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ÁRBITROS

Dr. Roberto Rico Martínez
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Instituto Politécnico Nacional

Manuel Mendoza Carranza

Dr. Ernesto Velázquez-Velázquez
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Instituto de Ciencias Biológicas
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Dept. Hidrobiología

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ÍNDICE AL VOLUMEN 33, NÚMERO 3 • 2023

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Efecto agudo del carbofuran en juveniles de *Chirostoma humboldtianum* (Valenncienes 1835) (Atheriniformes: Atherinopsidae)

Acute effect of carbofuran in *Chirostoma humboldtianum* juveniles (Atheriniformes: Atherinopsidae)

Octavio Abeja-Pineda[1], Gerardo Figueroa-Lucero[1], María Cecilia Hernández-Rubio[2] y Liliana Favari Perozzi[3]

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[1] Planta Experimental de Producción Acuícola, Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco 186, Col. Vicentina. Ciudad de México, 09340. México.

[2] Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n. Col. Santo Tomás. Ciudad de México, 11340. México.

[3] Departamento de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Av.Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Ciudad de México, 07360. México

***Corresponding author:**
Gerardo Figueroa-Lucero: e-mail: gfiguer@xanum.uam.mx

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RESUMEN

Antecedentes: Las poblaciones de Chirostoma han disminuido por pérdida de su hábitat, la introducción de especies exóticas y la contaminación. Chirostoma humboldtianum (Valenciennes, 1835) se registró en la cuenca de México, por última vez, en 1957. El carbofurán es un insecticida carbámico, sistémico de amplio espectro, por lo que su uso se ha prohibido en muchos países. Sin embargo, en México se utiliza ampliamente y sus efectos no se han evaluado en peces endémicos. **Objetivo:** Evaluar el efecto agudo del Carbofuran® en juveniles de *Chirostoma humboldtianum* a través de biomarcadores de neurotoxicidad y de estrés oxidativo en el cerebro, el hígado, las branquias y los músculos. **Método:** Se evaluó el efecto del carbofuran (0.0, 0.025, 0.05, 0.1, 0.2, 0.4 mg/L, n = 10 y tres réplicas) en un ensayo estático, sin recambio de agua durante 96 h, en juveniles de siete meses de edad. Los peces se obtuvieron por fertilización *in vitro,* de reproductores mantenidos en cautiverio, certificados morfológica y genéticamente. **Resultados:** La CL50 fue de 0.077 mg/L^{-1} (0.028 – 0.118 mg/L $^{-1}$, $\alpha = 0.05$) a las 96 h de exposición. La actividad de la acetilcolinesterasa (AchE) se inhibe en el cerebro y en el hígado. El nivel de lipoperoxidación (LPO) fue significativamente mayor en las branquias que en el hígado y los músculos ($p < 0.05$). Las enzimas superóxido dismutasa (SOD) y glutatión peroxidasa (GPx) presentan un patrón similar de activación en las branquias y el hígado. **Conclusiones:** El carbofuran causa daño neurotóxico y oxidativo, en juveniles de *C. humboldtianum* en concentraciones menores a las registradas en otras especies de peces.

Palabras clave: Carbofuran, estrés oxidativo, lipoperoxidación, neurotoxicidad.

ABSTRACT

Background: *Chirostoma* populations have diminished because of loss of their habitat, introduction of exotic species and pollution. *Chirostoma humboldtianum* (Valenciennes, 1835) was recorded in Mexico basin, for last time in 1957. Carbofuran is a carbamic inseticide, broad spectrum systemic; its use is prohibited in many countries. However, in Mexico, it is widely used and its effects has not been evaluated in endemic fish. **Goals**: Evaluate acute effect of Carbofuran® in juveniles of Chirostoma humboldtianum (Valenciennes, 1835) through neurotoxicity biomarkersf and oxidative stress on brain, liver, gills and muscles. **Methods**: Effect Carbofuran (0.0, 0.025, 0.05, 0.1, 0.2, 0.4 mg/L) was evaluated in a static essay during 96 h without renewal water on seven-months old juveniles. Fish were obtained by *in vitro* fertilization, from breeders in captivity, morphologically and genetically certified. **Results**: CL_{50} was 0.077 mg/L at 96 h (0.028 – 0.118 mg/$_L$ $^{-1}$, $\alpha = 0.05$). Acetylcholinesterase activity (AChE) is inhibited in gills and liver. Lipoperoxidation level (LPO) was significantly higher in gills than liver and muscles ($p < 0.05$). Superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes present a similar pattern in gills and liver. **Conclusions**: carbofuran causes neurotoxic and oxidative damage even at lower concentrations than those reported in other fish species.

Keywords: Carbofuran, lipoperoxidation, neurotoxicity, stress oxidative.

INTRODUCCIÓN

Uno de los grupos de contaminantes de mayor incidencia en los sistemas acuáticos y terrestres, lo constituyen los plaguicidas. Durante las últimas décadas, su uso se ha incrementado en todo el mundo, principalmente en países en desarrollo, con el fin de proteger los cultivos y al ser humano, de plagas y enfermedades (Lal *et al*., 2013). Aunque algunos de ellos presentan una toxicidad selectiva en los sistemas terrestres, su uso generalizado ocasiona graves efectos nocivos en otras especies no blanco, incluyendo daño genético (Lal *et al*., 2013; Nikoloff *et al*., 2012).

Los sistemas acuáticos son uno de los reservorios finales de los plaguicidas, los que, por su composición química, concentración y sus metabolitos, pueden ocasionar efectos adversos sobre las respuestas fisiológicas, retraso en el crecimiento, reproducción o incrementar la mortalidad según el período de desarrollo de los animales (Dobšíková 2003; Ghazala *et al*., 2014). Los efectos sinérgicos o las consecuencias de las interacciones entre dos o más contaminantes en el mismo organismo acuático son todavía pobremente entendidas (Pelletier 1986; Arcagni *et al.,* 2017; Amoatey *et al*., 2019).

Las respuestas a los contaminantes en los organismos se han evaluado comúnmente a través de la mortalidad acumulada, medida como $CL_{50.}$ En décadas recientes, se ha incorporado el uso de biomarcadores, como una alternativa útil para estimar los efectos de las sustancias tóxicas sobre los peces. Los biomarcadores son indicadores sensibles para detectar o diagnosticar efectos subletales, que demuestran que las sustancias tóxicas han ingresado a un organismo y se han distribuido en sus tejidos, produciendo daños al ADN, al sistema endócrino, cambios en la función reproductiva, en la disminución del crecimiento, que se reflejan en la sobrevivencia (Livingstone, 2003; Van der Oost *et al*., 2003; Young *et al*., 2005).

El Carbofuran (2,3-dihidro-2,2-dimetil-7benzofuranol N-metil carbamato) es un insecticida carbámico sistémico de amplio espectro; se utiliza comúnmente para eliminar plagas agrícolas, presenta una toxicidad selectiva sobre insectos, ácaros y nemátodos por contacto e ingestión (Ensibi *et al.,* 2012; Barbieri *et al*., 2017). Sin embargo, su uso generalizado ocasiona graves efectos, que incluye daño genético en otras especies no objetivo, como peces e invertebrados acuáticos, abejas, aves y mamíferos (Collective SPA, 2002; Clasen *et al*., 2014; Moreira *et al.,* 2015). Debido a que es uno de los insecticidas con mayores implicaciones negativas globales sobre la vida silvestre, ha sido prohibido por la USFDA en los Estados Unidos de América y Europa (Gupta 2009; USFDA 2014), pero se utiliza ampliamente en México.

El carbofuran es altamente tóxico para los peces, diversos estudios con diferentes periodos de exposición han reportado CL50 menores a 1 mg/L (Trotter *et al*., 1991). De acuerdo con la EPA (2002), *Oncorhynchus mikiss* y *Cyprinos carpio* presentan CL50 entre 0.8-0.9 mg/L. Por otro lado, se han obtenido CL50 de 0.2245 mg/L en *Poecilia reticulata* después de 96 h de exposición. En *Carassius auratus* no se observaron muertes después de 48 h con 0.5 mg/L de carbofuran. Sin embargo, esta concentración representa la CL50 a 96 de exposición para *Cyprinus carpio* (Bretaud *et al*., 2000; Dobšíková 2003). Esto indica que la tolerancia al carbofuran es variable y específica.

En México, el carbofuran se utiliza ampliamente en los cultivos agrícolas y representa un riesgo de contaminación para la biota acuática. El efecto más importante ocasionado por los plaguicidas carbámicos sobre los peces, es el daño en el sistema nervioso, éste se debe principalmente a la inhibición de la enzima acetilcolinesterasa (AChE) (Casida & Quistad, 2004), la cual sobre estimula la actividad neurológica de los peces (Iannacone *et al*., 2011). Por otro lado, en las sinapsis nerviosas y uniones neuromusculares, la inhibición de la enzima AChE, causa una hiper-activación de los receptores de acetilcolina (muscarínico y nicotínico) y puede causar la muerte debido a la tetanización del músculo, además de insuficiencia respiratoria (Ray & Ghosh, 2006). La intoxicación aguda en virtud de la inhibición reversible (carbamilación) de la AChE es una de las alteraciones mayormente reportada para el carbofuran sobre los peces (Casida 1963; O›Brien, 1967).

La peroxidación lipídica es otra forma de intoxicación por los radicales libres; en este sentido, se han estudiado algunos insecticidas y herbicidas como inductores de estrés oxidativo, que originan efectos negativos sobre los lípidos, las proteínas de las membranas celulares, inhiben la actividad y la integridad estructural de las enzimas antioxidantes, comprometiendo la eficacia del sistema inmune de los peces (Lushchack, 2011; Ranjbar, 2014). Sobresalen por sus efectos, la superóxido dismutasa (SOD), glutatión peroxidasa (GPx) y catalasa (CAT) por lo que han sido las más estudiadas; entre las no enzimáticas, el ácido ascórbico (vitamina C), el α-tocoferol (vitamina E), el glutatión (GSH), los β-caroteno (vitamina A) y los flavonoides (Ochoa & González, 2008).

El género *Chirostoma* Swainson, 1839 es endémico de México, pertenece a la familia Atherinopsidae y conforma un conjunto de especies con un origen monofilético, que se originó en un tiempo muy corto, en un área geográfica relativamente pequeña, cuya diversificación y especiación se remonta a 0.520 millones de años en la Mesa Central de México (Echelle & Echelle, 1984; Bloom *et al*., 2013).

Chirostoma humboldtianum (Valenciennes, 1835) es la primera especie íctica descrita por la ciencia en México. Es una de las especies de peces blancos que conserva el patrón de distribución más antiguo del género *Chirostoma* y se considera la especie basal de los peces blancos (Barbour 1973; Paulo-Maya *et al*., 2000; Bloom *et al*., 2013; Campanella *et al*., 2015).

Esta especie es de hábitats lacustres y se distribuye actualmente, de manera discontinua en sistemas lénticos, desde el Estado de México hasta Nayarit, a lo largo del sistema Lerma-Santiago. Esta región se caracteriza por un alto grado de desarrollo agrícola, urbanización e industrialización. Estos procesos afectan en diferente grado, la calidad del aire, del agua, del suelo y a las comunidades naturales que habitan en el mismo (Hernández-Rubio *et al*., 2006). Particularmente la cuenca del Sistema Lerma Santiago, se ha considerado una de las más contaminadas de México y sus efectos no sólo se restringen a las poblaciones de peces, sino que incluyen a las comunidades humanas que habitan las zonas ribereñas, entre otros (Priego *et al*., 2004).

En algunas especies del género, como *Chirostoma jordani* Woolman 1894, se han registrado cambios en la actividad enzimática (Dzul-Caamal *et al*., 2014), así como la inhibición de la acetilcolinesterasa por insecticidas organofosforados y la toxicidad del cloruro de cadmio en embriones de *C. humboldtianum* (Altamirano-Lozano *et al*., 2005).

El presente trabajo aborda el análisis del efecto del Carbofuran en juveniles de *C. humboldtianum*, a través de la mortalidad (CL50), de biomarcadores de neurotoxicidad y de estrés oxidativo de exposición directa, sobre el cerebro, el hígado, las branquias y los músculos. Así

mismo, se espera lograr conocimientos más amplios sobre el efecto de los insecticidas carbámicos sobre otras especies de peces, tomando como base a *C. humboldtianum,* especie con gran importancia, tanto ecológica como económica en México.

MATERIAL Y MÉTODOS

Tóxico. Se evaluó el efecto de cinco concentraciones (0.0, 0.025, 0.05, 0.1, 0.2, 0.4 mg/L) de Carbofuran (Furadan® 350L.) (2,3-dihidro-2,2-dimetilbenzofuran-7-il metilcarbamato; pureza de 33.21%, CAS: 1563-66-2) por dilución de una solución patrón de 137 μl en 100 ml de etanol absoluto (J.T.Baker ®); la cantidad del solvente en los acuarios fue mínima, por lo tanto no se consideró un grupo control para el solvente (Ensibi *et al.,* 2012). Las concentraciones se determinaron por bioensayos previos y tomando en consideración las CL_{50} ya establecidas para carbofuran, en otras especies de peces de agua dulce. Así mismo, se siguieron los protocolos establecidos en el marco de pruebas de toxicidad de la Agencia para la Protección Ambiental de los Estados Unidos de Norteamérica (USEPA 2002) y de la Organización para la Cooperación y el Desarrollo Económico (OECD 2012) para el desarrollo del experimento.

Organismos de prueba. Se utilizaron juveniles de *C. humboldtianum* de siete meses de edad, 7.45 ± 0.3 cm y 2.76 ± 0.2 g. Se obtuvieron por fertilización *in vitro*, de reproductores en cautiverio, certificados morfológica y genéticamente. Para la obtención de los huevos se emplearon 6 hembras y 12 machos. Los embriones se mantuvieron en agua con una dureza de 120 ± 20 mg/L^{-1} de $CaCo_3$, con una salinidad de 4 g/L NaCl, a 20 ± 1 ºC, pH 8 ± 0.5 y un fotoperíodo de 12:12 hasta su eclosión. Las larvas se alimentaron con *Brachionus plicatilis* (Müller, 1786) (Rotifera) y *Artemia franciscana* Kellog, 1906 (Anostraca); los juveniles se alimentaron con neonatos de *Daphnia pulex* Linnaeus, 1758 (Cladocera), larvas de *Chironomus plumosus* (Linneus, 1758) (Diptera), anfípodos Hyalella azteca (Saussure, 1858) (Amphipoda) y alimento balanceado hasta los siete meses de edad. Los juveniles de esta edad, se trasladaron a acuarios de 40 L, 24 h antes de ser utilizados en el bioensayo. No se les proporcionó alimento durante el tiempo de exposición al tóxico. Los peces fueron del tamaño adecuado para obtener la cantidad de tejidos (branquias, músculo, hígado y cerebro) necesarios para la cuantificación enzimática.

Todos los organismos se trataron de acuerdo con la guía ética de la norma oficial mexicana, que establece las especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio (NOM-062-ZOO-1999).

Diseño experimental. Se realizó un bioensayo agudo de tipo estático, sin recambio de agua, en acuarios de 40 L, con agua reconstituida con una dureza de 120 ± 20 mg/L^{-1} de $CaCO_3$ (EPA 1993), a 20 ± 1 °C, con aireación constante (4.0 ± 6.5 mg/L O_2), pH 8 ± 0.5 y fotoperiodo 12:12. Se emplearon diez organismos por tratamiento: 0.0, 0.025, 0.05, 0.1, 0.2, 0.4, mg/L^{-1} de carbofuran, con tres réplicas. Se realizó el registro de la mortalidad y efectos visibles durante las 96 horas de exposición, retirando los individuos muertos al momento de ser detectados.

