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Evaluación de la respuesta de biomarcadores de exposición a contaminantes en camarones de la costa de Campeche, México

Tesis

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PRESENTA

BIOL. GISELA AGUAYO DIONET

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Dedicatoria

A nuestro Señor Jesucristo.

A mi familia, en especial a mis padres Carlos y Gisela, y a mi hermano Hiram.

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RESÚMEN

El impacto de los contaminantes sobre los camarones juveniles de *Farfantepenaeus duorarum* se evaluó mediante biomarcadores de exposición en tres localidades pesqueras de la costa de Campeche, México. Se cuantificaron plaguicidas organoclorados (POC) en sedimento y tejido de camarón para analizar la relación entre estos y las respuestas de biomarcadores. Las concentraciones en sedimento y camarones en este estudio fueron 100 veces más altas que otros estudios registrados en las costas de Campeche entre el año 1993 al 2009. Los resultados en sedimentos superficiales mostraron que los plaguicidas rebasaron la guía de calidad de sedimentos estando por arriba del nivel de efecto probable PEL >0.05ppm. De acuerdo con los criterios establecidos por la administración de alimentos y medicamentos FDA y el codex alimentarius CODEX, todas las muestras (n=24) de tejido de camarón rosado se encontraron por arriba de los límites recomendados para consumo humano (>1ppm). Con respecto a los biomarcadores, se cuantificaron las actividades de enzimas de detoxificación (glutatión-s-transferasa GST, catalasa CAT y superóxido dismutasa SOD) y de la enzima de daño neurotóxico (acetilcolinesterasa AChE). La actividad de la enzima AChE se redujo en un 80% y pudiera relacionarse a la presencia de plaguicidas organofosforados OF, carbamatos y metales pesados. La actividad de SOD se incrementó significativamente un 15% en los meses de enero-febrero en dos sitios coincidiendo con el incremento significativo de actividad de la enzima CAT (>40%) en los mismos meses y localidades. Las actividades de las enzimas GST y CAT se incrementaron significativamente en un 50% durante la temporada de lluvias y podría deberse a los contaminantes que inhibieron la AChE de julio-septiembre y que coincide con el calendario agrícola primavera-verano. Este estudio reveló un desbalance en las enzimas de detoxificación en camarón rosado, que es un aviso temprano de daños fisiológicos y bioquímicos que pudieran presentar los crustáceos y podrían repercutir en los estadíos juveniles y por ende en la pesquería de camarón.

Palabras clave: camarón, plaguicidas, enzimas, biomarcador.

CAPÍTULO I.

INTRODUCCIÓN

Los plaguicidas sintéticos son un ejemplo de contaminantes xenobióticos (xeno=foráneo o extraño, bios=vida) que degradan los hábitats y pueden afectar los ecosistemas, incluyendo al hombre y otros consumidores al final de las cadenas tróficas (Albert y Benítez, 2005). Los plaguicidas pueden clasificarse de distintas formas: por su estructura química, objetivo del uso, modo de acción, persistencia o toxicidad (Albert y Benítez, 2005). Con respecto a la estructura química, se encuentran los plaguicidas organoclorados (POC), relevantes por el daño que causan a la salud humana, a la biota, y por su gran demanda de uso.

La estructura química de los plaguicidas les confiere alta estabilidad química, baja presión de vapor (poco volátiles), poco solubles en agua, y solubles en disolventes orgánicos (Ramírez y Lacasaña, 2001), esta última característica marca la tendencia de algunos plaguicidas para emigrar del agua hacia un disolvente no polar o hacia los tejidos de los organismos acuáticos, de esta relación se parte para calcular el coeficiente de partición octanol agua (octanol water partition coefficient, Kow), cuya medida refleja cómo se distribuye un agente químico entre un estado acuoso o hidrofílico a uno lipofílico (Albert y Benítez, 2005).

La presencia de plaguicidas organoclorados (POC) en los ecosistemas acuáticos es producto de su amplia utilización en las actividades agropecuarias e industriales, lo que ha generado preocupación debido a su toxicidad, acumulación, y persistencia en la biota acuática. La fuente principal de la contaminación por plaguicidas es la aplicación directa a la tierra y a los cultivos. En el suelo la sustancia puede ser retenida, transportada o degradada, y posteriormente al infiltrarse en las capas subterráneas llega a los mantos freáticos y viaja hasta ser vertida en zonas costeras donde se acumula en el sedimento. De esta manera los organismos están expuestos a sustancias químicas que pueden bioacumularse y causar alteraciones bioquímicas, fisiológicas y morfológicas (Rendón-von Osten *et al.,* 2005).

Cuando un organismo acumula un contaminante, las propiedades toxicocinéticas de absorción, distribución, metabolismo y excreción, determinarán el efecto sobre el organismo (Amiard-Triquet *et al.,* 2011). La incorporación de los contaminantes en la sangre de los organismos acuáticos tiene lugar a través de la piel, de las branquias o del tubo digestivo.

Cuando los plaguicidas se incorporan al organismo, provocan la formación de especies reactivas del oxígeno (ROS, por sus siglas en inglés), que resultan nocivas para los organismos cuando se producen en grandes cantidades, ya que dañan los constituyentes celulares e inducen la muerte celular (Lushchak, 2011).

Las ROS incluyen los radicales superóxido ($O_2^{\bullet -}$), hidroxilo ($OH^{\bullet -}$) y peróxido de hidrógeno (H_2O_2). Para protegerse frente a la producción de ROS los organismos disponen de un sistema antioxidante que convierten los agentes xenobióticos liposolubles en metabolitos hidrosolubles capaces de ser excretados, este proceso es denominado biotransformación, el cual se ha dividido a su vez en dos grupos de actividad enzimática: reacciones de fase I y fase II (Vamvakas y Anders, 1990).

La primera fase del metabolismo implica reacciones de oxidación y reducción o hidrólisis, con la finalidad de convertir los compuestos xenobióticos en metabolitos más polares, estas reacciones son catalizadas por las enzimas oxidasas de función múltiple, citocromo P450, citocromo b5 y el citocromo P450 nicotinamida adenina dinucleótido fosfato reductasa NADPH (Gold-Bouchot y Zapata-Pérez, 2004).

Las enzimas de biotransformación fase II, consisten en reacciones de conjugación o enlace del xenobiótico o metabolito derivado de la fase I, a compuestos más polares (glutatión GSH y ácido glucurónico GA) para facilitar la excreción (Rendón-von Osten, 2005). Las enzimas de fase II, incluyen el sistema glutatión (glutatión peroxidasa GPX, glutatión reductasa GR y glutatión-s-transferasa GST), superóxido dismutasa SOD y catalasa CAT (Rendón-von Osten, 2005).

La enzima SOD, neutraliza los radicales superóxido catalizando la conversión de estos en peróxido de hidrógeno y moléculas de oxígeno (Boelsterli, 2003), el peróxido de hidrógeno formado, puede ser neutralizado por la enzima CAT o por el sistema glutatión produciendo agua y oxígeno molecular (Berg *et al.,* 2008). Los niveles de las enzimas de fase II pueden verse afectados después de la exposición a contaminantes, por ello estas enzimas tienen una función importante tanto en la homeostasis, como en la desintoxicación y limpieza de compuestos xenobióticos del organismo (Van-der Oost, 2003), y por ello los niveles de actividad enzimática son utilizados como biomarcadores.

Un desequilibrio entre la generación y la neutralización de ROS por los mecanismos antioxidantes se denomina estrés oxidativo (Lushchak, 2011). La evaluación de biomarcadores de estrés oxidativo es una herramienta útil para detectar el efecto de los contaminantes en los organismos, los monitoreos ambientales deben incluir el análisis de biomarcadores, cuyo resultado permite tomar acciones correctivas y preventivas (Gold-Bouchot y Zapata-Pérez, 2004). Para hacer una evaluación de los efectos de los contaminantes en los ecosistemas se requiere un conjunto de biomarcadores (Van-der Oost, 2003).

La enzima acetilcolinesterasa (AChE) es un ejemplo de bioindicador neurotóxico; los plaguicidas organofosforados (OF) y carbamatos son inhibidores de la AChE. Esta enzima está implicada en la desactivación de la acetilcolina en las terminaciones nerviosas, evitando descargas nerviosos continuas (Lushchak, 2011). Debido a que los plaguicidas OF se utilizan en la agricultura tropical (Readman *et al.* 1992), en algún momento los organismos acuáticos estarán expuestos a dosis acumulativas de OF debido a que los cuerpos de agua o zona costera reciben estos contaminantes continuamente.

En México, los plaguicidas se han utilizado para combatir organismos nocivos para los cultivos agrícolas y para controlar los vectores que transmiten enfermedades al hombre o animales. Como consecuencia de estas actividades, los residuos están ampliamente distribuidos en los ambientes costeros y estuarinos (Albert y Benitez, 2005). En el sureste se empleó durante varios años el diclorodifeniltricloroetano (DDT) como medio para controlar a los mosquitos que transmiten el dengue, por lo que se llegó a emplear hasta 10 toneladas anuales de DDT en los estados de Tabasco y Campeche (Benítez y Barcenas, 1996).

