crassostrea iridescens*  
(Hanley, 1854) alimentados con  
tres dietas diferentes.

### ► ABSTRACT

The aim of the present manuscript was to determine the total Hg content in edible tissue of *Crassostrea iridescens* fed with three different diets (D1: Molasses/yogurt, D2: Sea water and D3: Microalgae: *Isochrysis* spp.) under laboratory conditions. After 114 days of experiment, the total Hg content in edible of oyster was similar (D1:  $1.07 \pm 0.780$   $\mu\text{g/g}$ , D2:  $1.041 \pm 0.078$   $\mu\text{g/g}$ , D3:  $0.964 \pm 0.214$   $\mu\text{g/g}$  d.w.). This tendency also was observed in each ingredient used for dietary treatments (*Isochrysis* spp.:  $2.591 \pm 0.280$   $\mu\text{g/g}$ , Molasses:  $2.320 \pm 0.174$   $\mu\text{g/g}$ , Yogurt:  $2.707 \pm 0.22$   $\mu\text{g/g}$ ). No significant differences were observed in Hg content in tissues and neither dietary ingredients ( $p > 0.05$ ). The consumption of oyster of this experiment do not represent a potential risk for human consumption, due to Hg values were below the limit established by Mexican Officially Norm 242.

**Keywords:** Hg, Diets, Potential risk, *Cassostrea iridescens*



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## ► RESUMEN

El propósito del presente manuscrito fue determinar el contenido total de Hg en el tejido comestible de *Crassostrea iridescens* alimentado con tres dietas diferentes (D1: Melaza/yogurt A, D2: Agua de mar, D3: Microalga: *Isochrysis* spp., bajo condiciones de laboratorio. Después de 114 días de experimento, el contenido total de Hg en los tejidos comestible del ostión fue similar D1:  $1.07 \pm 0.780 \mu\text{g/g}$ , D2:  $1.041 \pm 0.078 \mu\text{g/g}$ , D3:  $0.964 \pm 0.214 \mu\text{g/g d.w.}$ ). Esta tendencia se observó también en los ingredientes de los tratamientos dietarios *Isochrysis* spp.  $2.591 \pm 0.280 \mu\text{g/g}$ , Melaza:  $2.320 \pm 0.174 \mu\text{g/g}$ , Yogurt:  $2.707 \pm 0.22 \mu\text{g/g}$ ). No se observaron diferencias significativas en el contenido de Hg en los tejidos ni tampoco en los ingredientes dietarios ( $p > 0.05$ ). El consumo de ostión de este experimento no representa un riesgo potencial para el consumo humano, ya que los valores de Hg estuvieron por debajo de los límites establecidos por la Norma Oficial Mexicana 242.

**Palabras clave:** Hg, Dietas, Riesgo Potencial, *Crassostrea iridescens*

## ► INTRODUCCIÓN

Mercury (Hg) is a toxic element of biological and ecological concern in aquatic environment, by its high persistence, bioaccumulation and high potential of being biomagnified in food webs (Soto-Jiménez et al., 2023). Furthermore, Hg is a useful metal involved in anthropogenic activities such as mining for gold extraction, industrial and domestic water discharges, metallurgical industry, fluorescent lamps and fungicides among others (Jadaa & Mohammed, 2023).

The methylated-Hg could be magnified in food webs. Since the basal trophic position (accumulated by phytoplankton and seston) and transferred to upper predators (fishes with different feeding habits) until reach human populations (Valladolid-Garnica et al., 2023; Soto-Jiménez et al., 2023).



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The accumulation of Hg in bivalve mollusks is of great interest because they bioaccumulate high level of metals and are consumed by humans. Oysters as strong metal accumulators may act as an important vector transferring Hg to higher trophic levels, increasing the risk of biomagnification in food web. The aim of the present manuscript is to assess the bioaccumulation of Hg in the oyster *Crassostrea iridescens* fed with three different diets under laboratory conditions.

## ► MATERIAL AND METHODS

### Sampling

In September 2018, 39 oysters *C. iridescens* were collected by hand into the Mazatlan Bay, Mexico (SE Gulf of California). The bivalves were selected by similar size and location zone. The organisms were carried out in plastic buckets and once in the laboratory, they were confined into an aquaria with constant dissolved oxygen (5 mg/L), 35 ppt and 27 °C in order to depurate them for 72 h previous to experiment (Sami, 2024).

### Dietary treatments

Diet 1: Mixing of molasses and yogurt, ratio: 5 g and 40 g, respectively.

Diet 2: Sea water (contained of Phytoplankton, Zooplankton and Seston).

Diet 3: A concentrated of microalgae: *Isochrysis* spp.

### Experimental Design:

Thirteen oysters per diet were used to evaluate the bioaccumulation by dietary route and to determine the total Hg content in edible tissues of *C. iridescens*. Each group of specimens was placed in an aquarium.