Al finalizar el tiempo de exposición, los organismos sobrevivientes en cada tratamiento se sacrificaron por bloqueo medular, vía craneal a la altura del opérculo. Se extrajeron los tejidos (branquias, cerebro, hígado y músculo) que se mantuvieron a -80°Centigrados. Para el análisis bioquímico se utilizaron los tejidos de cinco individuos, se maceraron y homogeneizaron en agua tridestilada, con un homogeneizador eléctrico (Biospecific product, Inc. Model 398).

Determinación bioquímica de las enzimas. La actividad enzimática se determinó en alícuotas de homogeneizados al 10% (peso/volumen) de cada uno de los órganos, por duplicado y las lecturas se realizaron con un espectrofotómetro Spectronic 20-D ®. La actividad de la acetilcolinesterasa (AChE) se cuantificó en el cerebro y el hígado, mediante la técnica de Hestrin (1949). Se emplearon alícuotas de 1 mL del homogeneizado, al 10 % y a 4°C y se centrifugaron a 3500 revoluciones por minuto (rpm) por 15 minutos. Se tomó 1 mL del sobrenadante, se añadieron 2 mL de Tris-HCL (0.02 N, pH 7) y 1.0 mL de acetilcolina 8 mM. Se agitó y se incubó a 25 °C durante 20 minutos. Para detener la reacción, se agregó 1 mL de hidroxilamina alcalina 2 M, 1 mL de ácido clorhídrico 4 N, y 1 mL de cloruro férrico 0.37 M, disuelto en ácido clorhídrico 0.1 N. Se centrifugó a 3000 rpm por 5 min y se midió la absorbancia a 540 nm.

El nivel de lipoperoxidación (LPO) se determinó en las branquias, el hígado y el músculo, siguiendo el método de Buege & Aust (1978). Se empleó 0.5 g. de cada tejido y se homogenizaron por separado, con 5 mL de agua tridestilada. A 300 mL de cada homogeneizado, se agregaron 700 mL de Tris-HCL 150 mM (pH 7.4) y se incubaron a 37°C por 30 minutos. Posteriormente, se agregaron 2 mL de ácido barbitúrico (TBA) al 0.375% disuelto en ácido tricloroacético al 15%, a cada muestra y se mantuvieron a ebullición durante 45 minutos. La mezcla, una vez fría, se centrifugó a 3000 rpm por 10 min y la absorbancia se leyó a 532 nm. El nivel de lipoperoxidación se midió en nanomoles de malondialdehído/miligramo de proteína (MDA/mg proteína) con un coeficiente de extinción molar de 1.56 x 10^5/M cm.

La actividad de la enzima superóxido dismutasa (SOD) se determinó en las branquias, el hígado y el músculo por el método de Sun *et al.* (1988). Se emplearon alícuotas de 1.5 mL del homogeneizado al 10% y a 4°C; se filtró a través de una gasa y se centrifugó a 3000 rpm por 5 minutos. Se tomaron 0.5 mL del sobrenadante y se agregaron 1.5 mL de agua tridestilada. Posteriormente, se añadieron 8 mL de 3:5 cloroformo/etanol. Se centrifugó a 3000 rpm durante 30 min, a 4°C. Se transfirieron 0.5 mL del sobrenadante a 2.5 mL de la mezcla compuesta de xantina 0.1 mM, ácido dietilentriaminopentaacético (DETAPAC) 1 mM, 50 mg de albúmina sérica, nitroazultetrazoilo 25 μM, ácido disulfónico bathocuproine 250 μM y carbonato de sodio 40 mM (pH 10.2). La reacción se inició con la adición de 20 U/mL de xantina oxidasa y se incubó a 25°C durante 20 minutos. Se terminó la reacción con 1 mL de cloruro cúprico 0.8 mM; la absorbancia se midió a 560 nm y la actividad de la SOD se midió en unidades totales/mg de proteína.

La actividad de la enzima glutatión peroxidasa (GPx) se determinó en las branquias, el hígado y el músculo, mediante el método de Lawrence & Burk (1976) con hidroperóxido de cumeno como sustrato. Se filtró una alícuota de 1.5 mL del homogeneizado al 10%, a 4 °C y se centrifugó a 3000 rpm por 5 minutos. La mezcla de reacción se conformó por 200 mL del sobrenadante del homogeneizado, 2 mL de buffer de fosfatos 75 mM (pH 7.0), 50 mL de glutatión 60 mM, 0.1 mL de la glutatión reductasa 30 U/mL, 0.1 mL de sal disódica de EDTA 15 mM, 0.1 mL del fosfato de ß-nicotinamida adenina dinucleótido (NADPH) 3 mM, y 0.45 mL de H2O, con un volumen final de 3.0 mL. La reacción se inició con 0.1 mL de hidroperóxido de cumeno 45 mM. La conversión de NADPH a NADP+ se midió continuamente a 340 nm durante 4 minutos. La actividad de la GPx se expresa en nmol NADPH oxidado a NADP+/

min/mg de proteínas, con un coeficiente de extinción de 6.22x106 $cm^{-1}M^{-1}$. El contenido de proteínas se estimó por el método de Bradford (1976) y se utilizó albúmina sérica bovina como estándar, mismas que se emplearon en el procesamiento de la actividad enzimática.

Análisis estadístico. Una vez obtenida la relación de organismos muertos y vivos en cada concentración, se determinó la concentración letal media (CL_{50}) con la mortalidad acumulada en cada concentración, mediante el método probit XLstat v.101(Addinsoft, 2014). El efecto del carbofuran sobre la sobrevivencia final de los juveniles se evaluó a través de un ANOVA de una vía, tipo I (α = 0,05), previa normalización de los resultados (arcoseno Öpi).

Para determinar el efecto del carbofuran sobre la actividad enzimática en cada tejido, se aplicó un ANOVA de una vía ($p < 0.05$) y posteriormente, una comparación múltiple de medias, por el método de Tukey (Zar 1984).

Los resultados de la actividad de la AChE se expresaron en porcentajes de inhibición/estimulación de la Acetilcolinesterasa vs. el control y se calculó con base en la tasa de crecimiento específica (μ), de la muestra de prueba y el control, siguiendo la ecuación: $I\mu i = ((\mu c - \mu i)/\mu c)*100$ (ISO 8692, 2004).

donde:

Iμi = porcentaje de inhibición para la concentración de estudio de la prueba **i**

μi = tasa de crecimiento promedio para la concentración de estudio de la prueba **i**

μc = tasa de crecimiento promedio para el lote control

Un valor negativo de **Iμi** significa estimulación de la actividad de AChE; un valor positivo significa inhibición de la actividad de la AChE.

RESULTADOS

Concentración letal media CL50. La CL50 fue de 0.077 mg/L (0.028 - 0.118 $mg/_L^{-1}$, α = 0.05) a 96-h (Tabla 1). Se presentó el 100% de mortalidad en la concentración más alta de carbofuran (0.4 mg/L), por lo que estos individuos no se consideraron para el análisis enzimático. En el grupo control no se presentó muerte de organismos, no se observaron cambios en el nado ni lesiones musculares o en las aletas.

Inhibición/Estimulación de la Acetilcolinesterasa (AChE). El efecto neurotóxico del carbofuran en el cerebro de *C. humboldtianum*, evaluado mediante la inhibición/estimulación de la enzima acetilcolinesterasa, presentó un patrón inverso con respecto a las concentraciones de carbofuran. La mayor inhibición (41.30 %, 37.90%) se observó en las concentraciones de 0.025 mg/L y 0.05 mg/L. En las concentraciones más altas (0.1 mg/L y 0.2 mg/L), la inhibición fue de 28.22% y 5.62%, respectivamente (ANOVA $p<0.05$). En el hígado, la respuesta de la AChE se incrementó con el aumento en la concentración del tóxico (ANOVA $p < 0.05$). Se observó una estimulación de -21.77% de la AChE en la concentración 0.2 mg/L de carbofuran; en las concentraciones de 0.025 mg/L y 0.1 mg/L, la estimulación de la AChE fue de -0.073% y -3.002%. Sin embargo, en la concentración de 0.05 mg/L se presentó una inhibición en la actividad de acetilcolinesterasa de 7.48% (Fig. 1).

Nivel de Lipoperoxidación (LPO). En el nivel de LPO se observó un incremento proporcional al incremento de las concentraciones del carbofuran, en los tejidos analizados de *C. humboldtianum*. En las branquias se registraron los niveles más altos (0.33, 0.32, 0.49 y 0.84 nmol MDA/mg de proteína) respectivamente (ANOVA $p<0.05$). En el tejido de los músculos el nivel de LPO fue de (0.20, 0.29, 0.17 y 0.16 nmol de MDA/mg de proteína) (ANOVA $p<0.05$), lo cual indica que el nivel de LPO disminuye ligeramente en las concentraciones más bajas de carbofuran. Mientras que en el hígado, el nivel de peroxidación lipídica fue de 0.07 nmol de MDA/mg de proteína en la concentración de 0.2 mg/L; en las concentraciones menores de carbofuran, el nivel de LPO fue inferior a los valores registrados para el hígado de los organismos control (ANOVA $p < 0.05$) (Fig. 2).

Actividad de la Superóxido dismutasa (SOD). La respuesta antioxidante de la enzima SOD presentó una tendencia a aumentar con el incremento de las concentraciones del carbofuran (ANOVA $p < 0.05$). En las branquias se observ**ó** la mayor actividad de la enzima (0.0027, 0.0019, 0.0024 y 0.0024 Unidades/mg proteína); el hígado fue el segundo órgano en donde la actividad de la SOD fue de 0.0018, 0.0019, 0.0021 y 0.0024 Unidades/mg proteína. Finalmente, en el músculo se determinó una actividad menor: 0.0017, 0.0009, 0.0017 y 0.0019 Unidades/mg proteína (Fig. 3).

Actividad de la Glutatión peroxidasa (GPx). En el hígado, se presentó un incremento notable en la actividad de la GPx, con respecto al testigo, pero en relación inversa a las concentraciones del carbofuran (4.41, 3.10, 4.30, y 3.80 n mol NADPH/min mg de proteína, respectivamente). Una respuesta semejante se registró en los músculos con respecto al grupo control, aunque en las concentraciones menores del tratamiento (0.21, 0.26, 0.18, y 0.11 nmol NADPH/min mg de proteína). La actividad de la Gpx en las branquias, se observó un ligero incremento en relación al aumento de las concentraciones del carbofuran (0.56, 0.91, 0.60, 0.75 nmol NADPH/min mg de proteína) (ANOVA $p<0.05$) (Fig. 4).

Tabla 1. Toxicidad aguda de Carbofuran en *Chirostoma humboldtianum* (Valenciennes 1835)

Compuesto	Ecuación de la Regresión	Toxicidad aguda Intervalos de confianza 95%		Media LC50
	y = ax + b	Inferior	Superior	mg/L
		mg/L	mg/L	
Carbofuran	0.7613x + 1.3279	0.028	0.118	0.077
	R2 = 0.9912			

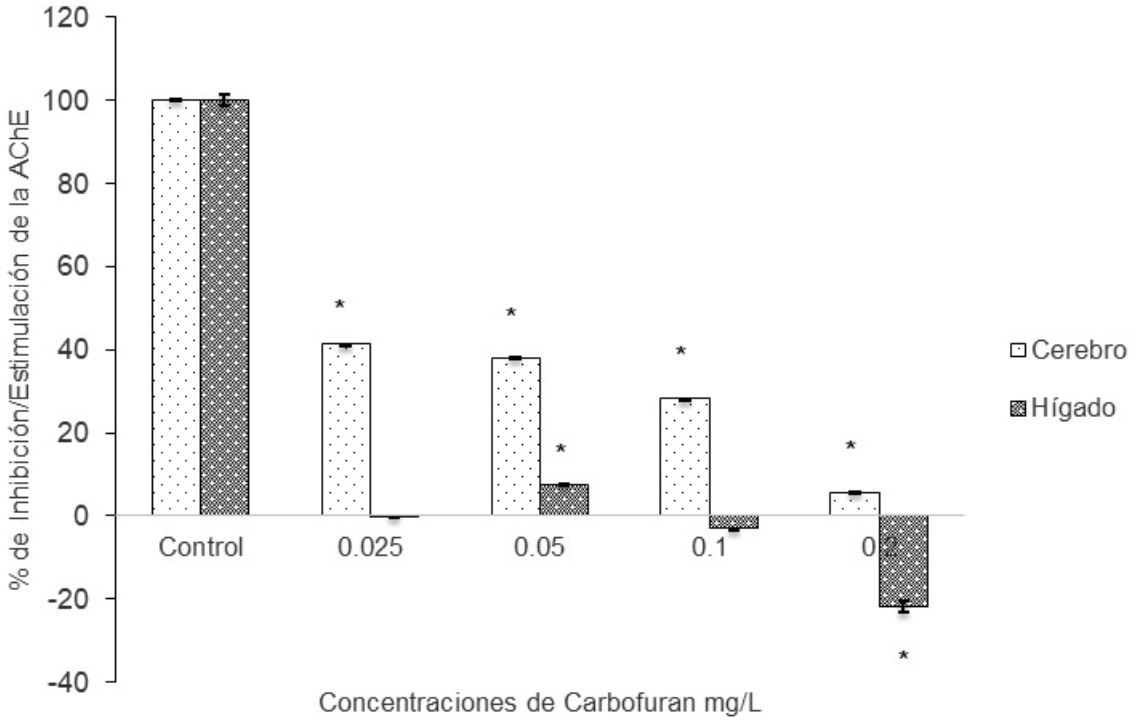

Figura 1. Inhibición/Estimulación de la acetilcolinesterasa (%) en el cerebro y el hígado de juveniles de *Chirostoma humboldtianum*, expuestos a diferentes concentraciones de carbofuran por 96 h. n=5, las barras muestran el error estándar, * diferencias significativas ($p < 0.05$).

DISCUSIÓN

Los plaguicidas son los compuestos que se encuentran en mayor concentración en los sistemas acuáticos, con mayor frecuencia, debido a su extenso uso en las actividades agrícolas, industriales y domésticas. Sin embargo, poco se sabe del efecto que estos agroquímicos ocasionan en las especies de peces endémicas de México. Los resultados obtenidos en este trabajo constituyen el primer estudio sobre la toxicidad del carbofuran en *C. humboldtianum*.

Los juveniles mostraron una sensibilidad muy alta al carbofuran, ya que la CL_{50}, (0.077 mg/L/96 h) se encuentra por debajo de los valores de CL_{50} determinados para otras especies de peces, como en la trucha arcoíris (*Oncorhynchus mykiss*) (22-29 mg/L/96 horas) y en la carpa dorada (*Carassius auratus*) (1.75 mg/L/96 h) (Yi *et al*., 2006).

Los resultados en la acetilcolinesterasa muestran que los carbamatos poseen la capacidad de inhibir o estimular la actividad de la enzima tanto en el cerebro como en el hígado de *C. humboldtianum*, cuyo efecto se expresa de forma distinta en cada órgano, lo cual se relaciona con las funciones que cada uno de ellos desempeña.

El primer paso en el proceso de inhibición de la AChE por un éster de un organofosforado o un carbamato, involucra la formación del complejo enzima-inhibidor, con la posterior carbamilación del hidroxilo de serina (Fukuto, 1990). Cuando se inhibe la actividad de la enzima, la acetilcolina no se descompone en colina y ácido acético, lo que ocasiona que se acumule en las uniones sinápticas o neuromusculares y altera el funcionamiento del sistema nervioso y, en consecuencia, afecta la locomoción y el equilibrio de los peces (Dutta & Arends, 2003). En *C. humboldtianum* se observó que la inhibición/estimulación de la acetilcolinesterasa no sólo causa la pérdida del equilibrio en el nado de los peces; sino que, como efecto de la tetanización y contracciones musculares, se producen lesiones en el músculo y las aletas que conlleva a necrosis del pedúnculo caudal.