En la zona costera de Campeche la hidrodinámica está influenciada por sus características físicas y factores climáticos (lluvias, vientos ciclónicos, mareas, formación de termoclinas y corriente del Lazo) (Posada-Vanegas *et al*., 2013), el viento y las lluvias producen un fuerte acarreo de contaminantes a las zonas costera y propician la dispersión de estos (Guzzella *et al*., 2005). Algunos estudios han reportado residuos de plaguicidas en especies que habitan las costas de Campeche (Tabla 1).

Tabla 1. Residuos de plaguicidas organoclorados en las costas de Campeche, México

Referencia	**Residuos de contaminantes**
Gold-Bouchot *et al.* (1993, 1995)	Concentraciones de 0.00069 ppm de ΣDDT en camarón blanco (*Litopenaeus setiferus*) y en 1995, concentraciones de 0.00025 ppm.
Rendón-von Osten y Memije 2001	Concentraciones de ΣDDT de 0.0000042 ppm en *L. setiferus.*
Díaz-González *et al.* (2005)	Niveles de plaguicidas organoclorados determinados en la biota (vegetación sumergida, pastos y peces).
Carvalho *et al.* (2009)	Concentración de Σendosulfán en muestras de agua filtrada de 11 sitios (<0.000001 ppm), ΣDDT en sedimento 0.00019 ppm (peso seco).

En la plataforma continental al sur del Golfo de México, destaca la Sonda de Campeche, región de alta biodiversidad en constante expansión urbana e industrial, que incluye puertos industriales, pesqueros y explotación petrolera (Yáñez-Arancibia y Sánchez-Gil, 1986). En la Sonda de Campeche se captura el camarón rosado (*Farfantepenaeus duorarum*). Esta pesquería es una de las más deterioradas, su captura pasó de 20,000 ton/año en los 70´s hasta capturas inferiores a las 2,000 ton/año en los 90´s (Ramírez-Rodríguez *et al.* 2003). Dentro de las posibles causas del decremento de la pesquería de *F. duorarum* se encuentran la sobrepesca, cambios en los patrones estacionales de temperatura y salinidad, y la degradación del hábitat (Ramírez-Rodríguez, 2002 y Ramírez-Rodríguez *et al.,* 2002). Lo anterior generó la necesidad de desarrollar un plan de manejo pesquero del recurso en el que se destaca promover el monitoreo de los hábitats costeros asociados al camarón y evaluar el impacto generado por la contaminación (Gracia, 1995; DOF, 2014).

La especie *F. duorarum* tiene un ciclo de vida corto que comienza con el desove en altamar, después los estadios de postlarvas y juveniles se desarrollan en estuarios que fungen como áreas de crianza, permanecen ahí hasta las etapas finales de juveniles y después migran a mar abierto (García y Le-Reste, 1986). La pesca de juveniles de *F. duorarum*, se lleva a cabo en la costa de Campeche y debido a la presencia de contaminantes en las costas de Campeche, es necesario evaluar si estas sustancias están ocasionando un efecto a nivel bioquímico que pueda tener consecuencias desfavorables para los estadíos juveniles de camarón rosado.

OBJETIVO GENERAL

Evaluar la respuesta de biomarcadores de exposición a plaguicidas organoclorados (POC) en camarones juveniles *Farfantepenaeus duorarum* de tres localidades pesqueras de la costa de Campeche, México

OBJETIVOS ESPECÍFICOS

- Determinar las concentraciones de los plaguicidas organoclorados (POC) en sedimentos y en tejido de camarones.
- Determinar la actividad de la enzima: acetilcolinesterasa (AChE) en respuesta a plaguicidas neurotóxicos.
- Determinar la actividad de las enzimas de detoxificación y estrés oxidativo.
- Determinar si existe una relación entre las concentraciones de contaminantes y la respuesta de biomarcadores.
- Establecer si hay diferencias entre las concentraciones de los contaminantes y las temporadas climáticas.

HIPÓTESIS

- Se espera detectar residuos de contaminantes como los POC en sedimentos y en camarones debido a su uso en las actividades agropecuarias.
- Debido a que posiblemente hay residuos de plaguicidas organofosforados OF y carbamatos CB, es probable que la actividad de la AChE se inhiba.
- Si existen contaminantes orgánicos en tejido de camarón las enzimas de destoxificación y de estrés oxidativo (CAT, GST y SOD) estarán activadas.
- Existe relación entre las concentraciones de contaminantes y la respuesta de biomarcadores.
- Existen diferencias entre las concentraciones de los contaminantes y las épocas climáticas.

CAPÍTULO II.

ARTÍCULO

Assessment of biomarker response to pollutant exposure in juvenile pink shrimp (*Farfantepenaeus duorarum)* off the coast of Campeche, Mexico.

Aguayo-Dionet G.[1], Rendón-von Osten J.[2], Pérez-Jiménez J.C.[1], Rojas-González R.I.[3]

[1]Laboratorio de Ecología Pesquera, El Colegio de la Frontera Sur (CONACyT), Av. Rancho Polígono 2-A, Ciudad Industrial, Lerma, C.P. 24500, Campeche, México (giaguayo@ecosur.edu; jcperez@ecosur.mx).

[2] Área de Contaminación e Impacto Ambiental, Instituto EPOMEX, Universidad Autónoma de Campeche, Av. Héroe de Nacozari No. 480, 24079 San Francisco de Campeche, Campeche, México (jarendon@uacam.mx).

[3]Centro Regional de Investigación Pesquera, INAPESCA, Carretera Campeche Lerma km. 5, C.P. 24500, Lerma, Campeche (ramón.rojas@inapesca.gob.mx).

Corresponding author: jcperez@ecosur.mx

ABSTRACT

Environmental quality and health status of juvenile *F. duorarum* were evaluated in three fishing sites in Campeche. Organochlorine pesticides (OCPs) found in sediment and shrimp tissue were quantified to analyze their relationship with biomarker responses. Results from superficial sediments showed that pesticide levels exceeded guidelines, being above the probable effects level (PEL). In accordance with the criteria established by the Food and Drug Administration (FDA) and the CODEX Alimentarius, all of the tissue samples of pink shrimp analyzed in this study had levels higher than those recommended for human consumption (>1 ppm). The biomarkers analyzed were detoxification enzymes, glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and a neurotoxicity biomarker, acetylcholinesterase (AChE). AChE activity was inhibited by 80%, which could be related to the presence of organophosphorus pesticides (OPs), carbamates and heavy metals. SOD activity was inhibited between the months of July and August, which could be explained by an excess of H_2O_2 in tissues. The increased activity in GST and CAT could have been caused also by the pollutants that inhibited AChE activity during the rainy season and the agricultural calendar in spring-summer. This study revealed an imbalance in the

detoxification enzymes of pink shrimp, which is an early warning sign of possible physiological and biochemical damage in the crustaceans, potentially affecting the juvenile population and in turn the fishery stock.

Keywords: pink shrimp, sediment, pesticides, enzymes, biomarkers

1. INTRODUCTION

The most ubiquitous environmental pollutants with harmful effects on man are the persistent organic pollutants (POPs), which are characterized by being highly toxic, persistent, bioacummulative and for their long-range transport upon evaporation (Shen and Wania, 2005). The presence of organochlorine pesticides (OCPs) in aquatic ecosystems is a result of their widespread use in agricultural and industrial activities, which has created concern due to their toxicity, accumulation and persistence in the aquatic biota. In Mexico, pesticides have been used to combat harmful organisms which attack crops and against vectors which transmit disease to man and animals (Albert, 1996).

When an organism accumulates a pollutant, the toxicokinetic parameters of absorption, distribution, metabolism and excretion will determine its effect on the organism (Amiard-Triquet et al*.,* 2011). One strategy for monitoring the effects of OCPs is analyzing activity of the enzymes responsible for providing cellular defense against pesticides (Wang et al., 2014); these ecotoxicology tools are known as biomarkers. A battery of biomarkers is required to assess the effects of pollutants in ecosystems (Van-der Oost, 2003).

Pesticides act as pro-oxidant stress factors which increase the intracellular generation of reactive oxygen species (ROS) (Karaca et al., 2014). The ROS include the superoxide anion radical ($^{\bullet}O_2^-$), hydroxyl ion (OH^-), and hydrogen peroxide (H_2O_2). As a defense mechanism against the production of ROS, organisms use enzymatic and non-enzymatic components; the main antioxidant defense enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST). These enzymes can be induced by ROS and can therefore be used as indicators of oxidative stress (Wang et al., 2014).

Oxidative stress is an imbalance between the generation of ROS and their neutralization through antioxidant mechanisms (Lushchak, 2011). Acetylcholinesterase (AChE) is an enzyme which is involved in the deactivation of acetylcholine in nerve endings, preventing continuous nerve discharges. The organophosphorus pesticides (OPs) and carbamates are AChE inhibitors (Lushchak, 2011), and for this reason AChE has been used as a neurotoxic biomarker of the presence of these pesticides.

Several economically important fisheries have been developed in the Gulf of Mexico, including the fishery for pink shrimp (*Farfantepenaeus duorarum*) in the Campeche Bank, southern Gulf of Mexico. However, the pink shrimp fishery has declined since the 1980's (Ramírez-Rodríguez et al., 2003). Hypotheses for the causes of the fishery collapse include overfishing by artisanal and industrial fleets, changes in seasonal temperature and salinity patterns and habitat degradation (Ramírez-Rodríguez et al., 2003).