In order to maintain water quality in culture, 100% water exchange was done after feeding. Furthermore, weekly cleaning was carried out, including remotion of organic matter of the wall of the aquariums and from the bottoms using a siphon to collect the major load of oxidized detritus and avoiding any deleterious effects by ammonia compounds. Subsequently, epibionts were removed using a plastic brush and rewashed in sea water to keep them free of any commensal or parasite organisms (Ostrensky et al., 2022). The period of the experiment was 114 days in order to observe the Hg biaccumulation in *C. iridescens*.



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## Total Hg content in *Crassostrea iridescens*

### Sample preparations

Once concluded the experiment, oysters were measured and dissected removing the whole organism from its shell. Pool samples were lyophilized to  $-40^{\circ}\text{C}$  and high vacuum environment ( $10^{-13}$  millibars for 72 h) in order to eliminate the humidity from samples. Additionally, dried samples were grounded in an agate mortar and pestle and kept them in plastic bottles with hermetic cap and ready for digestion process (Sánchez-Betancourt et al., 2024).

### Acid Digestion

For pre-digestion, 0.5 g of oyster tissue was confined into Sallivex digestion bomb and added 5 ml of  $\text{HNO}_3$  (trace metals grade) for 24 h (Quintero-Alvarez et al., 2012). Then, Savillex bombs were degassing and taken into graphite mod block unit with controlled temperature ( $120^{\circ}\text{C}$ ) for 3 h and transferred to clean polypropylene vial and diluted to 20 ml (Sánchez-Betancourt et al., 2024).

### Atomic Absorption Spectrophotometry

For Atomic Absorption Spectrophotometry, Loring & Rantala (1992) method of cold vapor generation atomic absorption spectrophotometry (CV-AAS) was used to quantify Total Hg content by an Hg analyzer (Buck Scientific 410).

All materials used in sampling and metal analysis were washed according to Moody & Lindstrom (1977), which consisted of washing and rinsing with distilled water and acid washed with 2 M HCl and 2 M  $\text{HNO}_3$ ; and finally, the material was rinsed with Milli-Q water. Certified reference NIST SRM 1566b (Oyster Tissue) was used for Total Hg with a satisfactory recovery percentage of 93.4 %. Blanks were used to check contamination and all tissues were analyzed in triplicate. The detection limit for Hg technique was  $0.01\text{ }\mu\text{g/g}$ .

Mercury concentrations expressed in dry weight were transformed to wet weight by dividing the dry weight by 4 (Sanchez-Betancourt et al., 2024), in order to compare the result with Mexican Officially Norm 242

### Statistical analysis:

Final longitude and Hg contents in edible tissues and between diets were analyzed using Sigmastat software. Analyses of variance were carried out to detect any difference between diets ( $p < 0.05$ ). All tests were carried out in the interval of 95% (Zar, 2010).



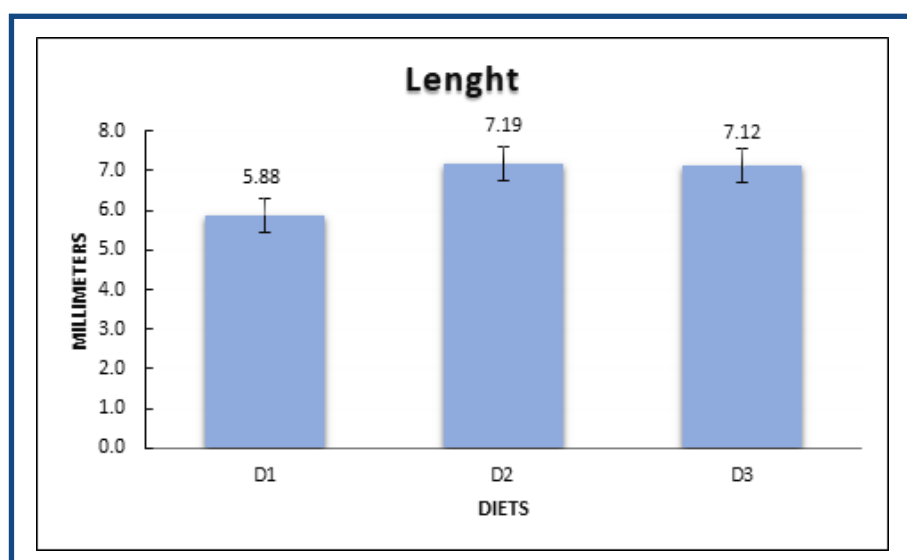
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## ▶ RESULTS

### Final Length

At the end of the experiment, similar final length was observed in Diet 2 and Diet 3, with similar values of  $7.19 \pm 0.89$  mm and  $7.12 \pm 0.82$  mm respectively, but the lowest length was found in Diet 1 with  $5.88 \pm 1.14$  mm being significantly different ( $p < 0.05$ ) to the rest of diets (Fig. 1).



**Figure 1.** Final length in oyster *Crassostrea iridescens* fed with three different diets. Different letters indicate significant differences ( $p < 0.05$ ).