Así mismo, se ha registrado que el carbofuran inhibe la actividad de la AChE en cerebro y en músculo de peces de agua dulce y de agua salobre (*Carassius auratus*, Bretaud *et al*., 2000; *Ciprinus carpio,* Dembelé *et al*., 2000; *Channa punctatus*, Jash & Bhattacharya, 1983). Por otro lado, Favari *et al.* (2003), reportan inhibición de la AChE en el hígado y el músculo de *Xiphophorus helleri* expuestos al efecto toxico del agua de un embalse contaminado por compuestos de uso agrícola y desechos municipales.

Otros estudios también han reportado variaciones en la actividad de AChE en diferentes órganos de peces expuestos a diferentes insecticidas en *Cyprinus carpio* con paraquat (Nemcsók *et al*., 1984), en *Ictaluros puntactus* con clorpirifos y paration (Straus & Chambers, 1995), en *Rhamdia quelen* con glifosato (Glusczak *et al*., 2007), en *Prochilodus lineatus* con el herbicida Roundupâ, un organofosforado (Modesto & Martínez, 2010).

La peroxidación lipídica es uno de los mecanismos más importantes de la acción toxica de los insecticidas ya sea organofosforados o carbamatos (Glusczak *et al*., 2007). La lipoperoxidación se inicia por las especies reactivas de oxígeno (ROS) que atacan a todas las mo-

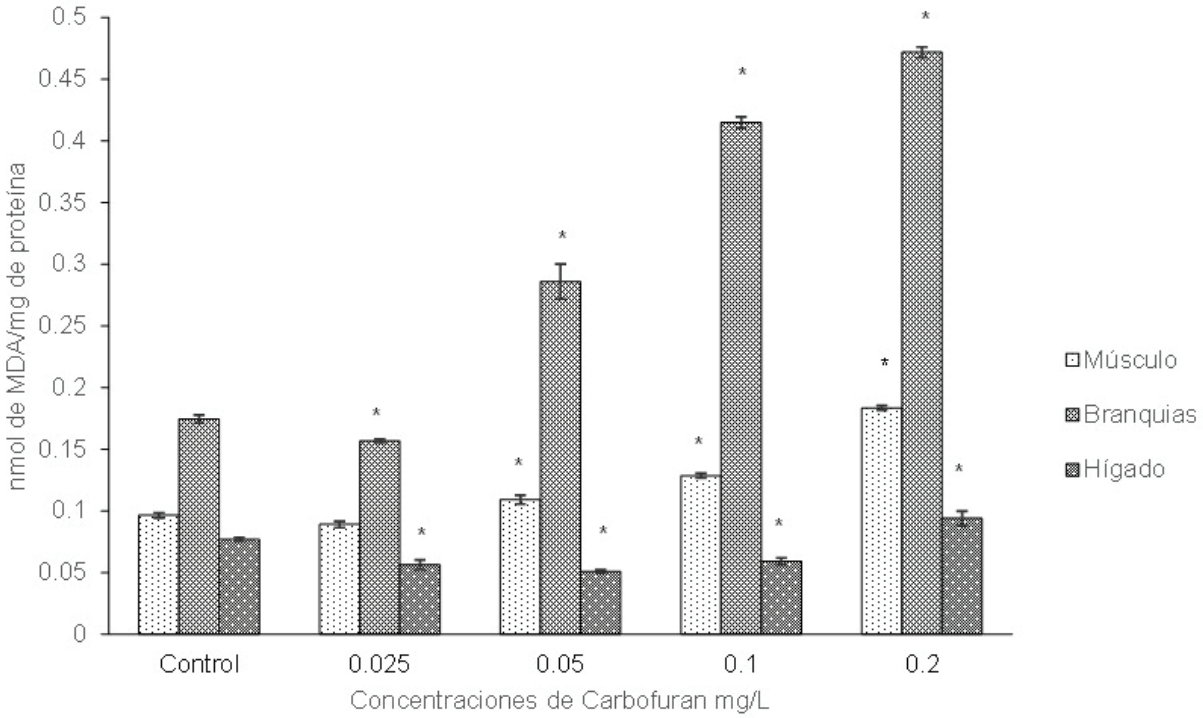

Figura 2. Nivel de lipoperoxidación en músculo, branquias e hígado de *Chirostoma humboldtianum*. n=5, las barras muestran el error estándar, *diferencias significativas ($p < 0.05$).

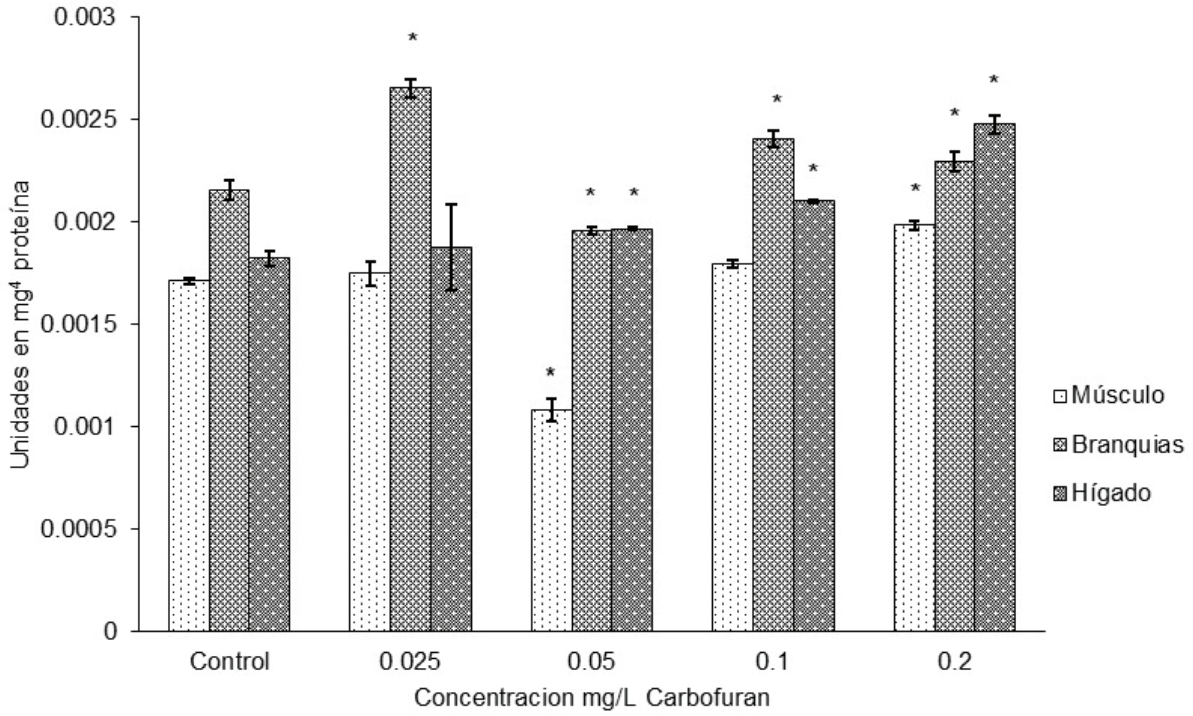

Figura 3. Actividad de la SOD en músculo, branquias e hígado de *Chirostoma humboldtianum*. n=5, las barras muestran el error estándar, *diferencias significativas ($p<0.05$).

léculas biológicas; incluyen a los ácidos grasos poliinsaturados de las membranas celulares, ya que extraen un hidrogeno y los convierten, por una serie de reacciones posteriores, en aldehídos reactivos como el malondialdehído (MDA), el principal producto de la peroxidación. Al presentarse en los lípidos de las membranas celulares, se altera su cohesión, fluidez, permeabilidad y función metabólica, que conduce a una inestabilidad de la membrana y causa daño y muerte celular (Halliwell & Chirico, 1993; Ochoa & González, 2008). Los resultados obtenidos en el presente estudio indican daño peroxidativo debido al carbofuran, el cual se reflejó en el incremento del nivel de la peroxidación lipídica en branquias y músculo, dos de los principales órganos afectados por las especies reactivas de oxígeno. Esto se debe a que la exposición directa de las branquias a los plaguicidas disueltos durante el intercambio gaseoso altera su función celular. Por otro lado, el tejido muscular de los peces puede ser afectado por la absorción cutánea al estar en contacto directo con el insecticida disuelto (Heath, 1995).

Sin embargo, en el hígado debido a su función de desintoxicación no se presentaron diferencias significativas en el nivel de LPO con respecto a los valores del control. Esto confirma la eficiencia de este órgano, para contrarrestar el impacto de los peróxidos y especies reactivas generados por la exposición a los contaminantes. En el hígado se genera la mayor cantidad de radicales libres, debido a las reacciones oxidativas y por consiguiente, presenta la mayor actividad enzimática antioxidante (Güll *et al*., 2004, Avci *et al*., 2005). Además, el hígado es el órgano más resistente al daño por radicales libres (Atli & Canli, 2010). De acuerdo con esto, la actividad enzimática hepática de la SOD y la GPx se incrementó posiblemente, como una reacción ante la presencia de peróxidos y las especies reactivas, lo cual evitó un nivel mayor de peroxidación lipídica en este órgano. Sin embargo, se debe considerar que cada una de las enzimas antioxidantes presenta mecanismos de reacción, en los que intervienen procesos de activación e inactivación de las enzimas, además de la carbonilación de las proteínas, debido a la acción directa de la formación de radicales libres que pueden causar la pérdida en la actividad de las enzimas y la alteración de las proteínas de las membranas (Modesto & Martínez, 2010).

La peroxidación lipídica está asociada a la presencia de radicales libres y un exceso de éstos, puede activar o inactivar a las enzimas antioxidantes como la SOD y la GPx, entre otras (Modesto & Martínez, 2010). La SOD es la responsable de catalizar al radical superóxido reactivo (O2-) en peróxido de hidrógeno (H2O2) y la GPx participa en la reducción de peróxido de hidrógeno, en oxígeno y agua (Atli & Canli, 2010).

La actividad de la SOD y la GPx indica la presencia de especies reactivas, generadas durante los procesos de biotransformación de los metabolitos y reaccionan de manera diferente en los tejidos. La SOD es la primera barrera del mecanismo de defensa antioxidante ante la presencia de iones superóxidos. Un abatimiento en la actividad de la SOD indica que la capacidad antioxidante de la enzima fue superada por productos superóxidos, como se observó en el músculo y en el hígado de *C. humboldtianum*. Lo anterior coincide con la disminución en la actividad de la SOD en tejidos de tilapias expuestas a oxyfluorfen y en *Carassius auratus* a triclorofenol señalados por (Peixoto *et al*., 2006, Li *et al*., 2007) respectivamente.

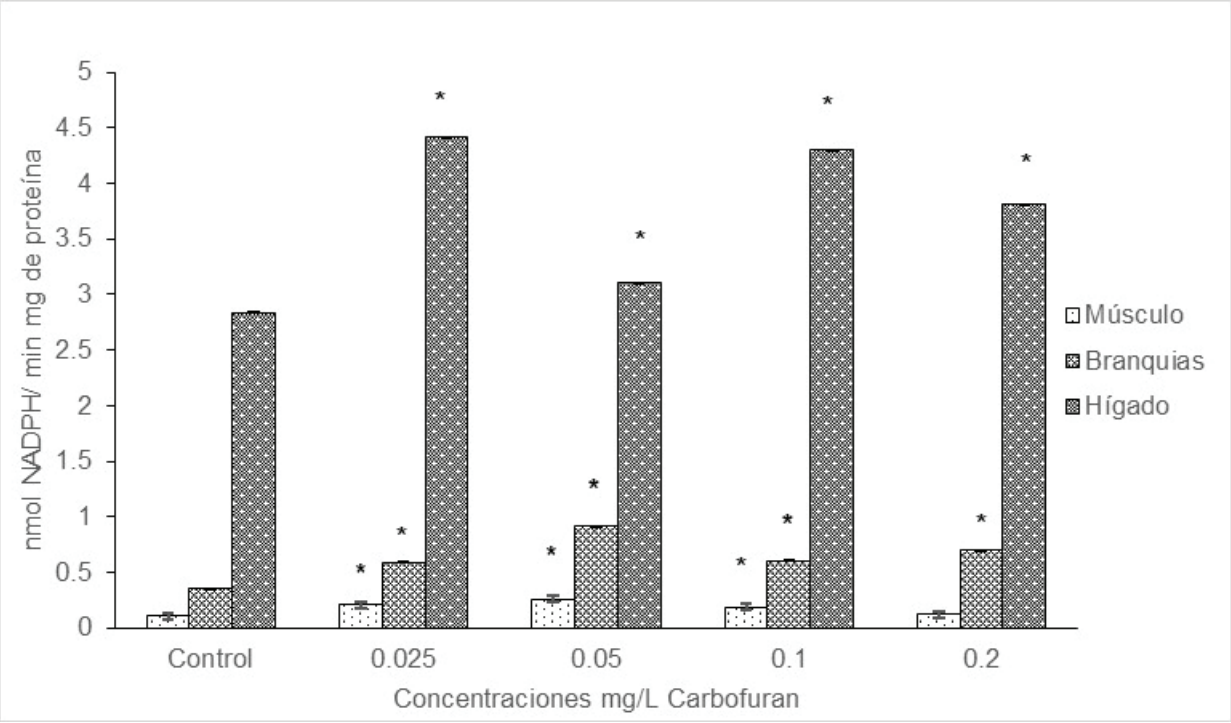

Figura 4. Actividad de la GPx en músculo, branquias e hígado de juveniles de *Chirostoma humboldtianum*. n=5, las barras muestran el error estándar, *diferencias significativas ($p<0.05$).

Así mismo, el aumento de la actividad de la GPx y la disminución en el nivel de LPO en el hígado de *C. humboldtianum*, muestra la eficacia de este órgano en la neutralización y transformación de los peróxidos e hidroperóxidos orgánicos para su fácil eliminación. Sin embargo, las branquias y el músculo no respondieron de la misma manera, ya que la baja actividad de la glutatión peroxidasa demuestra la incapacidad del sistema antioxidante en estos órganos, para la neutralización y detoxificación de los peróxidos, dando como resultado un aumento en el nivel de lipoperoxidación. Lo anterior coincide con los resultados de Ahmad *et al.,* 2000, donde señalan una capacidad menor de las branquias y los riñones para neutralizar el impacto de los peróxidos y especies reactivas generadas por contaminantes industriales en *Channa punctatus*.

El presente trabajo es uno de los primeros registros de la evaluación de la toxicidad por insecticidas en especies de peces nativos de México. Aún cuando todas las especies de *Chirostoma* presentan un alto grado de irritabilidad durante su manejo, que pudiera alterar su respuesta fisiológica ante el estrés, los resultados demuestran que las concentraciones probadas de carbofuran causan la muerte, además de daño neurotóxico y oxidativo en juveniles de *C. humboldtianum*, en concentraciones inferiores a las probadas en otras especies de peces.

Por otro lado, en este trabajo se analizaron los efectos del carbofuran sobre juveniles debido a que, en tallas menores, la cantidad de tejido no permite cuantificar el efecto, pero es de esperarse un efecto negativo mayor en larvas y juveniles más pequeños. Durante mucho tiempo, se ha argumentado que la disminución del tamaño de las poblaciones del género *Chirostoma* y su estatus en peligro de extinción, se debe a la falta de regulación de sus pesquerías y a la contaminación de los cuerpos de agua donde habitan. Sin embargo, no existen estudios que permitan demostrar lo segundo. En este caso, se demuestra la alta sensibilidad de *C. humboldtianum* al carbofuran y el efecto negativo de uno de los insecticidas de mayor uso en la agricultura en México y en el mundo. Las lesiones graves de este tóxico a los órganos blanco-probados demuestran la necesidad de desarrollar programas que permitan evaluar los efectos de los insecticidas sobre las especies de peces nativas y endémicas de importancia ecológica y económica, así como también, analizar exhaustivamente la calidad del agua de los sitios de distribución de las especies.