The pink shrimp *F. duorarum* has a short life cycle which begins with spawning in the ocean and development of post-larval and juvenile stages in estuaries, which function as nurseries until shrimps return to the ocean (García and Le-Reste, 1986). The juvenile stages are important, as they will recruit to the reproductive population in the ocean, and also represent economic value for artisanal fishermen.

Therefore, it is necessary to assess the biomarker responses for OCP exposure in juvenile shrimp being caught off the coast of Campeche, due to the presence in this region of hydrocarbon (Gold-Bouchot et al., 1993, 1995), pesticide (Díaz-González et al., 2005; Rendón-von et al., 2005) and polychlorinated biphenyls PCB residues.

2. MATERIALS AND METHODS

2.1 Study area and sampling techniques

Three sites were identified which represent the principal areas for pink shrimp fishing by artisanal fleets off the coast of Campeche: Seybaplaya (20°41'4.9"N 90°26'53.99"W) and Paraíso (19°21'51.84"N 90°43'21.35"W) in the municipality of Champotón, and Isla Arena (20°40.83N 90° 26.809W), in the municipality of Calkiní (Fig. 1). The communities surrounding the sample sites are sustained by agricultural activity and, to a lesser extent, livestock production. In the municipality of Champotón, rice, sugar cane and sorghum are cultivated, and cattle are raised (INEGI, 2015). The Paraíso community is influenced by mangrove ecosystems and by the Champotón River, because of the input of sediments and organic material. In the municipality of Calkiní, farming, livestock production and forestry are practiced (INEGI, 2015). The Isla Arena site is located within the Celestún Biosphere Reserve, which is one of the best conserved wetlands of the Yucatán Peninsula (DOF, 2002).

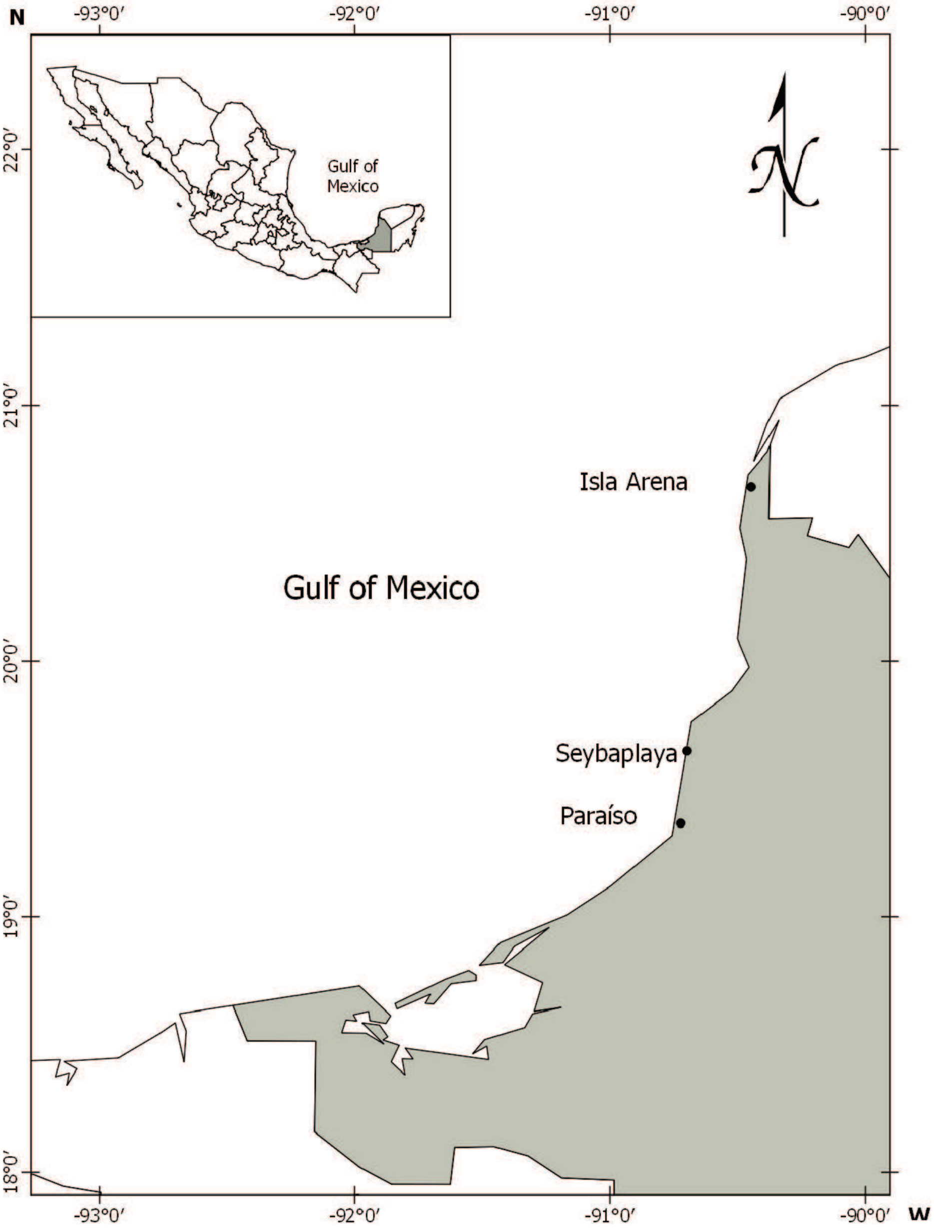

Figure 1. Study sites (Isla Arena, Seybaplaya and Paraíso) in southern Gulf of Mexico.

There are three climatic seasons in Campeche: winter cold fronts (October-March), dry (April-May), and rainy (July-September). However, based on precipitation records (CONAGUA, 2015), only two periods were considered for this study: dry (January-May) and rainy (June-September).

2.2 Field work

Samples were taken from January to September 2015, taking into consideration the spring-summer agricultural season and the health campaigns against mosquitos. At the Paraíso site, the samples were taken from January to September, for Seybaplaya from February to September, and for Isla Arena from March to September.

Ten shrimp were collected from each site with a "triángulo activo", a type of artisanal net (Wakida-Kusunoki et al., 2016), used by the local fishermen. The shrimps were wrapped in aluminum foil, placed in Ziploc bags and stored in cool-boxes. Sedimentary material from the surface layer of the bottom (5-10 cm) was obtained using a PVC tube of 1.5 m length and 2.5 cm diameter, with the aim of analyzing recently deposited pollutants. The water temperature, dissolved oxygen (DO), salinity and pH were taken using the mobile multi-parameter LDO 101 to determine if environmental variables influence the biomarkers.

2.3 Biochemical analysis

After identification of the shrimps as *Farfantepenaeus duorarum*, they were sexed, weighed and measured (from the anterior end of the rostrum to the posterior end of the telson). They were then dissected and the organs were placed in Eppendorf tubes, eyes for AChE assay and the gills for GST assay. Each hepatopancreas was divided into two parts, one for CAT analysis and the other for SOD. They were then conserved at -70°C until assay. The shrimp abdomens were wrapped in aluminum foil and stored in a refrigerator for pesticide analysis.

Before AChE, GST and CAT assays, samples were homogenized with a combined buffer for protein extraction (Schreck et al., 2009). AChE enzymatic activity was determined at 414nm by the method of Ellman et al. (1961) adapted to microplate (Guilhermino et al., 1996), using 0.05mL of homogenate and 0.250mL of the reaction solution 1mL of 5,50-dithiobis- 2-nitrobenzoic acid (DTNB) 10mM solution, 0.2mL of 0.075M acetylthiocholine solution and 30ml of 0.1M phosphate buffer.

GST activity was determined at 340nm by the method of Habig et al. (1974), adapted to microplate as described in Booth et al. (2000), using 0.1mL of homogenate and 0.200mL of the reaction mixture (GSH at 10mM and CDNB at 60mM).

The decrease of activity of CAT was determined at 240 nm according to Aebi (1984). The final concentrations of the assay chemicals were: 50 mM phosphate buffer (pH 7.0) and 10 mM hydrogen peroxide (H_2O_2). The activity of SOD was determined at 560 nm according to McCord and Fridovich (1969) adapted to microplate. Tissues were in 50 mM sodium phosphate buffer with 1 mM ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2-EDTA) (pH 7.8) and centrifuged at 15 000g for 15 min at 4 °C. The final concentrations of the assay chemicals were: 50 mM sodium phosphate buffer with 1 mM Na_2-EDTA (pH 7.8), 0.043 mM xanthine, 18.2 l M cytochrome c and 0.3 U mL_1 xanthine oxidase. One unit of SOD was defined as the amount of enzyme to inhibit the rate of reduction of cytochrome c by 50%.

All enzymatic activities were determined in quadruplicate and expressed as units (U) per mg of protein. A U is a nanomol of substrate hydrolyzed per minute for AChE, GST and CAT. Protein concentrations before and after each enzymatic analysis were determined in quadruplicate by the Bradford method (Bradford, 1976) using bovine c-globulin as standard. A Thermo Spectrum Multiskan microplate reader was used for all determinations.