### Total Hg Content in oysters:

The range of Hg content in oysters fed on molasses/yogurt diet, was  $0.951 - 1.136 \mu\text{g/g}$ , and fed on sea water was  $0.313 - 2.165 \mu\text{g/g d.w.}$ , meanwhile in organisms fed with microalgae was  $0.712 - 1.179 \mu\text{g/g}$  (Table 1).

The Hg mean concentrations in edible tissues of oysters did not detect any significantly differences between treatments ( $P > 0.05$ ) (Table 1).



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| Pool sample | Molasses/<br>yogurt | Sea Water | <i>Isochrysis</i> spp. |
|-------------|---------------------|-----------|------------------------|
| 1           | 1.014               | 0.313     | 0.864                  |
| 2           | 1.061               | 2.165     | 0.712                  |
| 3           | 0.951               | 0.947     | 1.100                  |
| 4           | 1.136               | 0.873     | 1.179                  |
| AVG         | 1.041a              | 1.074a    | 0.964a                 |
| SD          | 0.078               | 0.780     | 0.214                  |

Different letters indicate no significant differences were detected ( $p > 0.05$ ).

**Table 1.** Total Hg content ( $\mu\text{g/g}$  d.w.) in the edible tissue of *Crassostrea iridescens* fed with different diets.

## Total Hg content in ingredients of diets:

The Hg mean content was  $2.591 \pm 0.280 \mu\text{g/g}$  for *Isochrysis* pp., followed by molasses with  $2.320 \pm 0.174 \mu\text{g/g}$  and  $2.707 \pm 0.22 \mu\text{g/g}$  for yogurt. Neither, significantly differences were detected in the ingredients used in diets ( $p > 0.05$ ).

## DISCUSSION

The Hg content in diets did not affect the final length at the end of the experiment. According to Beiras & His, (1994), found that dissolved Hg ( $4 \mu\text{g/L}$ ) in aquaria under laboratory condition provoked deleterious effect in growth of *Crassostrea gigas*. In spite of not analyzing the Hg content in sea water of aquaria, we used the reference value for dissolve Hg in Mazatlan harbor ( $1 \mu\text{g/g}$ ) (Jara-Marini et al., 2008), being a lower concentration than reported to Beiras & His (1994) and without deleterious effects in oysters. For this reason, low dissolve Hg did not show deleterious effects in length of shells of *C. iridescens*.

The lowest length detected in oyster fed on Diet 1 is due to a nutritional imbalance in molsasses/yogurt diet. According to Palmonari et al. (2020), molasses contains a 97-98% of dry matter (d.m.), very low protein (6.7% d.m.) and high levels of sugar (saccharose:48.8 d.m.).



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Since the point of view of a nutritionist, molasses/yogurt diet is made up of fiber and sugar, these nutrients are not required for oyster cultivation (in order to promote growth) and neither are suitable for organism under metallic stress condition.

In Addition, molasses is more suitable for water quality than a nutritional diet (. The inclusion of molasses in super intensive cultures is used to avoid any deleterious effect by ammonia compounds (Panjaitan & Manullang, 2024).

On the other hand, better lengths were observed in oyster fed on microalgae and sea water diets. This is the result of the nutritional profile of microalgae *Isochrysis* spp, being lipids 12-14%, proteins 50-56%, carbohydrates 10-17% (Milledge, 2011). For this reason, oysters fed with microalgae and sea water diets shown better length, compared to carbohydrates rich diet.

The Hg content recorded in edible tissues of *C. iridescens* came from diets and from sea water. The level of Hg in diets ranged from 2.32 -2.70 µg/g and was transferred from diets to edible tissue of oyster, concluding in biotransference and biaccumulation of Hg in *C. iridescens* bivalve.

We did not determine dissolved Hg in seawater; however, we used the Hg reference values for Mazatlan harbor. This value was determined considering rainy and dry season of Hg dissolved in seawater (1 µg/g) (Jara-Marini et al., 2008). It is important to mention that there are living organisms in seawater as phytoplankton (which bioconcentrate Hg by diffusion) (Levy et al., 2007), zooplankton (by foraging macroalgae) and organic-inorganic seston which in turn provide an extra load of Hg to oysters in addition of sea water. Although this, we could not find any difference between tissues of oyster.

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Under this critical scenario, oyster used the osmoregulation physiological process to deal with the Hg load in the aquaria, reducing the internal Hg content compared with its outer metallic load (although Hg contents in diets and seawater), oysters were capable to reduce the Hg in their tissues, confirming their ability to concentrate and reduce the metal in their tissues. The period of the experiment was enough to elucidate the capability of osmoregulation in oyster.

Whether Hg content recorded in edible tissues of oyster was a potential risk for human consumption we compared our results with Hg value proposed by Mexican legislation (NOM-242; 1 mg/kg wet weight). Transforming Hg content to wet weight, we conclude that the mean concentration of Hg in edible tissues (0.241  $\mu\text{g/g}$ , 0.251  $\mu\text{g/g}$  and 0.268  $\mu\text{g/g}$  w.w.) did not exceed the safe level recommended by NOM-242, indicating that consuming these oysters are not of concern and neither pose a risk for human consumption.

## ► ACKNOWLEDGEMENT

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