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Diversity, abundance, and fish assemblages in the Sontecomapan lagoon system, Veracruz, Mexico

Diversidad, abundancia y asociaciones de peces en el sistema lagunar de Sontecomapan, Veracruz México

Silvia Díaz-Ruiz[1]*, Arturo Aguirre-León[2] and Mariela Gazca-Castro[1]

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ABSTRACT

Background. The Sontecomapan Lagoon is a protected natural area and is part of the Los Tuxtlas Biosphere Reserve on the south coast of the State of Veracruz, with significant fishing productivity and little ecological knowledge about its fish community. **Objectives.** Analyze space-time variations in diversity, richness, abundance, dominant species, community inhabitants, fish assemblages, and trophic groups. **Methods.** The fish collections were made between 2014 and 2020, in 10 stations with a trawl net. The sampling effort was validated using non-parametric estimators. Analysis of variance was used to indicate statistically significant differences in community ecological parameters between sites and months. Canonical correspondence analysis (ACC) was performed to determine species-habitat relationships. **Results.** Fifty species, 35 genera and 23 families were identified, with 2 new records, *Chaetodipterus faber* (Broussonet, 1782) and *Pomadasys ramosus* (Poey, 1860) and 2 dominant species, *Diapterus rhombeus* (Cuvier, 1829) and *Cathorops aguadulce* (Meek, 1904). The diversity values (H´ = 0.84 to 2.07, D = 2.36 to 3.83, J´= 0.26 to 0.64) and abundance (density = 0.009 to 0.035 ind./m^2, biomass = 0.105 to 0.044 g/m^2, average weight = 5.91 to 16.72 g/ind), are considered low compared to other similar coastal systems. The spatial and temporal fluctuations of these parameters are related to the life cycles of the species present and with the environmental dynamics of the system. The ACC analysis indicated that fish assemblages are determined by changes in salinity, transparency, and depth. **Conclusion.** The ecological information analyzed represents a broad frame of reference for a little-studied coastal area that requires management and conservation strategies for its biotic resources.

Keywords: diversity, dominant species, community inhabitants, fish-habitats, Sontecomapan lagoon.

RESUMEN

Antecedentes. La Laguna de Sontecomapan es un Área Natural Protegida y forma parte de la Reserva de la Biosfera Los Tuxtlas en la costa sur del Estado de Veracruz, con productividad pesquera y escaso conocimiento ecológico sobre su comunidad de peces. **Objetivos**. Analizar las variaciones espacio temporales de diversidad, riqueza, abundancia, especies dominantes, componentes comunitarios, conjuntos de peces y grupos tróficos. **Métodos.** Las recolectas de los peces se realizaron entre 2014 y 2020, en 10 estaciones con una red de arrastre. El esfuerzo de muestreo se validó utilizando estimadores no-paramétricos. Se utilizó análisis de varianza para indicar diferencias estadísticas significativas de los parámetros ecológicos de la comunidad entre sitios y meses. Se realizó análisis de correspondencia canónica (ACC) para determinar las relaciones especie-hábitat. **Resultados.** Se identificaron 50 especies, 35 géneros y 23 familias, con 2 nuevos registros, *Chaetodipterus faber* (Broussonet, 1782) y *Pomadasys ramosus* (Poey, 1860) y 2 especies dominantes, *Diapterus rhombeus* (Cuvier, 1829) y *Cathorops aguadulce* (Meek, 1904). Los valores de diversidad (H´ = 0.84 a 2.07, D = 2.36 a 3.83, J´= 0.26 a 0.64) y abundancia (densidad = 0.009 a 0.035 ind./m^2, biomasa = 0.105 a 0.044 g/m^2, peso promedio = 5.91 a 16.72 g/ind), se consideran bajos comparados con otros sistemas similares. Las fluctuaciones espaciales y temporales de estos parámetros tienen relación con los ciclos de vida de las especies presentes y con la dinámica ambiental del sistema. El análisis ACC indicó que los conjuntos de peces están determinados por los cambios de salinidad, transparencia y profundidad. **Conclusión.** La información ecológica analizada representa un amplio marco de referencia para un área costera poco estudiada que requiere estrategias de manejo y conservación para sus recursos bióticos.

Palabras clave: diversidad, especies dominantes, componentes comunitarios, peces-hábitat, Laguna de Sontecomapan.

[1] Laboratorio de Ictiología y Ecología Costera, Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa. Av. San Rafael Atlixco No. 186, Col. Vicentina, Iztapalapa, Ciudad de México, 09340. México.

[2] Laboratorio Ecología Costera y Pesquerías, Departamento El Hombre y su Ambiente, Universidad Autónoma Metropolitana-Xochimilco. Av. El Hueso 1100, Col. Villa Quietud, Coyoacán, Ciudad de México 04960, México.

***Corresponding author:**
Silvia Díaz-Ruiz: e-mail: sdr@xanum.uam.mx

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INTRODUCTION

The estuarine lagoon ecosystems are recognized as complex ecological structures derived from diverse aquatic environments, because mainly the exchange of water and organisms with the sea and the discharge of fresh water from the rivers that drain into their micro-basins (Snedden *et al.*, 2013; Aguirre-León *et al.*, 2014). These conditions generate a high primary productivity derived from phytoplankton, mangroves, and submerged vegetation because of the subsidies they receive through fluvial discharges and tidal exchange (Paerl & Justic, 2013). These factors determine the magnitude of secondary production by various faunal groups, including an important diversity of coastal fish (Díaz-Ruiz *et al.*, 2018; Aguirre-León *et al.*, 2020).

In these systems, studies on spatial and temporal variations of the ichthyofauna and their relationship with environmental variables have highlighted their importance as breeding, feeding, protection or reproduction areas for the development of the life cycles of coastal fish, many of which are fishing resources (Rodríguez-Varela *et al.*, 2010; Castillo-Rivera *et al.*, 2017; Díaz-Ruiz *et al.*, 2018; Raz-Guzman *et al.*, 2018; Aguirre-León *et al.*, 2014, 2021). The ecological behavior of species and its relationship with environmental variables are also reflected in changes in species richness, distribution and abundance in space and time (Barletta *et al.*, 2005, 2008; Aguirre-León *et al.*, 2018).

The coast of Veracruz has several coastal lagoons (Lara-Domínguez *et al.*, 2011), some are part of Protected Natural Areas or Ramsar Sites that place them in an ecological condition of high priority regarding the management and conservation of their biodiversity. Among these, the Sontecomapan lagoon belongs to the "Los Tuxtlas" Biosphere Reserve (CONANP, 2006), is also a Ramsar site (FIR, 2004). This lagoon is characterized by its biological richness, since it is used by a wide variety of fish during their life cycles, many of which are marine and freshwater fish for local consumption (Rodríguez-Varela *et al.*, 2010; Aguirre-León *et al.*, 2018; Beltrán-García *et al.*, 2019). Despite the economic importance of these resources, ecological studies on the fish community in this system are currently still scarce. Therefore, this study complements and enriches the information on the knowledge of the fish community in the Sontecomapan Lagoon and provides greater understanding to harmonize its use and conservation as soon as possible in the Tuxtlas region.

The focus of this study encompasses a spatio-temporal analysis of the fish community, for which the following objectives were proposed: 1) Analyze the richness diversity, evenness, and community dominance, 2) Determine the density, biomass, and average weight in the system, 3) Identify the types of fish inhabitants present in the system and the trophic categories of the community, 4) Analyze the environmental factors that condition the ecological behavior of fish assemblages.

MATERIALS AND METHODS

Study Area: The Sontecomapan lagoon, located south of Veracruz (018º 32' N, 095º 02' W) (Fig. 1) belongs to the Los Tuxtlas Biosphere Reserve (CONANP, 2006). It was declared a Ramsar Site in 2004 (FIR, 2004) due to the degree of conservation of its mangroves and recognized worldwide as a Biosphere Reserve within the "Man and the Biosphere" program of UNESCO (MAB) in October 2006 (MAB, 2010). The system is approximately 12.0 km long and 1.5 km wide, with an area of 8.9 km^2 and a complex geomorphology. It is shallow (average 2.0 m) with mixed sediments from sand to silt-clay. It communicates with the sea through a tidal channel and its sea mouth that is 5.0 m deep and 137.0 m wide (Esquivel & Soto, 2018). A considerable volume of water from the Coxcoapan, Yuhualtijapan, Basura, La Palma, and El Sábalo rivers drain into the system (FIR, 2004). The system presents a saline gradient with values from 34.6 to 0.0 UPS from the marine inlet to the headwaters of the rivers. The water temperature varies from 21.0 to 30.7 ºC. Dissolved oxygen changes from 2.0 to 8.6 mg/L. The depth from 1.0 to 5.5 m and the transparency from 0.2 to 2.9 m (Aguirre-León *et al.*, 2018). The climate is warm-humid, with an annual rainfall of 3 000 to 4 000 mm. The average extension of the climatic seasons goes from June to September for the rainy season, from October to February for the cold fronts called "Nortes" and from March to May for the dry season (Soto-Esparza & Giddings, 2011). The system presents varied aquatic environments from surrounding vegetation of *Rhizophora mangle* and *Avicennia germinans* in brackish and low salinity areas and submerged vegetation of *Ruppia maritima* (Carmona-Díaz *et al.*, 2004).

Sample collections: Data from this study were collected in October 2017 (O17), February 2018 (F18), October 2018 (O18) and January 2020 (J20), and combined with a data set from a previous work conducted in October 2014 (O14), February 2015 (F15), October 2015 (O15) and February 2016 (F16) (Aguirre-León *et al.* 2018). In both studies, the fish collection was carried out in 10 sites of the system (Fig. 1), with a shrimp trawl net 5.0 m long, with a working mouth of 2.5 m and a mesh size of 1.8 cm. These catches were made by trawling for 10 minutes as a standard effort at an average speed of two knots and covering an average trawl area of 1,500 m^2. The same methodology was used for samples collected from 2014 to 2020, two repetitions for each site and month were made, for a total of 160 captures.

Fish samples were preserved with a 10% formaldehyde solution in labeled plastic bags for later processing in the laboratory. Collected specimens were deposited as vouchers at the fish collections of Coastal Ecology and Fisheries Laboratory (UAM-Xochimilco). In addition, the physicochemical parameters at the surface and at the bottom were recorded at each sampling site using a YSI 85 multiparameter, these were: salinity (UPS), temperature (°C), and dissolved oxygen (mg/L), as well as transparency and depth (m).

Laboratory activities: Taxonomic identification of the species was carried out using identification sheets and descriptions based on the work of Castro-Aguirre *et al.* (1999), Carpenter (2002) and Miller *et al.* (2009). The taxonomic arrangement of the species was based on Nelson *et al.* (2016) and the authority and current nomenclature of these was corroborated in Fricke *et al.* (2021). Each species was counted and weighed with an Ohaus digital balance (precision 0.01 g). The parameters of diversity H´ (Shannon-Weaver, 1963), species richness D (Margalef, 1969), evenness J´ (Pielou, 1966), as well as those of abundance, density (ind./m^2), biomass (g/m^2) and average weight (g/ind.) of the community were estimated as described by Aguirre-Léon *et al.* (2020). The dominant species were determined using the Importance Value Index (IVI= N%+ P%+F%) with the ANACOM program (De la Cruz-Agüero, 1994). The different fish inhabitants of the community were identified following the criteria of Elliott *et al.* (2007) and Potter *et al.* (2015), which are classified as: ES = estuarine species, MED = marine estuarine-dependent, MEO = marine estuarine-opportunist and FS = freshwater species. The fish were grouped into trophic categories,

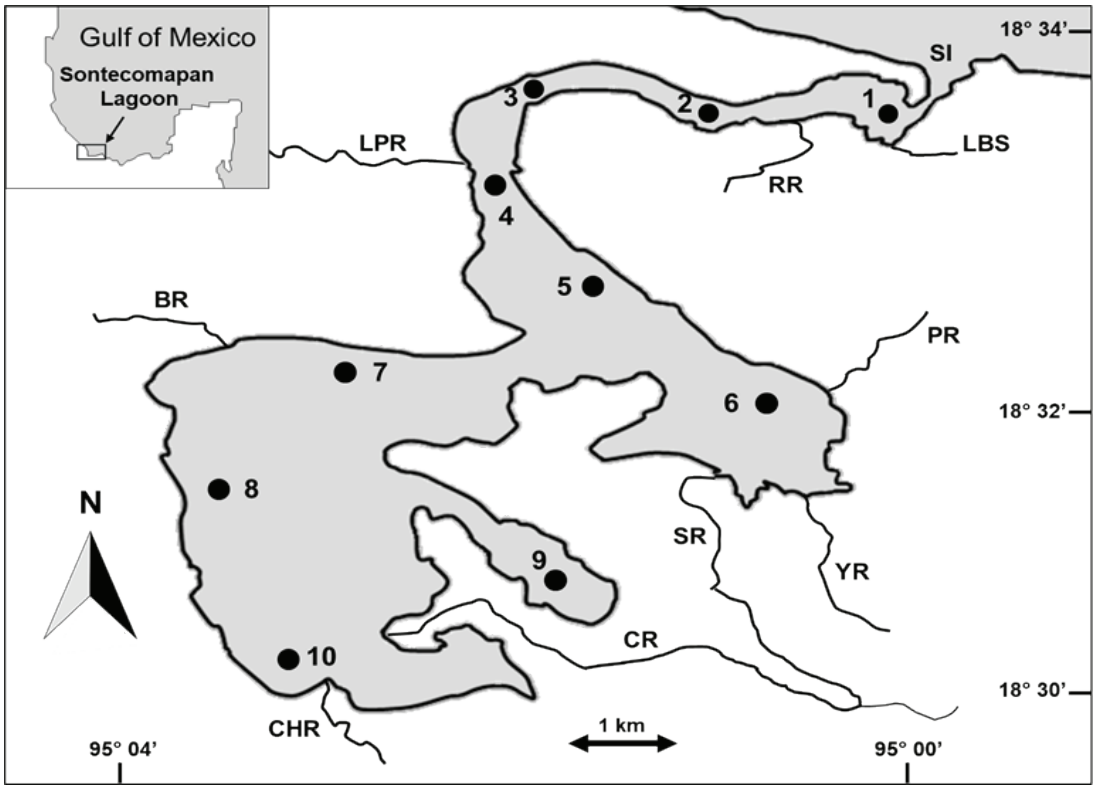

Figure 1. Sontecomapan Lagoon System, Veracruz. The location of sampling stations (•), the main physiographic features of the study area and rivers draining into the system are noted. Palma River (LPR), Basura River (BR), Chuniapan River (CHR), Coxcoapa River (CR), Sábalo River (SR), Yuhualtijapan River (YR), Pollos River (PR), La Boya Stream (LBS), Sontecomapan Inlet (SI).

their diet was examined through the literature, and they were classified following the criteria proposed by Elliott *et al.* (2007) and Froese & Pauly (2022) in: zooplanktivorous (ZP), species that feed predominantly on plankton; omnivores (OM), species that feed on plants, epifauna and infauna; detritivores (DE), species that feed mainly on detritus and /or microphytobenthos; piscivores (PI), fish that feed on nektonic invertebrates and finfish; zoobenthivore (ZB), fishes that feed on invertebrates associated with the substratum.