2.4 Pesticide analysis

Organochlorine pesticide (OCP) analysis was carried out on sediment and tissue samples. Cuticles were removed from the abdomens of the 10 shrimps from each site and grouped into a single sample, denoted *pool*. Each sample was then weighed and placed in Petri dishes, dehydrated in an oven at 45°C until dry, then grind. Sediments samples were defrosted and excess of water was removed, dried in an oven at 45°C, then grind and sieved using a 250 μm mesh.

Extraction and purification processes were carried out on the samples before being analyzed using gas chromatography. Extraction was carried out using a dichloromethane-hexane mix (1:1) and a Mars Xpress microwave. Purification was done using column chromatography packed with silica gel, florisil and layer of sodium sulfate. The extract was then eluted with hexane and the fraction obtained was completely evaporated. The resulting evaporated was resuspended with hexane to be analyzed in a Varian 3800 gas chromatograph, equipped with an electron capture detector (ECD) with a nickel source (Ni-63), and an HT8 capillary column (60 m x 0.25 mm; 25 m of film) (SGE Analytical Science, USA).

A certified standard containing 23 compounds was used (SUPELCO 47426-U CLP Organochlorine Pesticide Mix), which were divided into 7 groups according to their chemical characteristics: ΣHCHs (alpha HCH, beta HCH, gamma-lindane HCH, delta HCH), ΣHeptachlors (heptachlor, heptachlor epoxide, ΣDrines (aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone), ΣChlordanes (trans-gamma-chlordane, alpha-cis-chlordane), ΣDDTs (pp-DDD, pp-DDE, pp-DDT), metoxichlor and ΣEndosulfans (endosulfan I, endosulfan II, endosulfan sulfate). Concentrations were expressed in µg/g (micrograms per gram) o ppm (parts per million). One milliliter of a 200 ng/mL pesticide mix (SUPELCO) was added to one of each set of ten samples to calculate the recovery percentage. Pollutants were extracted and processed following the same methods used for the rest of the samples. The limits of detection (LOD) for sediments were between 0.00157 and 0.00363 ppm, and for tissue samples were between 0.00285 and 0.00462 ppm. Percentages of recovery were > 85%.

2.5 Statistical analysis

In order to distinguish between recent and historical sediment pesticide deposition, several ratios were calculated for DDTs, HCHs and endosulfans: pp´DDE+pp´DDD/ΣDDT, where values >0.5 indicate historical DDT deposition; and pp´DDT/pp´DDD+pp´DDE to assess the level of DDT degradation in sediment, where a value of >1 suggests low DDT degradation (Sarkar et al., 2008). The β-HCH/ΣHCH ratio indicates a historical deposition of HCH when >0.5; endosulfan ratios were determined for each metabolite divided by the sum of its metabolites; values close to one were considered recent depositions.

Because the biomarker variables (AChE, GST, CAT and SOD) and OCPs did not meet assumptions of normality and homogeneity of variance, the nonparametric Kruskal-Wallis test was used to compare results between sites and climatic seasons. Principal component analysis (PCA) was used to establish whether an association exists between physicochemical variables and pollutant concentration with the biomarker response. The statistical analyses were carried out using Statistica software (Statsoft Inc., 2005).

3. RESULTS

3.1 Pollutant determination

3.1.1 Tissue and sediment

Organochlorine pesticides were detected in shrimp tissue and sediments. From the mix of 23 pesticide standards, 20 compounds of all the OCP groups were detected from May onwards in both sediment and tissue samples (Table 1). In sediment samples, all of the pesticides were detected only in May for Paraíso and Isla Arena sites, where the highest values were recorded (>100 ppm). In tissue samples, all of the pesticide groups were detected in May, August and September; but only in August for all of the sites. The highest values were detected in August for the Paraíso and Seybaplaya sites (>100 ppm) (Table 1).

The results from the recent and historical pesticide depositions in sediment samples indicate historical depositions of the β-HCH isomer at the three sites, recent depositions and low degradation of DDT at Paraíso and Isla Arena sites, and recent depositions of endosulfans at Paraíso and Isla Arena sites (Table 2). In the Seybaplaya site, endosulfans was not detected, and DDT was only detected in May.

3.1.2 Pesticide concentrations in tissue and sediment with respect to climatic seasons

In the sediment samples, the drines, DDTs and endosulfans had the highest concentrations during the dry season (>200 ppm) in Paraíso and Isla Arena; whereas in the rainy season, concentrations did not reach >2ppm in any of the three sites. In Seybaplaya, only DDT was detected in the dry season, with a value of 1.9 ppm (Fig. 2). In tissue samples, the concentrations of all of the pollutants were higher in the rainy season than in the dry season. In the dry season the highest concentration (83.1 ppm) was for DDTs in Paraíso. The HCH, heptachlor

Table 1. Concentrations of organochlorine pesticides (OCP) in sediment and tissue (µg|g o ppm, dry weight) at three sites off Campeche (P = Paraíso, S = Seybaplaya and I = Isla Arena), from May to September 2015.

	Dry season			Rainy season											
	May			June			July			August			September		
Pesticides	P	S	I	P	S	I	P	S	I	P	S	I	P	S	I
							Sediment								
ΣHCH´s	112.9	*	144.4	0.4	0.3	*	*	0.1	*	*	*	*	*	*	0.3
Σheptachlors	123.5	*	39.5	*	*	0.8	*	0.1	*	*	*	1.5	1.1	*	*
ΣChlordanes	9.82	*	87.9	0.1	*	*	0.1	0.2	0.2	0.1	0.1	0.5	0.1	0.1	*
Σdrines	479.4	*	353.6	0.2	*	*	0.04	0.1	*	*	0.1	0.6	*	*	*
ΣDDT´s	376.5	1.88	369.3	*	*	*	*	*	*	*	*	*	*	*	*
Metoxichlor	109.35		20.03	*	*	0.78	*	*	*	*	*	0.94	1.12	*	*
ΣEndosulfans	222.5	*	216.4	*	*	*	*	*	*	*	*	*	*	*	*
							Tissue								
ΣHCH´s	16.55	4.1	2.5	*	*	0.4	*	*	0.3	176.3	109.2	7.8	8.4	3.7	3
Σheptachlors	18.19	3.6	2	5.5	*	*	*	5.7	*	246.5	262.7	28.8	82.3	30.8	13.5
ΣChlordanes	16.46	2	1.5	0.3	*	*	*	*	*	57.2	29.2	1.5	1.9	1.1	1.2
Σdrines	12.97	16.6	18.2	4	3.1	1.2	*	2.7	1.1	111.8	39.4	0.6	1.9	0.2	0.5
ΣDDT´s	83.00	8.1	1.2	0.3	*	*	0.2	*	*	154.8	49.1	5.3	3.2	*	0.6
Metoxichlor	8.9	*	*	5.05	*	*	*	5.68	*	167	198	22.17	73.53	28.24	11.9
ΣEndosulfans	13.55	3	0.5	0.6	*	0.2	0.9	0.01	0.2	37.1	21.1	0.9	1.1	2.9	1.5

and metoxichlor groups presented the highest concentrations in the rainy season (>150 ppm) at Paraíso and Seybaplaya sites. The lowest concentrations were at Isla Arena site for both seasons (Fig. 2).

Table 2. Historical and recent depositions of pesticides in sediment samples.

Sites	βHCH/ ΣHCH	ppDDD+ppDDE/ ΣDDT	ppDDT/ ppDDD+ppDDE	Endo II/ Σendosulfan
Paraíso	0.91	0.22	3.4	0.5
Seybaplaya	0.8	-	-	-
Isla Arena	0.9	0.2	4	0.6

Abbreviations: ΣHCH, sum of α-HCH, β-HCH, γ-HCH, δ-HCH; ΣDDT, sum of pp DDT, DDE and DDD; ΣENDO, sum of endosulfan I, endosulfan II and endosulfan sulfate.

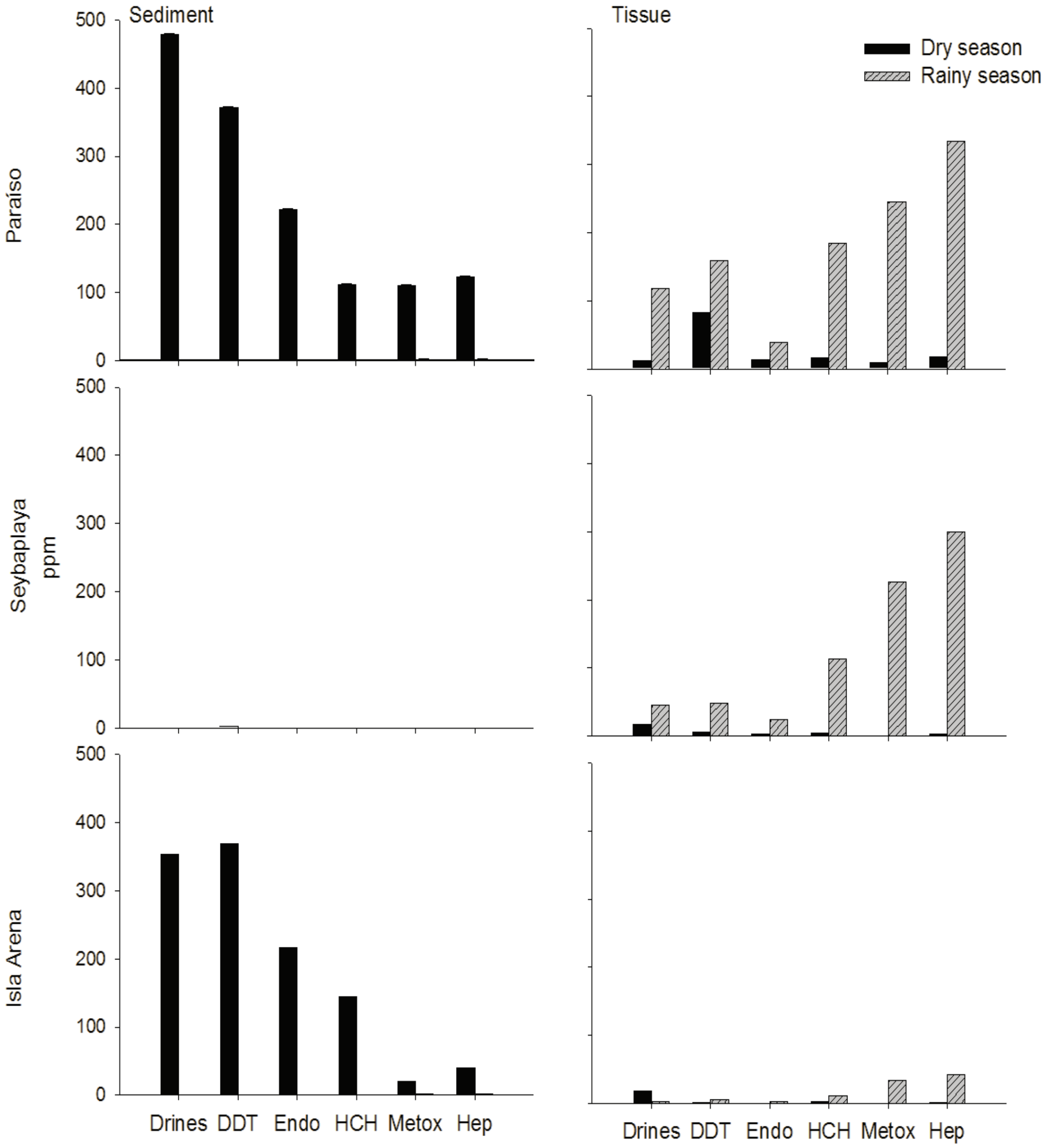

Figure 2. Pesticides concentrations (ug|g o ppm) in sediment and tissues by climatic seasons at the three sampling sites off Campeche. Endo= endosulfans, Metox= metoxichlor and Hep = heptachlor.

3.2 Biomarker determination

The morphometric variables (size and weight) presented significant differences between sample sites, with the smallest shrimp at the Seybaplaya site (Table 3). There was no correlation between biomarker responses (AChE, GST, CAT and SOD) with size and weight of the shrimps (*p*>0.05 for all tests).

Table 3. Morphometric variables (mean ± standard deviation) of shrimps at the three study sites.

	Paraíso	Seybaplaya	Isla Arena	Statistics
Size (mm)	68.3±13.7	60.70±9.3	67.40±8.0	KW:H = 19.8, *p*<0.01
Weight (g)	3.2±1.5	1.8±0.5	2.7±1.0	KW:H = 44.1, *p*<0.01

Significant differences were found between study sites for the enzymes AChE, GST and CAT (AChE: KW-H= 16.9, GST: KW-H= 7.2, CAT: KW-H= 8.2, *p*<0.01 for all tests), and no significant differences were found between study sites for SOD (KW-H= 4.5, *p*= 0.1) (Fig. 3). The AChE enzyme had a 70% inhibition at Isla Arena with respect to the other sites; GST presented a similar behavioral activity in all three sites. A 30% induction of CAT was suggested in Paraíso and Seybaplaya in relation to Isla Arena.

There were differences in enzyme activity between months (Fig. 4). In all three sites, AChE presented an inhibition >80% for all months, except June at Paraíso and Seybaplaya. GST presented a significant 50% induction of activity during July-September in all study sites in relation to the other months (KW-H= 108.4, *p*<0.001). CAT presented a significant >40% induction of its activity in January at Paraíso, and a significant 50% induction in August at all three sites, in relation to the studied months (KW-H= 96.5, *p*<0.001). SOD presented a significant induction activity of 15% in January at Paraíso, and 15% in February at Seybaplaya (KW-H= 70.1, *p*<0.001), while Isla Arena presented >70% induction in July in relation to the other months.

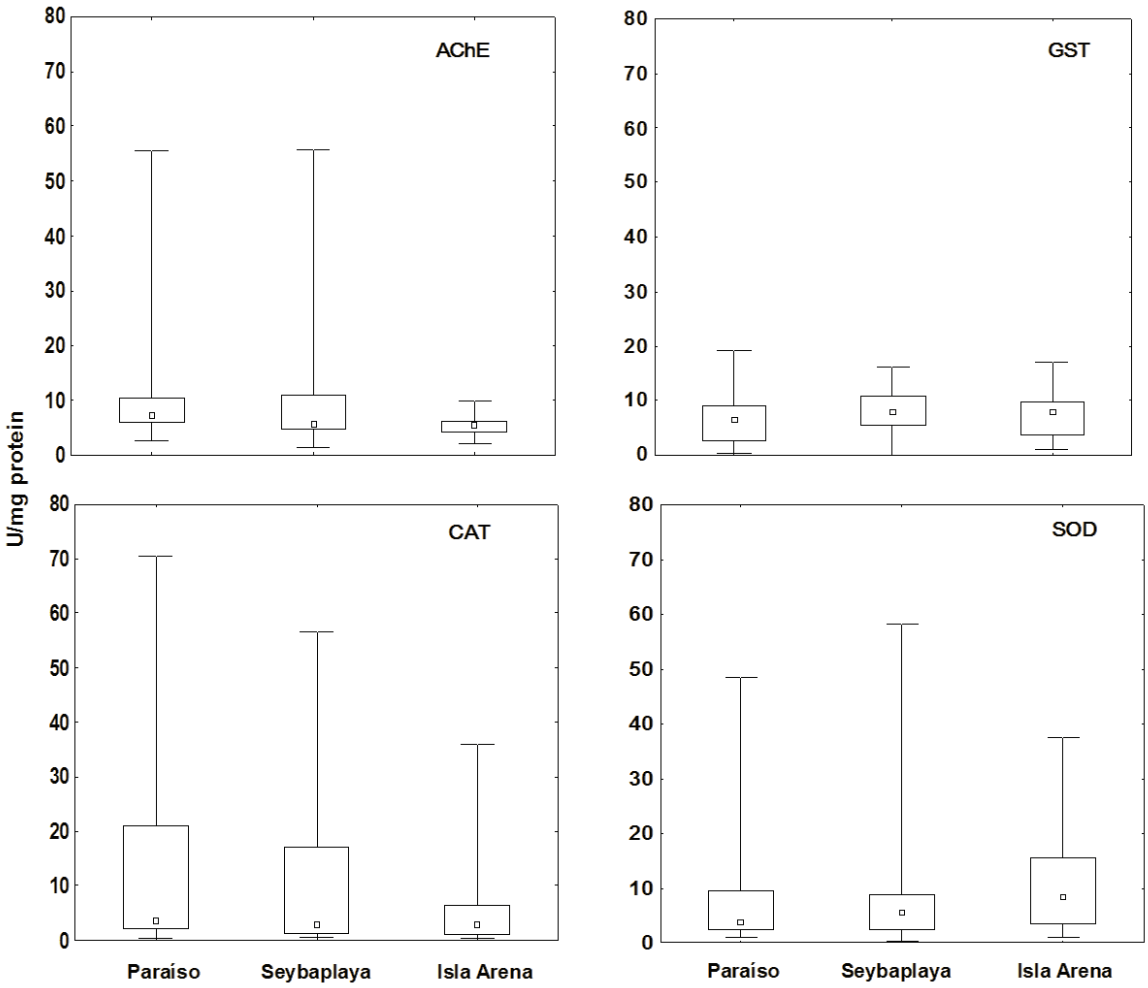

Figure 3. Enzymatic activity (AChE, GST, CAT and SOD) in pink shrimp tissues at three sampling sites off Campeche. Median; box: 25%-75%; range: min-max.

3.3 Relationship between physiochemical parameters and enzyme response

No associations were found between physiochemical parameters and the biomarker response in either climatic seasons (Fig. 5). Water temperature at the Paraíso and Seybaplaya sites had similar seasonal fluctuations. Salinity was higher during the dry season (January-May) than in the rainy season (June-September) in the Paraíso and Seybaplaya sites. Values of dissolved oxygen (DO) remained relatively constant at Paraíso and Seybaplaya sites. The pH showed constant values in the study sites, with generally alkaline values during the entire sampling season (Table 4).