Statistical analysis: The ecological parameters of the community were compared between sites and months of study. Its average and standard deviation on these scales were calculated for each parameter. Parametric and non-parametric analysis of variance was used to indicate statistically significant differences. Previously, the assumptions of normality were evaluated using the D'Agostino Omnibus test, as well as the homogeneity of variances using the Modified Levene Equal Variance test (Levene, 1960). When necessary, logarithmic transformations were performed. When the assumptions of normality and equality of variance were met, the ANOVA-F test was used, and the Tukey test to determine between which sites or months there were differences. When these assumptions were not met, the Kruskal-Wallis-H test and the Z multiple test were used to determine these differences (Zar, 2010). These analyses were performed using the STATISTICA 13 program (StatSoft, Inc., 2013). A significance level of $P < 0.05$ was considered for all these analyses (Heiman, 2014).

The sampling effort was validated by estimating species accumulation curves based on the non-parametric estimators Chao2, Jackknife1, Jackknife2 and Bootstrap (Magurran, 2004). These estimators were selected for their precision in evaluating the total species richness, based on species presence-absence records, and because they allow estimating the rarity of species based on incidence (unique and duplicate species) (Willott, 2001). An abundance database of species by sites and sampling months was used. The construction of these curves was made with 9 999 randomizations using the statistical program Primer-E (Magurran *et al.,* 2011; Clarke & Warwick, 2014).

Canonical correspondence analysis (CCA) was used to define the fish assemblages and their correlation with the environmental factors of the system, for which matrices were constructed with the relative numeric abundance of the species and the average values of physicochemical parameters (salinity, temperature, dissolved oxygen, depth, and transparency) by sites and months of sampling (ter Braak & Verdonschot (1995). The inter-set correlations derived from this analysis were used to determine the environmental variables that had the greatest effect on species composition, distribution, and abundance (McGarigal *et al.*, 2000; ter Braak & Smilauer, 2002). This analysis was configured with the MVSP program (Multivariate Statistical Package), version 3.22 (KCS, Inc., 2013).

RESULTS

Fish community composition: A total of 6 916 individuals were captured during the study for an overall of 23 families, 35 genera and 50 species identified. Five families had the highest number of species, Carangidae and Gerreidae with six each and Engraulidae, Centropomidae and Lutjanidae with four each, (Table 1). For this study *Chaetodipterus faber* (Broussonet, 1782) (Ephippidae) and *Pomadasys ramosus* (Poey,1860) (Haemulidae) collected in February and October 2018 respectively, are new records for the system. According to the non-parametric species accumulation curves (Fig. 2) the expected average richness could be 66 species. The representativeness of the sampling varied between 56 (65%) for Bootstrap and 73 (92%) for Jackknife2 of the expected species. Therefore, 16 species would be missing to complete the current list of this study.

Spatial and temporal variation of diversity: The spatial analysis of average values of diversity H', richness D and evenness J', is integrated in Figure 3 (A, B, C). A similar trend was observed in the three mentioned indices with higher average values towards the tidal channel zone of the system (H´= 1.52±0.26, D = 1.92±1.22; J´= 0.82±0.34), and the lowest towards the interior of the system and areas of greater fluvial influence (H´= 1.07±0.43, D = 1.43±0.53, J´= 0.53±0.18). The spatial trend of these indices was related to the low number of species and the dominance of some of them in the system, there were no significant differences between sites for these parameters ($P > 0.05$). The average temporal changes of H', D and J' are indicated in Figure 3 (D, E, F). The temporal trend in the three indices was similar with few variations between months. The highest averages of H' (1.51±0.41), D (2.15±0.67) and J' (0.76±0.14) occurred in February 2018, October 2018, and January 2020, respectively. The values of H´ per month tend to be low, less than 2.0, with statistically significant differences, $F(7,150) = 2.88$, $P = 0.0102$, Tukey test indicated that they were between February 2016 - February 2018 ($P = 0.0403$) and February 2016 - January 2020 ($P = 0.0457$). The D index was equally low, with statistically significant differences between months, $F(7,150) = 4.23$, $P = 0.0006$), Tukey test indicated that they were between February 2014 - October 2018 ($P = 0.0215$), February 2016 with October 2017 ($P = 0.0191$), with February 2018 ($P = 0.0120$) and with October 2018 ($P = 0.0013$).

Spatial and temporal variation of abundance: On a spatial scale, the average behavior of density, biomass, and average weight of the community is shown in Figure 4 (A, B, C). The highest average density value (0.062 ind./m^2±0.09) was recorded at site 10, biomass (0.43 g/m^2±0.30) at site 8 and average weight (32.9 g/ind.±27.3) at site 1. The lowest were density (0.002 ind./m^2±0.002) and biomass (0.067 g/m^2±0.08) at site 2 and average weight (8.9 g/ind.±8.7) at site 1. Significant statistical differences were found for the density, $H(9,160) = 47.68$, $P < 0.001$, the Z test showed that they were between the sites 1-8 ($P = 0.0103$), 1-9 ($P = 0.0031$) y 1-10 ($P = 0.0064$). Also, between sites 2-7 ($P = 0.0430$), 2-8 ($P = 0.0067$), 2-9 ($P = 0.0020$) y 2-10 ($P = 0.0041$). And between the sites 3-9 ($P = 0.0212$), and 3-10 ($P = 0.0398$). Likewise for biomass $H(9,160) = 29.36$, $P = 0.0006$, the Z test indicated that they were between the sites 1-8 ($P = 0.0414$), 2-5 ($P = 0.0475$) and 2-8 ($P = 0.0321$). The changes of these parameters in temporal scale are observed in Figure 4 (D, E, F). The average trends of these three parameters are similar over time. The highest averages in density (0.055 ind./m^2 ±0.08) and biomass (0.379 g/m^2±0.24) were presented in October 2015 and October 2018 respectively and for the average weight (22.2 g/ind.±21.8) in February 2015 and (22.4 g/ind.±27.46) in October 2017. No significant differences were found between months for these parameters ($P > 0.05$).

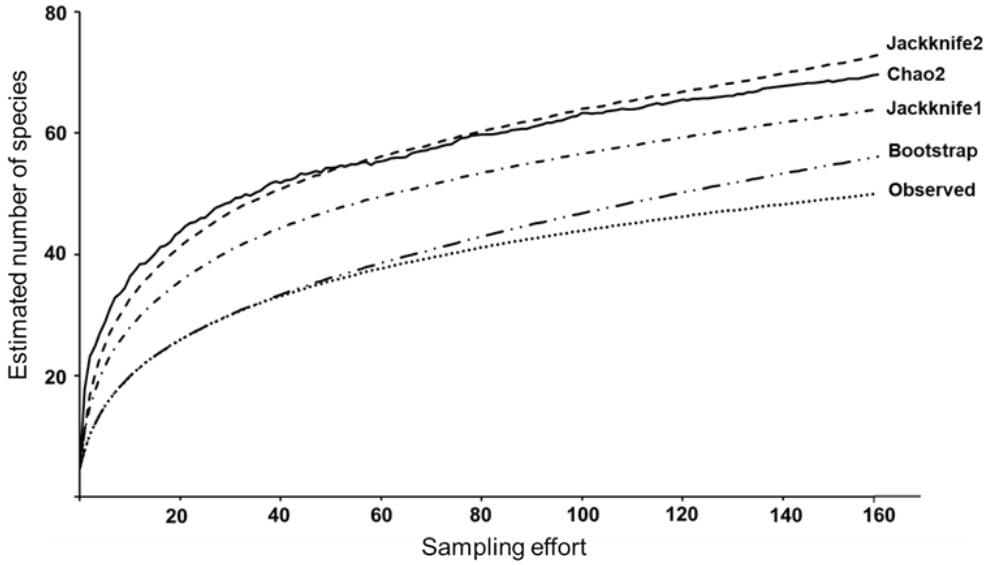

Figure 2. Estimated accumulation curves of fish species caught in the Sontecomapan Lagoon System. The trends of the different statistical tests calculated are indicated.

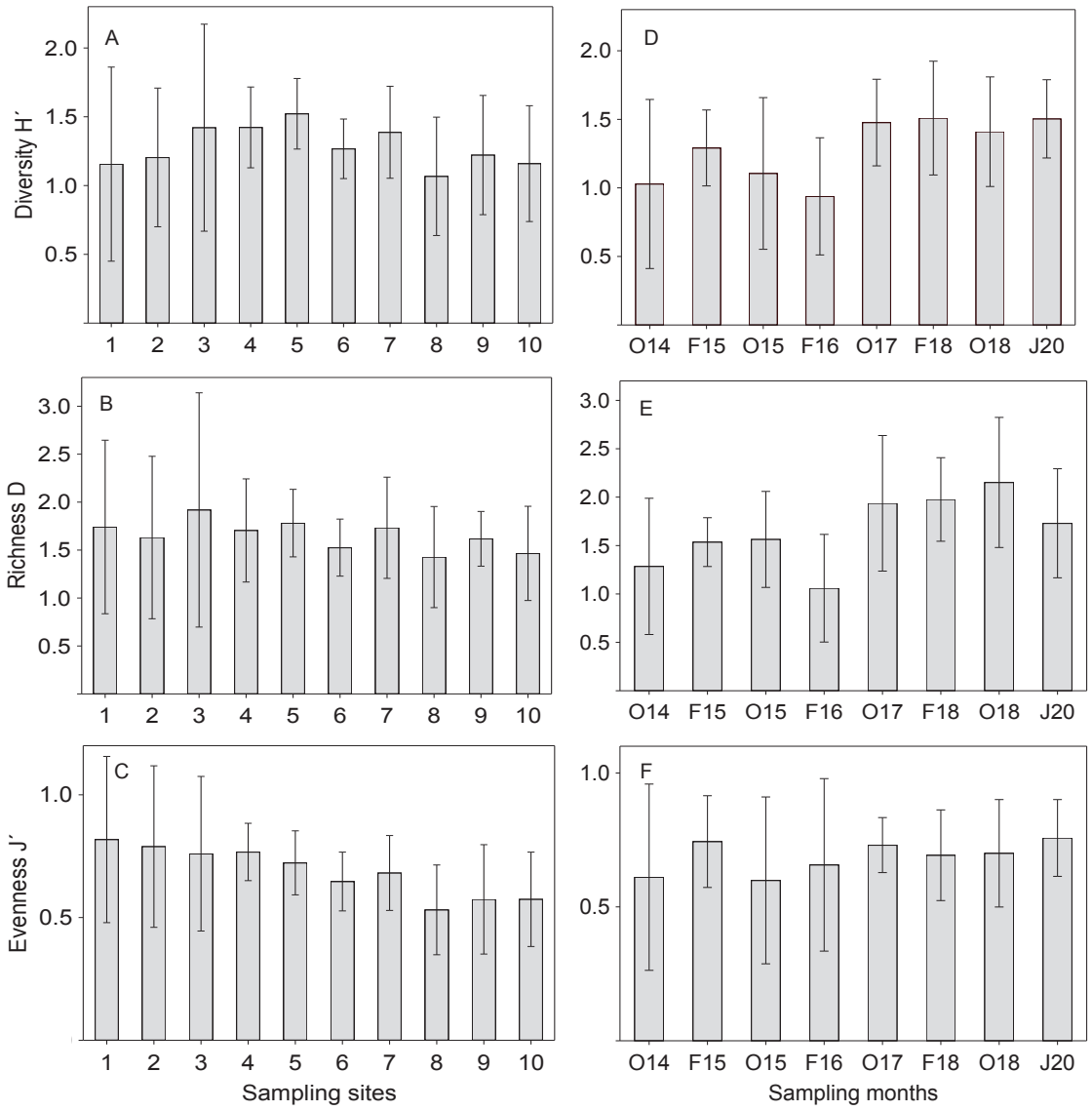

Figure 3. Spatial and temporal average values (± 1 SD) of the diversity indices H', D and J' for the fish community in the Sontecomapan Lagoon System.

Dominant species, community inhabitants and trophic categories: Two species were dominant, *Diapterus rhombeus* (Cuvier, 1829) (IVI = 92.7%) and *Cathorops aguadulce* (Meek,1904) (IVI = 55.9%) together represented 69.2% in number (4 789 individuals) and 53% in weight (40. 2 kg) of the total catch of the community, with frequencies of occurrence of 63 to 65% (Table 1). Other less abundant and smaller species that also contributed significantly to the community were *Eugerres plumieri* (Cuvier, 1830) and *D. auratus* Ranzani,1848. The behavior of the ecological inhabitants of the community is related to the environmental dynamics of this system, indicating that 23 species (46%) were marine estuarine-opportunists (MEO) such as *Anchoa lamprotaenia* Hildebrand, 1943, and 22 (44%) marine estuarine-dependent (MED) being the best represented *D. rhombeus* for its wide distribution in this lagoon. The estuarine species (ES) was represented by 4 species (8%) where *C. aguadulce* stands out with the widest distribution in the system. The only freshwater species (FS) collected was *Mayaheros uropthalmus* (Günther, 1862).

The fish community was composed of five trophic categories, 3 planktivorous species (6%) (ZP) being the most abundant *Anchoa lamprotaenia* Hildebrand, 1943, 1 detritivorous (DE) (2%) which was *Dormitator maculatus* (Bloch, 1792), 5 omnivores (OM) (10 %) such as *Gobionellus oceanicus* (Pallas, 1770) and *Archosargus probatocephalus* (Walbaum, 1792). Also 9 piscivores (PI) (18%), standing out for their abundance *Caranx crysos* (Mitchill, 1815) and *Hemicaranx amblyrhynchus* (Cuvier, 1833), as well as 32 zoobenthivores (ZB) (64%) such as *Achirus lineatus* (Linnaeus, 1758), *Centropomus parallelus* Poey, 1860 and *Lutjanus griseus* (Linnaeus, 1758).

Table 1. List of the fish species collected in the Sontecomapan lagoon system, Veracruz.