Table 4. Physicochemical variables (mean ± standard deviation) by season (dry and rainy) at the three sampling sites off Campeche.

	Paraíso		Seybaplaya		Isla Arena	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
Temperatura °C	26.9±2.8	30±0.9	27.6±2.5	30±01.1	29.2±1.0	29.0±2.0
*DO mg/L	7.3 ±3.3	7.2±3.0	7.3 ±1.7	6.9±01.1	9.1±0.8	8.3±0.8
Salinity ppm	32.2±2.1	25.2±1.3	34.3±1.1	26.2±12.1	36.6±0.5	36.5 ±1.9
pH	8.1 ±0.1	7.8 ± 0.4	8.8±0.3	8.7±0.8	8.2±0.5	8.3±0.8

*DO = dissolved oxygen

3.4 Relationship between pollutant concentration and biomarker response

No associations between biomarker response and pesticide concentrations were found in the dry season (Fig. 6a). However, in the rainy season associations were found in tissue samples between AChE and the HCH pesticide, and between the catalase (CAT) enzyme and the ∑Heptachlors, ∑Chlordanes, ∑Drines, ∑DDTs and ∑Endosulfans pesticides (Fig. 6b). Using the associations suggested by the PCA, non-parametric correlation tests were carried out. All tests showed significant correlations (AChE with ∑HCH, r= -0.52; CAT with ∑heptachlor, r= 0.45, ∑Chlordanes r= 0.54, ∑Drines r= 0.39, ∑DDT r= 0.72, ∑Endosulfans r= 0.47; p<0.05 for all tests).

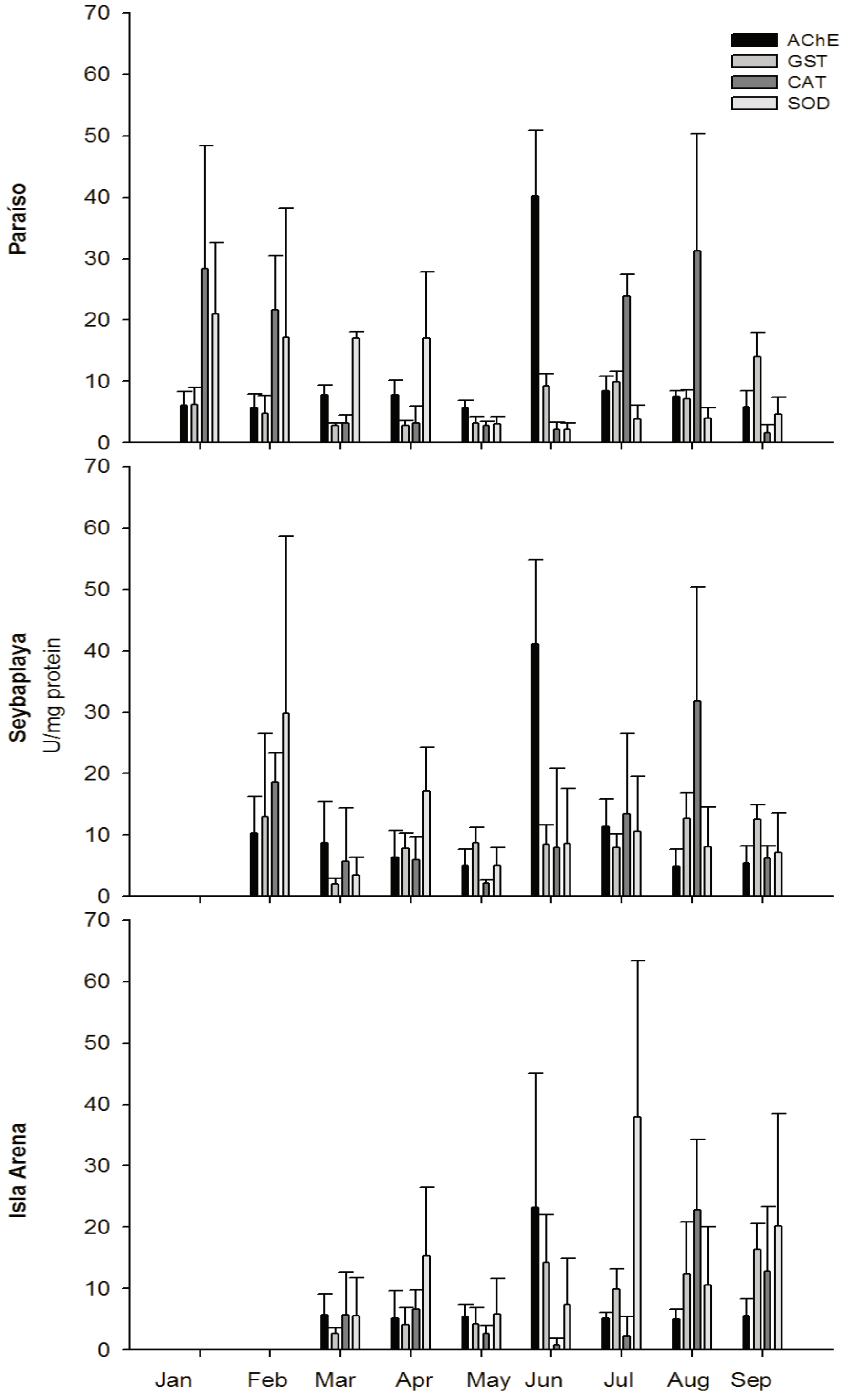

Figure 4. Enzymatic activity (mean±SD) by months (AChE, GST, CAT and SOD) in pink shrimp tissues at three sampling sites off Campeche.

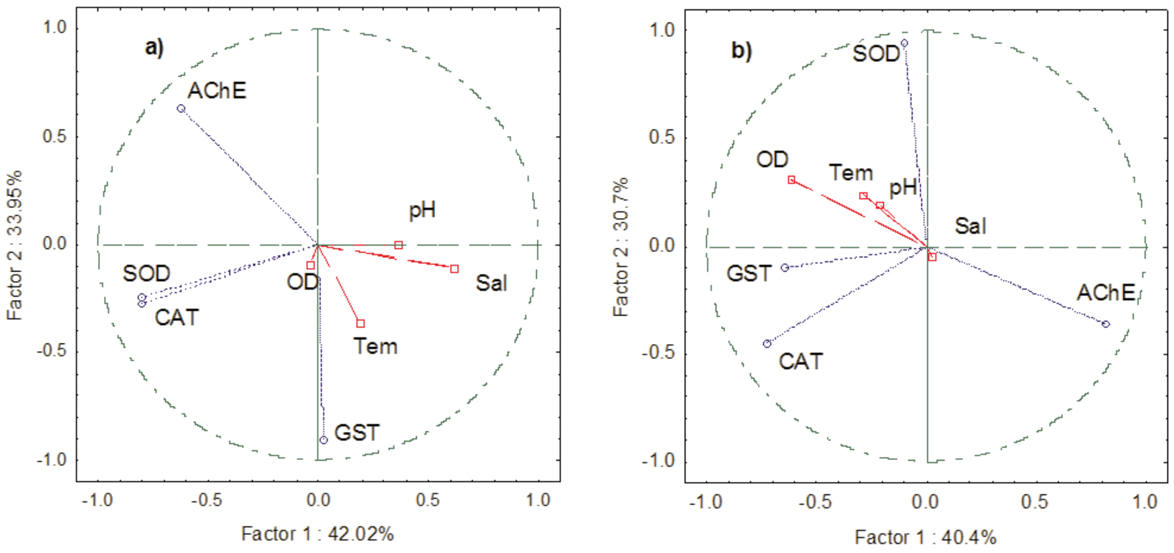

Figure 5. PCA for physicochemical variables and biomarkers by season, a) dry and b) rainy.

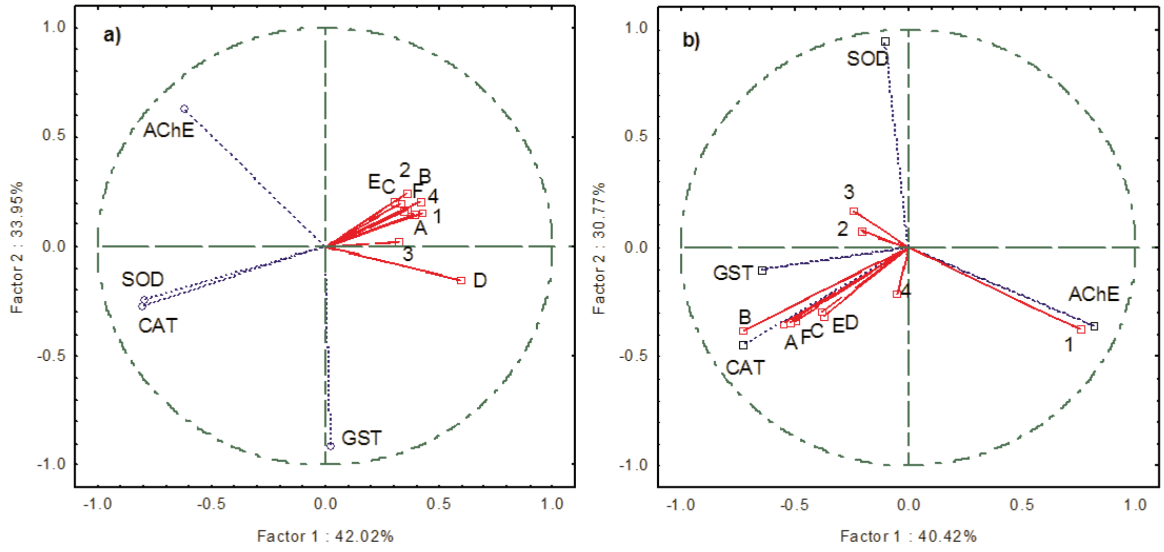

Figure 6. PCA for pesticides and biomarkers by season, a) dry and b) rainy. Pesticides: 1=HCH, 2=heptachlor, 3=chlordanes, 4=drines, 5=DDT and 6=endosulfans. Letters A-F are tissue samples (A=HCH, B=heptachlor, C=chlordanes, D=drines, E=DDT, F=endosulfans).

4. DISCUSSION

Pesticide concentrations in sediment and shrimp tissue in the present study were 100 times higher than in other studies along the coast of Campeche (Gold et al., 1993, 1995; Rendón-von and Memije, 2001; Carvalho et al., 2009). In this study, all sediment pesticide concentrations exceeded the marine/estuarine sediment quality guidelines (Burton, 2002), being above the probable effects level (PEL, 0.05 ppm); while DDT and endrin were over the severe effect level (>1.3 and 0.2 ppm, respectively). With the exception of ΣDDT, the present study detected concentrations 5 times higher in shrimp than those registered by Hinojosa-Garro et al. (2016) in fish from two lagoons of the Champotón municipality, and 60 times higher than those reported from sediment samples in southern Gulf of Mexico by other authors (Table 5).

Sediment pesticide concentrations were 100 times higher in the dry season than in the rainy season. The highest levels of pesticides were found at Paraíso and Isla Arena sites. The Paraíso site has organic matter input from the Champotón River and the Isla Arena site from the mangrove system. Burton (2002) mentions that pesticides adhered to organic matter and remain in the sediment for long periods; which could partially explain the high concentrations at Paraíso and Isla Arena. In the rainy season the surf could have controlled the resuspension, mixing and transport processes of the sediments (Lu et al. 2015) and caused a reduction of pesticide concentrations (<2 ppm). Also, changes of wind direction between seasons could disperse pollutants as suggested by Guzzella et al. (2015).

Precipitation of 35-50 mm in April caused by cold fronts (CONAGUA, 2015) could have caused the run-off of pollutants and their posterior detection in May. Moreno-González et al. (2013) mention that sporadic rains facilitate sediment transportation from agricultural to coastal zones, and increase the presence of pollutants. The presence of pesticides in January-April off Campeche is not dismissed, but they are below detectable levels.

Regarding to recent or historical pesticide depositions in sediment, the ratio of β-HCH/ΣHCH was >0.8, indicating a historical pesticide depositions; β-HCH has a low vapor pressure that causes low volatility with respect to the other isomers of HCH. The high vapor pressure of α and γ-HCH causes the opposite reaction, explaining their low concentrations in sediment compared to β-HCH and δ-HCH. The ratio of DDE+DDD/ΣDDT was <0.5 indicating recent depositions; however, the DDT/ppDDD+ppDDE correlation was >1 for the Paraíso and Isla Arena sites, suggesting low degradation of DDT, in agreement with Sarkar et al. (2008); which potentially increase its effects on the marine biota.

Table 5. POC concentrations (dry weight ppm) in sediment and aquatic organisms in southeastern Mexico.

Sampling site	Species or sediment	ΣHCH	ΣHepta	ΣClord	ΣDrines	ΣDDT	ΣEndo	Reference
Mocú lagoon	*Cichlasoma salvini*	1.16	7.3	6.2	2.5	142	1.6	1
	Thorichthys meeki	0.2	23.5	3.3	2.8	68.8	3.4	
Xnohá lagoon	*Orechromis nilóticus*	4.02	13.1	27.8	13.9	435.2		1
	Cichlasoma salvini	3.22	79	7.7	5.6	177.8	3.3	
	Thorichthys meeki			19.4	8.9	144.3	1.7	
Sabancuy lagoon	Sediment					0.0039	0.00196	2
Champotón River	Sediment	<1	0.1		1.5	0.4		3
Mangrove system	Sediment	1	0.25		0.2	0.3		3
Coatzacoalcos River	Sediment	0.16				0.24		4
Off Campeche	Sediment	0.003	<0.01		<0.01	<0.01		5

References= 1) Hinojosa-Garro et al. (2016), 2) Ramírez-Elías et al. (2016), 3) González-Jáuregui et al. (2014), 4) Espinosa-Reyes et al. (2013), 5) Rendón-von et al. (2005).

Pesticide concentrations in tissue samples were lower in the dry season than in the rainy season, when high concentrations (>100 ppm) of the heptachlor, HCH and DDT groups were detected. This could be due to their physiochemical properties, as DDT and heptachlors have high octanol/water partition coefficient (logK_{ow} >5.5) in comparison to the other OCPs, causing their high lipophilicity (Shen and Wania, 2005); while HCH has a logK_{ow} between 3.9-4.1, causing higher water solubility in comparison to heptachlor and DDT (Willett et al., 1998). Metoxichlor has a log K_{ow} of about 4.5 (Finizio et al. 1997), however, their behavior and effect are low understood. Endosulfan has a K_{ow} between 3.6-3.8, similar to HCH, and its occurrence in tissue samples was >37% in the three sites, which could be due to its bioavailability. Osuna-Flores and Riva (2002) also found high concentrations of endosulfan and heptachlor in sediments and shrimps, which was correlated with the benthic habits of the shrimp and their diet.

It seems that the molt process play a role in the bioaccumulation of pesticides. Juvenile crabs (*Cancer magister*) exposed to metoxichlor were more sensitive during the molt process causing a 38% of mortality (Amstrong et al., 1976). Furthermore, Cripe (1994) demonstrated that postlarval specimens of *F. duorarum* were more sensitive to toxins after molting, causing their death even up to 72 hrs after exposure to OF, pyrethroids and heavy metals. In this study, sampling took place during the new moon period, when shrimps could have been in post-molt process, detected by the soft texture of the specimens. Other studies indicate that specimens at specific stages of molting, such as pre-molt and post-molt, do not feed and are characterized by water absorption activity (Fernández et al., 1997; Lipcius and Herrnkind, 1982). Considering this characteristic, along with the bioavailability of pollutants and their desorption from the sediments, it is possible to explain the reason for the bioconcentration of pesticides in shrimp.

Due to the persistence of pesticides, residual compound limits which do not represent risk to human health (maximum residue limits, MRL; acceptable daily intake, ADI), have been established by the Food and Drug Administration (FDA) and the CODEX Alimentarius (Table 6). According to the criteria established by the FDA and the CODEX Alimentarius of the United Nations Food and Agriculture Organization (FAO), the pesticides found in the tissue of the pink shrimp in this study (aldrin, chlordane, heptachlor and lindane) exceeded more than 30 times the limit recommended for human consumption, and the pesticide DDT exceeded more than 10 times the limit. Moreover, the Official Mexican Standard NOM-029-SSA1-1993 (DOF, 1995) specifies that crustaceans must be free of these pollutants. Based on the aforementioned, the consumption of juvenile pink shrimp could damage human health.

Table 6. Maximum residue limits (MRL ppm) of pesticides in fish and meat established by the FDA and CODEX.

Pesticide	FDA (Fish)	CODEX (Meat)*
Aldrin/Dieldrin	0.3	0.2
ΣChlordanes	0.3	0.05
ΣHeptachlor	0.3	0.2
ΣDDT/DDE	5.0	5.0
Lindano (γ-HCH)	0.1	2

*non-marine animals.

Mexican regulation prohibits the use of drines and chlordanes; the heptachlor group is not registered in the country (meaning that is an un-authorized pesticide in Mexico), HCH and DDT are restricted to the Secretary of Health, and endosulfan is authorized for agricultural use (COFEPRIS, 2010). Of those pesticides, HCH, heptachlors, drines, chlordanes, endosulfans and DDT have been internationally prohibited since the Stockholm convention due to their adverse effects to human health (PNUMA, 2001). This study confirms the presence of those pesticides in the Campeche coast which are being used illegally.

On the other hand, the results of this study showed that shrimp from the three sites presented an inhibition of AChE activity (> 80%). This indicates a possible discharge of OP pesticides or carbamates to the Campeche coast. In this region, chlorpyrifos residues have been detected in water samples of rivers flowing into the Términos Lagoon, Campeche (Carvalho et al., 2009). Kumar et al. (2010) mention that shrimp within the influence of agricultural zones can be exposed to concentrations of OP high enough to cause effects at the population or community level. Among the effects to the exposure of aquatic organisms to OP pesticides are the hyperactivity, spasm and abnormal swimming (Bonansea et al. 2016).