Families	Species	AB	Number	N %	Weight	W %	Freq. %	IVI (%)	CI	CT
Dasyatidae	*Hypanus sabinus* (Lesueur, 1824)	Hs	9	0.13	4946.65	6.51	1.03	7.67	MED	ZB
Engraulidae	*Anchoa hepsetus* (Linnaeus, 1758)	Ah	1	0.01	4.75	0.01	0.13	0.15	MED	PL
Engraulidae	*Anchoa lamprotaenia* Hildebrand, 1943	Aa	39	0.56	54.03	0.07	1.42	2.06	MEO	PL
Engraulidae	*Anchoa mitchilli* (Valenciennes, 1848)	Am	47	0.68	70.01	0.09	1.94	2.71	MED	ZB
Engraulidae	*Cetengraulis edentulus* (Cuvier, 1829)	Ce	19	0.27	45.20	0.06	0.26	0.59	MEO	ZP
Ariidae	*Ariopsis felis* (Linnaeus, 1766)	Af	117	1.69	2734.97	3.60	5.81	11.11	MED	ZB
Ariidae	*Cathorops aguadulce** (Meek, 1904)	Ca	891	12.88	22353.0	29.42	13.57	55.87	ES	ZB
Synodontidae	*Synodus foetens* (Linnaeus, 1766)	Sf	2	0.03	90.53	0.12	0.26	0.41	MEO	PI
Batrachoididae	*Opsanus beta* (Goode & Bean, 1880)	Ob	8	0.12	2263.20	2.98	1.03	4.13	MED	ZB
Mugilidae	*Mugil curema* Valenciennes, 1836	Mc	7	0.10	329.84	0.43	0.39	0.92	MED	OM
Belonidae	*Strongylura marina* (Walbaum, 1792)	Sm	4	0.06	229.15	0.30	0.39	0.75	MED	ZB
Belonidae	*Strongylura notata* (Poey, 1860)	Sn	7	0.01	68.45	0.09	0.13	0.23	MEO	ZB
Scorpaenidae	*Scorpaena plumieri* Bloch, 1789	Sp	1	0.01	286.90	0.38	0.13	0.52	MEO	ZB
Centropomidae	*Centropomus ensiferus* Poey, 1860	Cn	4	0.06	70.70	0.09	0.26	0.41	MEO	PI
Centropomidae	*Centropomus parallelus* Poey, 1860	Cp	58	0.84	1576.26	2.07	3.49	6.40	MED	ZB
Centropomidae	*Centropomus pectinatus* Poey, 1860	Ct	16	0.23	470.02	0.62	0.90	1.75	MED	ZB
Centropomidae	*Centropomus undecimalis* (Bloch, 1792)	Cu	26	0.38	984.20	1.30	1.94	3.61	MED	ZB
Carangidae	*Caranx crysos* (Mitchill, 1815)	Cc	21	0.30	310.06	0.41	1.03	1.75	MED	PI
Carangidae	*Caranx hippos* (Linnaeus, 1766)	Ch	1	0.01	22.14	0.03	0.13	0.17	MEO	PI
Carangidae	*Caranx latus* Agassiz, 1831	Cl	1	0.01	22.54	0.03	0.13	0.17	MEO	PI
Carangidae	*Chloroscombrus chrysurus* (Linnaeus, 1766)	Cy	2	0.03	3.40	0.00	0.26	0.29	MEO	PI
Carangidae	*Hemicaranx amblyrhynchus* (Cuvier, 1833)	Ha	7	0.10	24.37	0.03	0.78	0.91	MED	PI
Carangidae	*Selene vomer* (Linnaeus, 1758)	Sv	4	0.06	60.70	0.08	0.39	0.53	MEO	ZB
Lutjanidae	*Lutjanus analis* (Cuvier, 1828)	La	5	0.07	208.67	0.27	0.65	0.99	MEO	ZB
Lutjanidae	*Lutjanus apodus* (Walbaum, 1792)	Lp	3	0.04	52.90	0.07	0.39	0.50	MEO	ZB
Lutjanidae	*Lutjanus griseus* (Linnaeus, 1758)	Lg	34	0.49	1865.94	2.46	3.62	6.56	MED	ZB
Lutjanidae	*Lutjanus synagris* (Linnaeus, 1758)	Ls	8	0.12	136.34	0.18	0.65	0.94	MEO	ZB
Gerreidae	*Diapterus auratus* Ranzani, 1848	Da	622	8.99	3955.10	5.21	6.33	20.53	MEO	ZB

Families	Species	AB	Number	N %	Weight	W %	Freq. %	IVI (%)	CI	CT
Gerreidae	*Diapterus rhombeus** (Cuvier, 1829)	Dr	3898	56.36	17629.3	23.20	13.18	92.74	MED	ZB
Gerreidae	*Eucinostomus argenteus* Baird & Girard, 1855	Ea	64	0.93	349.43	0.46	1.81	3.19	MED	ZB
Gerreidae	*Eucinostomus gula* (Quoy & Gaimard, 1824)	Eg	42	0.61	581.87	0.77	1.94	3.31	MED	ZB
Gerreidae	*Eucinostomus melanopterus* (Bleeker, 1863)	Em	138	2.00	992.04	1.31	7.75	11.05	MED	ZB
Gerreidae	*Eugerres plumieri* (Cuvier, 1830)	Eu	505	7.30	9110.53	11.99	9.17	28.47	MED	ZB
Haemulidae	*Rhonciscus crocro* (Cuvier, 1830)	Rc	1	0.01	105.16	0.14	0.13	0.28	MEO	PI
Haemulidae	*Pomadasys ramosus* (Poey, 1860)	Pr	1	0.01	124.70	0.16	0.13	0.31	MEO	ZB
Sparidae	*Archosargus rhomboidalis* (Linnaeus, 1758)	Ar	1	0.01	59.20	0.08	0.13	0.22	MED	OM
Sparidae	*Archosargus probatocephalus* (Walbaum, 1792)	Ap	4	0.06	381.29	0.50	0.39	0.95	MEO	OM
Polynemidae	*Polydactylus octonemus* (Girard, 1858)	Po	14	0.20	267.54	0.35	0.78	1.33	MED	ZB
Sciaenidae	*Bairdiella ronchus* (Cuvier, 1830)	Br	10	0.14	457.48	0.60	0.65	1.39	MED	ZB
Cichlidae	*Mayaheros urophthalmus* (Günther, 1862)	Mu	1	0.01	14.00	0.02	0.13	0.16	FS	ZB
Eleotridae	*Dormitator maculatus* (Bloch, 1792)	Dm	3	0.04	19.23	0.03	0.13	0.20	ES	DE
Eleotridae	*Eleotris pisonis* (Gmelin, 1789)	Ep	1	0.01	0.50	0.00	0.13	0.14	ES	PI
Eleotridae	*Gobiomorus dormitor* Lacepède, 1800	Gd	2	0.03	121.40	0.16	0.26	0.45	ES	ZB
Gobiidae	*Gobionellus oceanicus* (Pallas, 1770)	Go	46	0.67	599.36	0.79	3.23	4.68	MEO	OM
Ephippidae	*Chaetodipterus faber* (Broussonet, 1782)	Cf	2	0.03	197.45	0.26	0.13	0.42	MEO	OM
Paralichthyidae	*Citharichthys spilopterus* Günther, 1862	Cs	152	2.20	1057.56	1.39	7.49	11.08	MED	ZB
Paralichthyidae	*Etropus crossotus* Jordan & Gilbert, 1882	Ec	1	0.01	3.43	0.00	0.13	0.15	MEO	ZB
Achiridae	*Achirus lineatus* (Linnaeus, 1758)	Al	64	0.93	612.85	0.81	4.26	6.00	MED	ZB
Achiridae	*Trinectes maculatus* (Bloch & Schneider, 1801)	Tm	7	0.10	29.60	0.04	0.52	0.66	MEO	ZB
Tetraodontidae	*Sphoeroides spengleri* (Bloch, 1785)	Ss	1	0.01	28.05	0.04	0.13	0.18	MEO	ZB
TOTALS			6916	100	75982.0	100	100	300.00		

Importance Value Index (IVI): * dominant species. Community inhabitant (CI): MED = marine estuarine-dependent, MEO = marine estuarine-opportunist, ES = estuarine species, FS = freshwater species. Trophic category (CT): PL = planktivore, OM = omnivore, DE = detritivore, PI = piscivore, ZB = zoobenthivore. Abbreviations (AB) used in Figure 5 are shown.

Fish-habitat assemblages: The canonical correspondence analysis (CCA) (Fig. 5) shows the relationship between abundance of species, environmental variables, and sampling sites. The first two ordination axes had a total accumulated variance of 52%: axis 1, 40% and eigenvalue of 0.263 and axis 2, 12% and eigenvalue of 0.081. The most important factors in the distribution of the species for axis 1 were transparency (r = 0.881), depth (r = 0.664) and salinity (r = 0.639). Those that had the highest correlation with axis 1 corresponded to the estuarine-opportunist and estuarine-dependent marine inhabitants (Table 1) with minimal abundance associated with site 1 (marine inlet) of the system as *Scorpaena plumieri* Bloch, 1789, *Caranx hippos* (Linnaeus,1766) and *Archosargus rhomboidalis* (Linnaeus,1758). Another group of species from the two previous inhabitants with lower to intermediate abundance were distributed in sites 2 to 4 (tidal channel) such as *Sphoeroides splengeri* (Bloch, 1785), *A. lamprotaenia*, *Synodus foetens* (Linnaeus, 1766), *C. faber* and *Strongylura marina* (Walbaum, 1792), as well as the four species of the genus *Lutjanus*, the most abundant being *L. griseus*. Also, the species of the genus *Eucinostomus*, highlighting *E. melanopterus* and others as *A. lineatus* and *Citharichthys spilopterus* Günther, 1862. For axis 2, the main factors were salinity (r = -0.528) and dissolved oxygen (r = 0.479). The species that showed correlation with axis 2 correspond to the marine estuarine-dependent and estuarine inhabitants with minimum to maximum abundance, as the dominant species *D. rhombeus* and *C. aguadulce*. Some species of this assemblage with low abundance were associated with sites 5 and 7 with intermediate to low salinities such as *Centropomus pectinatus* Poey, 1860, *C. parallelus*, *Bairdiella ronchus* (Cuvier, 1830), *Opsanus beta* (Goode & Bean, 1880), *Polydactylus octonemus* (Girard,1858) and *P. ramosus*. Other species distributed on the site 8 with intermediate dissolved oxygen values were *Gobiomorus dormitor* Lacepède,1800, *Trinectes maculatus* (Bloch & Schneider, 1801), *Cetengraulis edentulus* (Cuvier, 1829), *Strongylura notata* (Poey, 1860) and *D. maculatus*. Sites 6 and 9 associated with axis 1 and site 10 with axis 2, represent the environment with less salinity, transparency, and depth due to fluvial influence in which marine dependent or opportunist and freshwater species were distributed with minimal abundance such as *C. latus* Agassiz, 1831, *A. hepsetus* (Linnaeus, 1758), *Mugil curema* Valenciennes, 1836, *Etropus crossotus* Jordan & Gilbert, 1882 and *M. uropthalmus*.

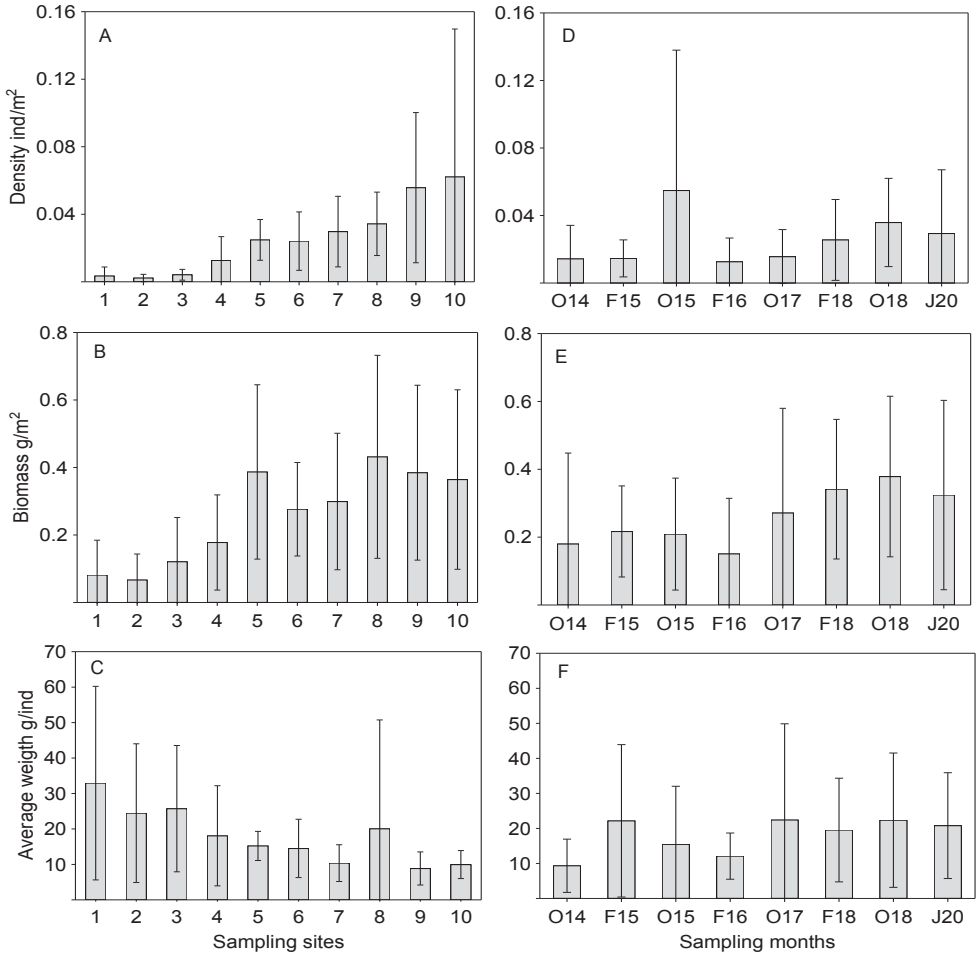

Figure 4. Spatial and temporal average values (± 1 SD) in density, biomass, and average weight for the fish community in the Sontecomapan Lagoon System.

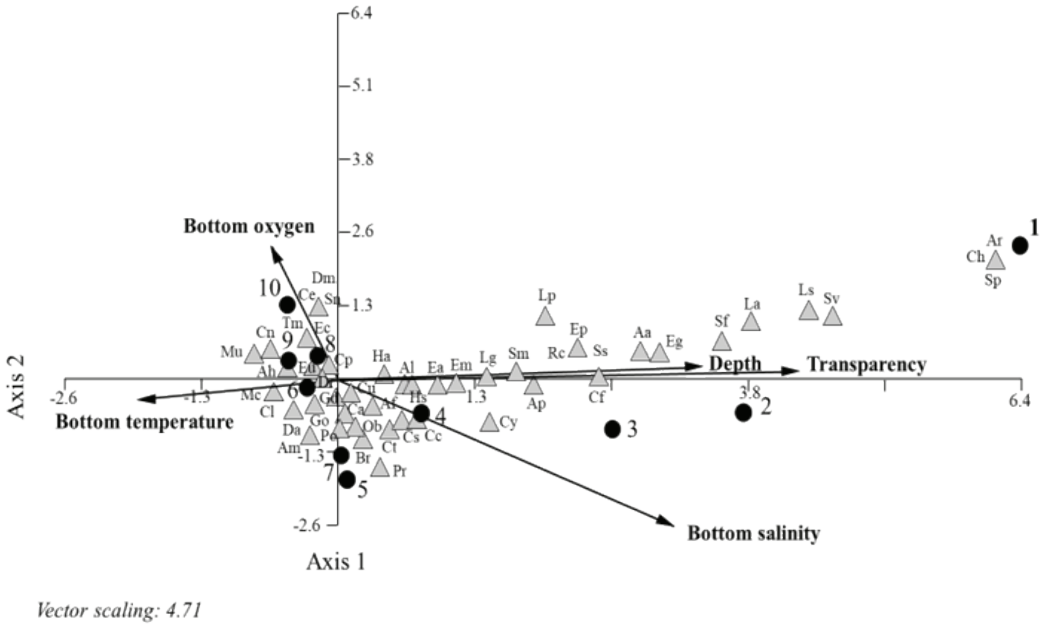

Figure 5. Canonical Correspondence Analysis Plot of species abundance, environmental variables, and sampling stations in the Sontecomapan Lagoon System. Arrow length and direction indicate the relative importance of environmental variables. Species abbreviations (AB) are listed in Table 1.

DISCUSSION

This paper integrates ecological information related to previous studies that have described the composition of fish species in the Sontecomapan Lagoon in different environments of this system (Aguirre-León *et al.*, 2018). This study not only updates species richness, but also provides new estimates of species distribution, diversity, and abundance at spatial and temporal scales. In addition, multivariate statistical methods are used to determine the influence of physicochemical variables on the spatial patterns of the parameters just mentioned, as well as to establish the behavior of species assemblages. This determined the importance of carrying out complementary studies on the ecological structure of the community in this coastal system.

The studies that have been carried out in Sontecomapan from 1983 to 2017 have only integrated lists of the species present with some annotations on the hydrology of the system without further ecological analysis (Rodríguez-Varela *et al.*, 2010; Castillo-Rivera & Lara-Domínguez, 2018). In this way, the present study increases the current knowledge about the richness of species in the Sontecomapan lagoon system, adding two new records to the system, *C. faber* and *P. ramosus*, so that the total reaches more than 120 species identified in 37 years. According to the models of the accumulation curves, the richness of species in this lagoon showed that the number of species collected during the sampling period was close to the estimated average value, which validates the fishing effort used in this study. However, the results suggest that the number of species in the Sontecomapan Lagoon can be increased by carrying out systematic sampling during the dry and rainy months, since a great variety of species use different habitats of the system for various biological activities and that they are related with the environmental changes in the different climatic seasons. In several studies it has been observed that environmental variables such as salinity and temperature have a great influence on the composition of species for different times of the year (Barletta *et al.*, 2003, 2005; Arceo-Carranza *et al.*, 2010; Díaz-Ruiz *et al.*, 2018). Therefore, it is suggested to continue with additional studies in this lagoon, integrating environmental factors with ecological and biological aspects of the species to have a more complete interpretation of the results obtained here.

In tropical lagoon systems, the use of various habitats by fish species is known, determined by complex responses to environmental and biological factors, which is reflected in seasonal changes in community structure such as diversity, abundance, and biomass of fish (Barletta *et al.*, 2005; Bouchereau *et al.*, 2008; Vilar *et al.*, 2011; Cowan *et al.* 2013; Aguirre-León *et al.*, 2020).

In this study, it was observed that the changes in diversity and species richness were similar with high values in the connection channel and the middle part of the system, where the greatest number of euryhaline and stenohaline species of the families Carangidae, Gerreidae, Engraulidae, Centropomidae and Lutjanidae occurred, which represent 48% of the species in the community. This behavior is linked to the influence of the tide and salinity variations, which was reflected in the decrease in diversity with the salinity gradient towards shallow and turbid areas of fluvial influence, but with high values in the abundance of species such as *D. rhombeus* and *C. aguadulce*. Authors such as Barletta *et al.* (2005), Velázquez-Velázquez *et al.* (2008), Vaslet *et al.* (2010), Cowan *et al.* (2013) have also observed this inverse relationship between evenness and dominance in other similar tropical systems. Diversity is also related to depth, freshwater discharge and precipitation that condition the hydrology and transparency of the lagoon (Aguirre-León *et al.*, 2014; Castillo-Rivera *et al.*, 2017; Molina *et al.*, 2020). During the months of study, the behavior of the diversity and richness of

species was also related to the seasonal variations of salinity and temperature, as well as the exchange of species of the marine component. This behavior of the mentioned indices was reflected in the significant differences found for H and D between the months of this study. This behavior is like that reported in other coastal lagoons of the Gulf of Mexico, where it has been found that the temporality of the diversity indices is also related to changes in the tide between day and night, the dynamics of nutrients and an increase in primary production (Díaz-Ruiz *et al*., 2003; Arceo-Carranza *et al*., 2010; Castillo-Rivera *et al*., 2017). It is considered that the values of diversity and richness of species in Sontecomapan are low or similar when compared to other coastal systems of Veracruz such as the Tamiahua lagoon (Díaz-Ruiz *et al*., 2003), the Chica-Grande lagoon (Aguirre-León *et al*., 2014), the La Mancha lagoon (Díaz-Ruiz *et al*., 2018) and the El Ostion lagoon (Aguirre-León *et al*., 2020). This downward trend in the values of the diversity indices of fish communities has been observed in the last 20 years on the coast of Veracruz (Aguirre-León *et al*., 2018).

Variations in abundance are also influenced by changes in the environmental conditions of the system and by seasonal migrations of the species during their life cycles (Arceo-Carranza *et al*., 2010; Medina-Gómez *et al*., 2015; Díaz-Ruiz *et al*., 2018; Aragón-Flores *et al*., 2021). Other authors such as Azevedo *et al*. (2007) and Arceo-Carranza *et al*. (2010), have reported similar abundance patterns in other coastal systems related to environmental conditions and the seasonal migration of species linked to their life cycles. In the present study, salinity, temperature, and dissolved oxygen were related to variations in the abundance of fish populations. In the Sontecomapan lagoon, the average values of density and biomass showed a similar trend in space with an inverse relationship to the salinity gradient. This behavior of the indicated density and biomass was reflected in the significant differences found for these parameters between sampling sites. This is due to the dominance of *D. rhombeus* and *C. aguadulce* in low salinity areas, in addition to other species with intermediate abundance such as *D. auratus*, *E. melanopterus*, *E. plumieri* and *C. spilopterus*, which together incorporate greater biomass to the environment of fluvial influence. In studies carried out in estuarine lagoon systems, a similar behavior has been observed in the abundance of fish species, indicating that salinity variations act as a "control" factor that determines the presence of dominant species (Barletta *et al*., 2008; Molina *et al*., 2020; Sosa-Lopez *et al*., 2007; Aguirre León *et al*., 2020; Castillo-Rivera & Morgado-Dueñas, 2022). The average weight was inverse to the previous indicators, in this case towards the environments with the highest salinity where species of greater size and weight with less abundance were recorded, such as *H. sabinus*, *A. felis* and several species of the Centropomidae family that use them depending on the biological stages that return to the system. Azevedo *et al*. (2007), Arceo-Carranza *et al*. (2010), Perera-García (2011), Hernández-Vidal *et al*. (2014), have reported similar abundance patterns in other coastal systems related to environmental conditions and the seasonal migration of species linked to their life cycles. On a temporal scale, it was observed that during the months of October the highest density is represented by *D. rhombeus* and *C. aguadulce* and the highest average weight by *E. plumieri*, *A. felis*, *H. sabinus* (Lesueur, 1824) and *O. beta* with individuals of large sizes and greater weight. The variations in abundance observed are due to the use of the different habitats regulated by climatic changes that control the biology of the species, so there are patterns of preferential use in environments with the greatest saline influence and freshwater influence, which is reflected in the structure and dynamics of the community (Able, 2005; Cowan *et al*., 2013; Perera-Garcia *et al*., 2011; Reyes-Ramirez *et al*., 2017).

The CCA analysis made it possible to integrate and interpret information on the ecological behavior of the different fish species that use the system during their life cycles linked to hydrological changes. The spatial and temporal distribution and abundance of fish is an expression of the adaptations that they have developed, whether they are dominant or rare species. The fish-habitat analysis shows how the different fish components respond to the environment, largely related to the gradients of environmental variables that they optimize at the different stages of their biological cycles, as well as to the specific nutritional needs of the fish (Barletta *et al*., 2005; Pérez-Rufaza *et al*., 2007; Cowan *et al*., 2013, Potter *et al.,* 2015). In Sontecomapan lagoon, studies on fish assemblages and their relationship with the environment are very scarce (Aguirre-León *et al*., 2018). In the present study, the information and ecological analysis on these is expanded, observing that of the 50 species that make up the community, the dominance of *D. rhombeus* and *C. aguadulce* prevails as marine estuarine-dependent and estuarine inhabitants, respectively, with zoobenthivores feeding habits. There is no retrospective information on the dominant species of Sontecomapan as part of their fish assemblages, however in other coastal systems of the Gulf of Mexico, these two species have been dominant in previous studies in recent decades (Aguirre-León & Díaz-Ruiz, 2000 y 2006; Pineda-Peralta *et al.,* 2016; Castillo-Rivera *et al*., 2017; Aguirre-León *et al*., 2020). The environmental factors that determine to a greater extent the structure of fish assemblages in Sontecomapan are salinity, transparency, and depth, to a lesser extent, dissolved oxygen, and temperature. The fish assemblages that are distributed according to the environments for the lagoon included in different proportions the different types of fish inhabitants recognized for coastal systems (Elliott *et al*., 2007; Potter *et al*., 2015). However, those of marine estuarine-opportunist and estuarine-dependent predominate and, to a much lesser extent, estuarine and freshwater species. These inhabitants are grouped into the five trophic categories present in the system, with omnivores and zoobenthivores predominating. Some species of the assemblages of this lagoon that characterized the environment of greater salinity and transparency like marine inlet and tidal channel, were the marine stenohaline and occasional species, *A. lamprotaenia*, *S. plumieri*, *S. spengleri*, and other marine euryhaline species with low abundance such as *Lutjanus apodus* (Walbaum, 1792), *L. synagris*, *Eucinostomus gula* (Quoy & Gaimard, 1855) and *Rhonciscus croco* (Cuvier, 1830). These species mainly belong to piscivores, zoobenthivores, and zooplanktivores consumers. In the environment with intermediate salinities and less transparency, the dominant species *D. rhombeus* and *C. aguadulce* predominated, and a significant proportion of marine euryhaline species where the species *C. parallelus* and *C. undecimalis* (Bloch, 1792) stand out as zoobenthivores. In the environment with the greatest fluvial influence, a combination was recorded mainly of estuarine, marine estuarine-dependent species and scarcely the freshwater ones, being omnivorous and detritivorous consumers. The presence of such fish assemblages with similar proportions among community components, consumer types and dominant species have also been observed in several coastal systems of the Gulf of Mexico (Sheridan & Minello, 2003; Pérez-Rufaza *et al*., 2007; Arceo-Carranza *et al*., 2010; Aguirre-León *et al*., 2014; Arceo-Carranza & Chiappa-Carrara, 2015; Castillo-Rivera *et al.,* 2017; Díaz-Ruiz *et al*., 2018; Aguirre-León *et al*., 2020).

The analysis of the results in the Sontecomapan lagoon allows us to visualize changes in the structure and function of the fish community related to the use of habitats at different times by the species. These patterns of use during their life cycles are related to the hydrological and climatic changes of the ecosystem. It is recognized that knowledge of the integrated environmental and ecological processes is extremely important to understand the health status of the system. This is based on spatial and temporal patterns of richness, diversity, and abundance, especially for this lagoon that lacks systematic information, despite being part of a Biosphere Reserve. Therefore, the Sontecomapan lagoon system requires more information that contributes to the conservation of fish diversity in the future as a protected natural area.

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Diatom diversity and species composition in phytoplankton, sediment traps, and surface sediments from a warm monomictic tropical lake

Diversidad de diatomeas y composición de especies en fitoplancton, trampas de sedimento, y sedimentos superficiales de un lago tropical cálido monomíctico

Eloy Montero[1], Margarita Caballero[2*], Gabriela Vázquez[1], Mario E. Favila[3]

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[1] Red de Ecología Funcional, Instituto de Ecología A.C. Carretera Antigua a Coatepec 351, El Haya, Xalapa, Veracruz, 91070. México.

[2] Laboratorio de Paleolimnología, Instituto de Geofísica, Universidad Nacional Autónoma de México. Ciudad Universitaria, Ciudad de México, 04510. México.

[3] Red de Ecoetología, Instituto de Ecología A.C. Carretera Antigua a Coatepec 351, El Haya, Xalapa, Veracruz, 91070. México.

***Corresponding author:**
Margarita Caballero: maga@igeofisica.unam.mx

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ABSTRACT

Background: Diatom assemblages in sediments are frequently used as water quality and paleoenvironmental indicators. However, sedimentary diatom assemblages may present taphonomic biases due to processes occurring in the water column and water-sediment interface. **Objective**: The present study aimed to determine if in tropical deep lakes, the differences between water column, sediment trap and surface sediment samplings were large enough to provide antagonistic interpretations. It also aimed to determine if diversity metrics would be statistically different between the three kinds of samples. **Methods**: This study was performed in Lake Alberca de Tacámbaro, Michoacan, Mexico, and involved the comparison of diatom species composition and diversity between phytoplankton, sediment trap and surface sediment samplings. **Results**: Nearly 80% of the diatom species, including the five most abundant taxa, were present in the three kinds of samples. Phytoplankton and sediment trap samplings documented the seasonal dynamics and indicated that the changes in species composition and diversity metrics were associated with the mixing and stratification processes of the water column. Unexpectedly, phytoplankton and sediment trap samples had relatively high percentages (ca. 20%) of benthic taxa (*Achnanthidium minutissimum* and *Brachysira vitrea*), which behaved as tychoplanktonic. Surface sediment samples showed a higher species richness and Simpson's diversity, but the three kinds of samples had similar Shannon diversities. **Conclusions**: In spite of the differences between the sampling methods, they did not provide antagonistic results on the condition of the lake. Surface sediment samples showed richer and more equitable assemblages, including diatoms from different habits, with an average-time window of about two years. The discrepancies between the phytoplankton and surface sediment diatom assemblages are an indication of recent changes in the diatom flora of this lake.

Keywords: diatom community, diatom species richness, climatic change, eutrophic lake, Mexico.

RESUMEN

Antecedentes: La composición de diatomeas en sedimentos es una herramienta para evaluar calidad del agua y en la reconstrucción paleoambiental. Sin embargo, puede presentar sesgos tafonómicos debido a procesos en la interfaz agua–sedimentos. **Objetivo**: Determinar si en lagos tropicales profundos las diferencias entre muestreos de columna de agua, sedimentos de trampas y superficiales serían lo suficientemente grandes para aportar interpretaciones contradictorias sobre la condición del ecosistema y establecer si existen diferencias significativas en las métricas de diversidad. **Métodos**: En la Alberca de Tacámbaro, Michoacán, México, se comparó de la composición y métricas de diversidad de diatomeas en muestras provenientes de los tres tipos de muestreo. **Resultados**: Casi el 80% de las especies, incluyendo las cinco más abundantes, estuvieron presentes en los tres tipos de muestreo. El fitoplancton y trampas de sedimentos documentaron la dinámica estacional, con cambios asociados con la mezcla y estratificación de la columna de agua. ILas muestras de fitoplancton y de trampas de sedimento tuvieron un porcentaje elevado (~20%) de especies de bentónicas (*Achnanthidium minutissimum* y *Brachysira vitrea*), que se comportan como ticoplanctónicas. Los sedimentos superficiales mostraron una mayor riqueza de especies y diversidad de Simpson, los tres

métodos tuvieron valores similares de diversidad de Shannon. **Conclusiones**: Los tres tipos de muestreo no aportaron interpretaciones antagonistas sobre la condición del lago. Los sedimentos superficiales mostraron una riqueza de especies mayor y más equitativa con diatomeas de varios hábitos y zonas del lago, integrando una ventana de tiempo promedio de aproximadamente dos años. Las discrepancias entre las muestras del fitoplancton y los sedimentos superficiales parecen ser una indicación de cambios recientes en la flora de diatomeas de este lago.

Palabras clave: comunidad de diatomeas, riqueza de especies de diatomeas, cambio climático, lago eutrófico, México.

INTRODUCTION

Diatoms are eukaryotic algae with siliceous cell walls (frustules) that inhabit a wide range of lacustrine habitats, where they may occur as planktonic, benthic, or tychoplanktonic organisms (Battarbee *et al.*, 2001; Cohen, 2003). Their frustules can be preserved when they are incorporated into lake sediments, making them one of the most valuable fossils for paleolimnological studies (Bradbury & Krebs, 1995; Cohen, 2003). Due to their specific preferences and tolerances for environmental conditions, sedimentary diatoms have been extensively used as bioindicators of water quality in freshwater ecosystems (Halland & Smol, 2010; Kireta *et al.*, 2012) and have also been used to track long-term ecological perturbations such as deforestation, lake eutrophication, climate change, and surface water acidification (Winder *et al.*, 2009; Quillen *et al.*, 2013; Battarbee *et al.*, 2014; Caballero & Vázquez, 2019; Liao *et al.*, 2020), among others.

In recent decades there has been an increasing interest in using paleolimnological data sets to address long-term ecological processes in lakes (Gregory-Eaves & Beisner, 2011). This approach, however, arises questions about how faithful are sedimentary diatom assemblages reflecting changes in the diatom community of the water column, to allow for coupling studies of modern and fossil diatom assemblages (Battarbee *et al.*, 2005), as it is well known that sedimentary diatoms may be altered by different processes occurring in the water column – sediment interface, such as zooplankton grazing, variable settling rates, lateral transport of sediments, and frustule fragmentation and dissolution (Kato *et al.,* 2003; Ryves *et al.,* 2003; Battarbee *et al.*, 2005; Ryves *et al.*, 2013). Furthermore, sedimentary diatom assemblages usually integrate not only planktonic diatoms but also taxa with different habitat types (Gregory-Eaves & Beisner, 2011), and therefore can give information on processes occurring not only in the water column, but also in other micro-environments within the lake. The potential of sedimentary diatoms to have a deeper time perspective of ecological processes justifies an in-depth comparative analysis of the diatom assemblages preserved in sediments compared to the diatom community present in the water column. This kind of comparison can be made using different sampling techniques such as phytoplankton, sediment traps, and surface sediment sampling (Battarbee *et al.*, 2005; Ryves *et al.*, 2013). These methods have their own advantages and limitations as each may present sampling or taphonomic biases (Behrensmeyer & Kidwell, 1985; Kato *et al.*, 2003; Dubelaar *et al.*, 2004; Battarbee *et al.*, 2014; Hassan & Diaz, 2023; Hofmann *et al.*, 2020) but, are these large enough so that they could provide antagonistic interpretations for the same ecosystem?