However, other pollutants such as heavy metals and antibiotics have been shown to have anticholinesterase properties (Richetti et al., 2011). In the Champotón river, central coast of Campeche, mercury (Hg) in sediments surpassed the 0.7 ppm PEL level by 40 times in the rainy season, while Cd and Hg in water samples were at the 0.01 ppm limit set by the NOM-001-ECOL-1996 (Rendón-von et al., 2010). Another study along the Champotón coast (SEMAR, 2015) quantified heavy metals in sediment. During the dry season cadmium just passed the 4 ppm PEL level with a concentration of 4.9 ppm; during the rainy season copper (Cu) and nickel (Ni) passed the 15 ppm threshold effect level (TEL) with 32 and 22 ppm, respectively. Because the metals are not chemically or biologically degradable, they accumulate in sediments and enter into the food chain, which represents a risk for long-term human health (Mohanty and Samanta, 2016). Kumar et al. (2010) indicate that an inhibition of AChE >20% is considered pesticide exposure and >40% causes detrimental effects to organisms. The 80% reduction of AChE activity seen in this study could be related to the presence of OP pesticides and carbamates, or a combination of these along with the presence of heavy metals, which could have physiological and behavioral repercussions in the population of *F. duorarum.*

Among the mechanisms of antioxidant enzymes, the first line of defense is initiated by SOD (Vijayavel and Balasubramanian, 2009). In this study SOD activity increased significantly by 15% in the months of January-February in Paraíso and Seybaplaya, coinciding with the significant increase in CAT activity (>40%) in the same months and sites. The SOD enzyme presented lower activity values (<10 U/mg-protein) during July and August at Paraíso and Seybaplaya in comparison to the Isla Arena site (10-35 U/mg-protein). Modesto and Martínez (2010) mention that an excess of H_2O_2 in tissue inhibits SOD activity; however, in this study was not possible to establish the causes for the low values of SOD activity.

The CAT enzyme showed a significant induction of 50% in August compared to March-June. In situations of oxidative stress, CAT activity increases due to the imbalance of reactive oxygen species (ROS), and is considered an early indicator of oxidative stress because of its response to the bioaccumulation of pollutants (Van-der Oost, 2003, Wang et al., 2014). In this study, CAT activity was significantly correlated with heptachlor, chlordane, drine, DDT and endosulfan pesticides; this could indicate that the shrimp are in a constant process of xenobiotic compound detoxification.

The GST enzyme showed a significant 50% induction in July-September compared to March and April. This agrees with the study by Tec (2005), which quantified GST in *F. duorarum* off the Campeche coast and reported an increase in GST activity during August, which was attributed to precipitation. Increased activity in GST has been associated with OCPs, heavy metals, antibiotics and polycyclic aromatic hydrocarbons (PAH) in aquatic organisms (He et al., 2012). Tu et al. (2010) reported high levels of GST that suggested the activation of detoxification processes, and found that lipid peroxidation, glutathione peroxidase, GST, AChE and CAT biomarkers were correlated with shrimp contaminated by endosulfan.

The increased activity seen in the GST and CAT enzymes during this study could be due to the pollutants that also inhibited AChE in July-September, and coincides with the rainy season when pesticides are transported by run-off. The study by Benali et al. (2015) associated the increase of CAT and GST in clams with the strong hydrodynamics of the region, which transport persistent organic pollutants and oils. In this study, the reported seasonal variations in antioxidant defense of GST and CAT also coincide with the spring-summer agricultural calendar, specifically the planting of cane, rice, corn and bean. More studies are needed to identify the bioaccumulation of pesticides and other pollutants in shrimps (including adult stages) and their biochemical responses.

The results from this study are relevant to coastal resource management. The conservation plan from the Eco-region of Peténes-Celestún-Palmar (northern Campeche coast) promotes the characterization of agrochemicals and the assessment of their effects to the environment (Acosta-Lugo et al., 2010). Also, this study contributes to the pink shrimp Fishery Management Plan, which establishes the need to carry out studies on the nursery areas (DOF, 2014). The shrimp exposure to pesticides and heavy metals may cause oxidative stress and probable irreversible damage, causing reduction of juveniles and affect their recruitment to the fishing stock. These results should be considered in future stock assessments for the pink shrimp.

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CAPÍTULO III

CONCLUSIONES Y RECOMENDACIONES

CONCLUSIONES

Se encontraron plaguicidas organoclorados en sedimentos y camarones como posible resultado de las actividades agropecuarias de la región así como del uso del DDT para el control de organismos vectores de enfermedades, estos residuos de plaguicidas pudieron ser transportados por las escorrentías hacia la zona costera y depositarse en el sedimento.

Las concentraciones de los plaguicidas en sedimento se encuentran por arriba del nivel de efecto probable PEL (0.05 ppm); y en particular, el DDT y el endrín superaron los valores de efecto severo TEL >1.3 y 0.1 ppm, respectivamente.

Se presentaron diferencias entre las concentraciones de plaguicidas en sedimento y tejido con respecto a las épocas climáticas. Las concentraciones de plaguicidas encontradas en sedimento fueron 70 veces más altas en época de secas que en lluvias, y puede relacionarse al arrastre de contaminantes ocasionado por las lluvias esporádicas en el mes de abril y porque ambos sitios reciben aporte de materia orgánica, la que aporta el río Champotón y la generada por el manglar en la localidad de Isla Arena; en la época de lluvias el oleaje pudo ocasionar que los plaguicidas se hayan desorbido de los sedimentos y dispersado a otros sitios.

Los residuos de plaguicidas aldrín, clordanos, heptacloros y lindano, detectados en muestras de tejido de camarón, rebasaron más de 30 veces los límites recomendados para consumo humano por la FDA y el CODEX alimentario; en particular el plaguicida DDT rebasó más de 10 veces éste límite. Además, la Norma Oficial Mexicana NOM-029-SSA1-1993 (DOF, 1995) especifica que los crustáceos deben estar exentos de estos contaminantes.

El desbalance en los valores de actividad de las enzimas de detoxificación (SOD, CAT y SOD) y la enzima de daño neurotóxico (AChE) en el tejido de camarón rosado se pudieron relacionar a la presencia de plaguicidas en la época de lluvias.

Los resultados mostraron que los camarones capturados de los tres sitios presentaron inhibición de la actividad de la AChE > al 80%, lo que se adjudica a que los plaguicidas organofosforados y carbámicos están siendo utilizados en las prácticas agropecuarias, o puede ser una sinergia entre estos contaminantes y los metales pesados, que también son anticolinesterásicos y que están presentes en la costa de Champotón.

El incremento de actividad en las enzimas GST y CAT puede deberse a los contaminantes que inhibieron la AChE de julio-septiembre y coincide con la temporada de lluvias, donde los plaguicidas son transportados por la escorrentía. Las variaciones estacionales de la defensa antioxidante referidas a GST y CAT también concuerdan con el calendario agrícola primavera-verano específicamente con la siembra de caña, arroz, maíz y frijol.

Los resultados muestran que utilizar una batería o conjunto de biomarcadores en el organismo, permite una visión más completa del efecto fisiológico de un contaminante dentro del organismo. La enzima acetilcolinesterasa AChE, mostró ser una buena herramienta para la evaluación de los efectos tóxicos causados por los plaguicidas organofosforados y carbámicos.

Los camarones al continuar expuestos a mayores cantidades de plaguicidas o metales pesados puede ocasionar estrés oxidativo y por ende daño que podría ser irreversible, teniendo consecuencias en la disminución de juveniles y como consecuencia afectar el reclutamiento al stock pesquero. Lo anterior debe tomarse en cuenta en las futuras evaluaciones de la especie.

RECOMENDACIONES

Para complementar estos resultados sería importante realizar estudios en condiciones controladas de laboratorio con *F. duorarum*, exponiéndolo a diferentes contaminantes, para precisar diferencias entre los biomarcadores y así eliminar la influencia de variables como la alimentación y procesos de muda en los camarones.

De acuerdo a las características de la región, es necesario implementar un programa de monitoreo ambiental para la cuantificación de plaguicidas organoclorados, organofosforados, metales pesados e hidrocarburos aromáticos policíclicos en la costa de Campeche, teniendo en cuenta realizar muestreos en las diferentes épocas del año y tratando de establecer la existencia de picos en la aplicación de estos compuestos, el monitoreo debería incluir más sitios de muestreo y una colaboración transversal entre las instituciones académicas y de gobierno.

Los monitoreos ambientales deben incluir biomarcadores de detoxificación fase I y fase II, indicadores de daño oxidativo, así como otros biomarcadores como la vitelogenina que puede ser usada para evaluar el efecto causado por ciertos compuestos estrogénicos, recordando que el uso de biomarcadores permite priorizar sitios que necesiten vigilancia e intervención debido a que, además de ser económicos, son la alerta temprana de contaminación.

Por último, aunque es una tarea difícil de cumplir, se debe ejecutar lo mencionado en las normas oficiales mexicanas, los productos de la pesca deben estar exentos de plaguicidas: crustáceos frescos-refrigerados y congelados (NOM-029-SSA1-1993), pescados frescos-refrigerados y congelados NOM-027-SSA1-1993, moluscos bivalvos frescos-refrigerados y congelados (NOM-031-SSA1-1993). Y se debe llevar a ley el proyecto de norma oficial mexicana PROY-NOM-000-SAG-FITO/SSA1-2013 que establecerá los límites máximos de residuos (LMR).

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