Most studies performed in inland waters comparing diatom assemblages from phytoplankton and sediments have been conducted in temperate zones. Only a few have been performed in tropical regions, where the lake mixing regime can lead to diatom populations dynamics that can be contrastingly different compared to temperate lakes (Caballero *et al.*, 2006; Vázquez & Caballero, 2013; Caballero *et al.*, 2016). Therefore, this work aimed to contribute to the evaluation and comparison of the diatom diversity and species composition observed from phytoplankton, sediment traps, and surface sediment samplings along two annual cycles in a deep tropical lake. We hypothesized that because surface sediment samples have a degree of temporal and spatial integration, their assemblages would include diatoms from different habits, leading to a higher species richness and Shannon diversity, but lower dominance when compared to the diatom assemblage from phytoplankton net samples or monthly sediment trap samples. The lake chosen for this study was Lake Alberca de Tacámbaro, a tropical crater in Western Mexico, which was sampled for two years. Previous studies have found that the diatom flora of this lake responds to changes in the environmental conditions and trophic status (Caballero *et al.*, 2016; Caballero & Vázquez, 2019; Montero *et al.*, 2021), making it an interesting lake to undertake comparative studies of data obtained from the living diatom community in the water column and from diatom assemblages in sediments

MATERIALS AND METHODS

Study site. Lake Alberca de Tacámbaro is located in the south-west region of the Trans-Mexican Volcanic Belt (19° 12' 40.56" N, 101° 27' 28.59" W, 1460 masl; Fig. 1). This is a eutrophic, freshwater crater lake that has a maximum depth of 28 m and presents a warm monomictic stratification pattern. The lake shows a short mixing period in winter (January-February), an early shallow stratification period in spring (March-June), a full stratification period in summer (June-September), and a late, deep stratification period in autumn (September-December) (Caballero *et al.*, 2016; Caballero & Vázquez, 2019; Montero *et al.*, 2021). The main morphometric and environmental characteristics of this lake are summarized in Table 1.

Fieldwork. Phytoplankton, sediment traps, and surface sediment samplings were conducted during winter (mixing), spring (early stratification), summer (full stratification), and autumn (late stratification) in 2018 and 2019 (Table 2), at the zone of maximum depth (25 - 28 m) around 10:00 am (UTC -06:00), as previously described in Caballero & Vázquez (2020) and Montero *et al.* (2021).

On each occasion, phytoplankton samples were collected at seven depths (0, 5, 8, 10, 15, 20 and 25 m depth) with a Van Dorn bottle, and then fixed with Lugol's iodine solution. Also, a system with two tubular sediment traps (aspect ratio: 12) supported by a buoy and separated from the bottom of the lake by about 1 m, was installed at the zone of maximum depth of the lake (> 25 m). The buoy of the sediment traps was always left 2 to 3 m below the lake surface to avoid vandalism or theft by visitors. When the traps were installed, the GPS (GARMIN 64S) location of the buoy was recorded, and a "rodeo" sampling technique was developed to recover them. This method used two inflatable boats, one of which was anchored at the GPS location of the sediment traps while the second circled the first one with a long rope that had equally spaced weights. The loops trapped the buoy and allowed to recover it

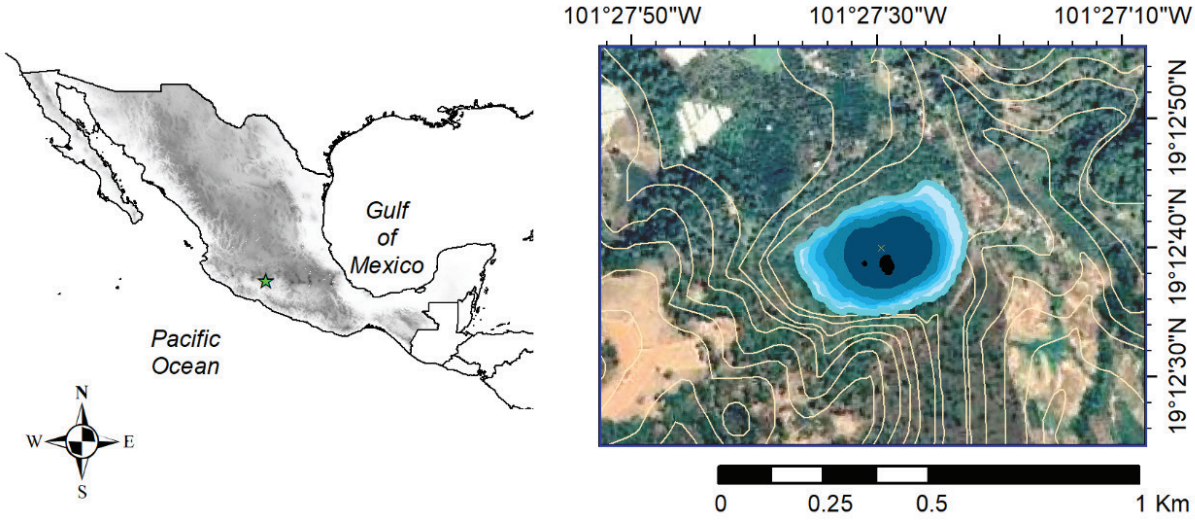

Figure 1 Digital elevation model of Mexico and satellite imagery with topography highlighting the location of Lake Alberca de Tacámbaro (green star) at the south-west region of the Trans-Mexican Volcanic Belt.

when pulling. The accumulated sediments were collected at time intervals ranging from 84 to 126 days from one of the two traps. In the second trap, the accumulated sediments were collected annually, from September 2018 to August 2019. Surface sediments from the central part of the lake were also collected on each occasion from the top 1 cm of the surface layer of sediments using an Ekman dredge. The dredge was recovered with a slow but constant upward movement to avoid sediment disturbance and/or washout. The information on the sampling dates and characteristics of samples from phytoplankton, sediment trap, and surface sediment, are shown in Table 1.

Diatom abundance (cells mL^{-1}) in phytoplankton samples was estimated following Utermöhl's method (Lund *et al*., 1958) by using sedimentation chambers (2.5 – 10 mL, 24 h) and an inverted microscope at 400x (maximum magnification available). When counting, there was no discrimination between living and dead diatoms. For each sampling, diatom abundances at the different strata of the water column were averaged to obtain an integrated phytoplankton sample that included all the sampled depths; cell densities were expressed as relative abundances (%).

Sediment samples (traps and dredges) were freeze-dried, and 0.5 g of dry sediment (gds) were cleaned with HCl (10%) and H_2O_2 (30%) until all carbonates and organic matter were eliminated. Diatom slides were made using Naphrax® as the mounting medium and minimum counts of 400 valves were made under the optical microscope (Olympus BX50) at 400x. Based on these counts, diatom valve concentration (valves per gram of dry sediment [v gds^{-1}]) and diatom species relative abundances were estimated.

Diatom species were identified based on specialized literature (Krammer & Lange-Bertalot, 1997; 1999; 2000); taxonomic classification was based on AlgaeBase (Guiry & Guiry, 2022). Due to the limited magnification in the inverted microscope, *Aulacoseira granulata* var. *angustissima* (O. Müller) Simonsen could not be distinguished from the nominal variety (*A. granulata* (Ehrenberg) Simonsen), and *Discostella pseudostelligera* (Hustedt) Houk & Klee could not be distinguished from *D. stelligera* (Cleve & Grunow) Houk & Klee; therefore, these forms were counted together and treated as species groups: *A. granulata* and *D. stelligera/pseudostelligera*. All diatom species were classified according to their habit into planktonic (centric taxa and needle shaped arraphids), tychoplanktonic (short, arraphid taxa) and benthic (mono and biraphid taxa).

Diversity metrics. Alpha diversity was evaluated using the effective number of species method, which is equivalent to Hill's numbers (Hill, 1973). The diversity of order 0 (^0D) is insensitive to species frequencies and represents species richness. The diversity of order 1 (^1D) is equivalent to the exponential of Shannon's entropy and accounts for the most common species in a community (Shannon's diversity). The diversity of order 2 (^2D) is equivalent to the inverse of Simpson's index and gives more weight to dominant species (Jost, 2006). The sample completeness for each sampling (and sampling method) was evaluated with the iNEXT package in R studio (Chao *et al*., 2014; Hsieh *et al*., 2016; R Core Team, 2019). Additionally, dominance-diversity curves were constructed to analyze and compare diatom abundance distribution patterns in phytoplankton, sediment trap, and surface sediment samples. Dominance-diversity figures were based on the eight most-abundant diatoms for each sampling.

Beta diversity (variation in species composition) for the different diatom assemblages was measured as the overall dissimilarity among sites using the multiple-site Sørensen dissimilarity index (β_{SOR}). This dissimilarity index ranges from 0 to 1, where 0 indicates a null dissimilarity among sites, and 1 indicates a total dissimilarity among sites. The β_{SOR} index can be partitioned into two components (Baselga, 2012): dissimilarity due to species replacement (or Simpson dissimilarity, β_{SIM}) and dissimilarity due to nestedness (β_{NES}); both dissimilarity components were calculated. Beta diversity was also evaluated using the Whittaker's method, which represents the number of different communities in the dataset (Whittaker, 1960). Beta diversity analyses were computed based on presence/absence datasets in the betapart package of R studio (Baselga & Orme, 2012; R Core Team, 2019).

Table 1 Main morphometric and environmental characteristics of Lake Alberca de Tacámbaro. Environmental parameters are shown as average values along the water column for the mixing and stratification periods during 2018 and 2019.

Morphometric parameter	
Crater major axis[a]	0.7 km
Crater minor axis[a]	0.5 km
Crater (catchment) area[a]	0.3 km^2
Lake area[a]	8.2 ha
Maximum depth[a]	28 m
Mean depth[a]	13 m

Environmental parameter	Period			
	Mixing	Early stratification	Full stratification	Late stratification
Trophic status[b]	Eutrophic - hypertrophic			
Euphotic zone depth (m)[b]	7.2	7.8	8.0	5.6
Water temperature (°C)[b]	18.1	19.7	20.6	19.7
Dissolved oxygen (mg L^{-1})[b]	3.5	3.1	2.6	3.3
pH[b]	7.1	7.2	7.4	7.1
Chlorophyll *a* (µg L^{-1})[b]	11.7	10.1	12.5	10.8
Dissolved inorganic nitrogen (µM)[b]	186	193	164	165
Soluble reactive phosphorus (µM)[b]	1.4	2.2	8.3	1.9
Silica (SiO_2 µM)[b]	866	894	857	870

aData obtained from Caballero *et al.*, 2016. bData obtained from Montero *et al.*, 2021.

Sediment characterization. Total carbon (TC) was determined using a Thermo Scientific™ Flash 2000 soil analyzer. The remaining percentage represents the silicate fraction composed of diatom frustules and terrigenous material and is used here to describe the sediment composition.

Statistical and numerical analysis. Analyses of variance (ANOVA) were performed to determine whether diversity measures (^0D, ^1D, ^2D) were significantly different in phytoplankton, sediment trap, and surface sediment samples; when significant differences were found, multiple comparison tests (Tukey test) were performed to identify the differences. Wilcoxon tests (95 % confidence level) were computed to determine whether the percentage of the silicate fraction, and valve concentration were statistically different between trap and surface sediments. A cluster analysis was conducted to compare the diatom assemblages from phytoplanktonic, sediment trap, and surface sediment samples. A dissimilarity matrix was calculated using the percentage similarity method, and the dendrogram was generated by the unweighted pair-group method using arithmetic averages (UPGMA) (Borcard *et al.*, 2018). Singletons were removed from the analysis (Legendre & Legendre, 1998). The cluster analysis and dendrogram were performed using the packages 'vegan' and 'ggdendro' in R Studio version 4. 1. 0 (R Core Team, 2019).

RESULTS

Diatom diversity, habit, and species composition. A total of 40 diatom taxa were recorded in all the samples, 11 were planktonic, 28 were benthic, and one was tychoplanktonic. Thirty-one of these taxa (80%) were present in the three kinds of samples (see Table 3 for taxa names and authors). Sample coverage was higher than 0.9 in all samples, which indicates that more than 90% of the individuals in the diatom community were represented in the samples.

Diversity metrics in phytoplankton samples indicated that diatom species richness (^0D) ranged from 14 to 19 (average 16) and Shannon's diversity (^1D) indicated that the number of equally common diatoms ranged from 1 to 6 (Fig. 2A). Simpson's diversity (^2D) highlighted that the number of dominant diatoms in phytoplankton varied from 1 to 3. Beta diversity (measured as β_{SOR}) was 0.55, and species replacement (or turnover) accounted for 85 % of the overall dissimilarity ($\beta_{SIM} = 0.47$). Beta diversity using Whittaker's method was 2.01, which indicates that two main alternating species associations were present in the phytoplankton. Regarding species composition, phytoplankton samples evidenced the seasonal dynamics of the planktonic diatom community, which was highly dominated by *Aulacoseira granulata* in the mixing and early stratification periods, where its abundance and the associated chlorophyll *a* concentration (>1000 cells mL^{-1} and > 20 µg L^{-1}, data not shown) were characteristics of algal blooms (Alcocer & Lugo, 2003; Chorus & Welker, 2021). On the other hand, *Discostella stelligera/pseudostelligera,* in association with *Pantocsekiella ocellata* and *Brachysira vitrea,* were the dominant diatoms in full and late stratification, and *Achnanthidium minutissimum* dominated once, during late stratification (Fig. 2B). The diatom assemblages from phytoplankton samples were dominated by planktonic species (82% on average), but they also included benthic taxa (17% on average of total abundance).

In sediment traps, diatom species richness (^0D) ranged from 17 to 24 (average 20), including the annual trap where 23 taxa were present. The number of equally common diatoms (Shannon's diversity ^1D) in sediment trap samples ranged from 1 to 6 (including the annual trap where it was 6), and the number of dominant diatoms (Simpson's diversity ^2D) ranged from 2 to 4 (including the annual trap where it was 4) (Fig.

2A). Beta diversity (β_{SOR}) was 0.49, with species replacement accounting for 83 % of the overall dissimilarity (β_{SIM} = 0.41). The beta diversity calculated with Whittaker's method (1.7) was slightly lower than in the phytoplankton. Diatom assemblages in samples from sediment traps were similar to those observed in the phytoplankton, with *A. granulata* being the dominant species in the intervals that included the mixing and early stratification periods, and with *D. stelligera/pseudostelligera*, in association with *P. ocellata*, and *B. vitrea* having high abundances during the intervals that included the full and late stratification period. *Achnanthidium minutissimum* was always present in these samples but it never reached the highest abundances (Fig. 2C). The annual sediment trap showed an assemblage where *A. granulata* was the most abundant species, closely followed by *D. pseudostelligera, B. vitrea, A. minutissimum,* and *P. ocellata,* which together contributed to approximately 95% of total valve concentration (Fig. 2C). The diatom assemblages in sediment trap samples were mostly constituted by planktonic species, which accounted on average for 80%; benthic and tychoplanktonic species were also present and accounted for 19% and 0.5% respectively.

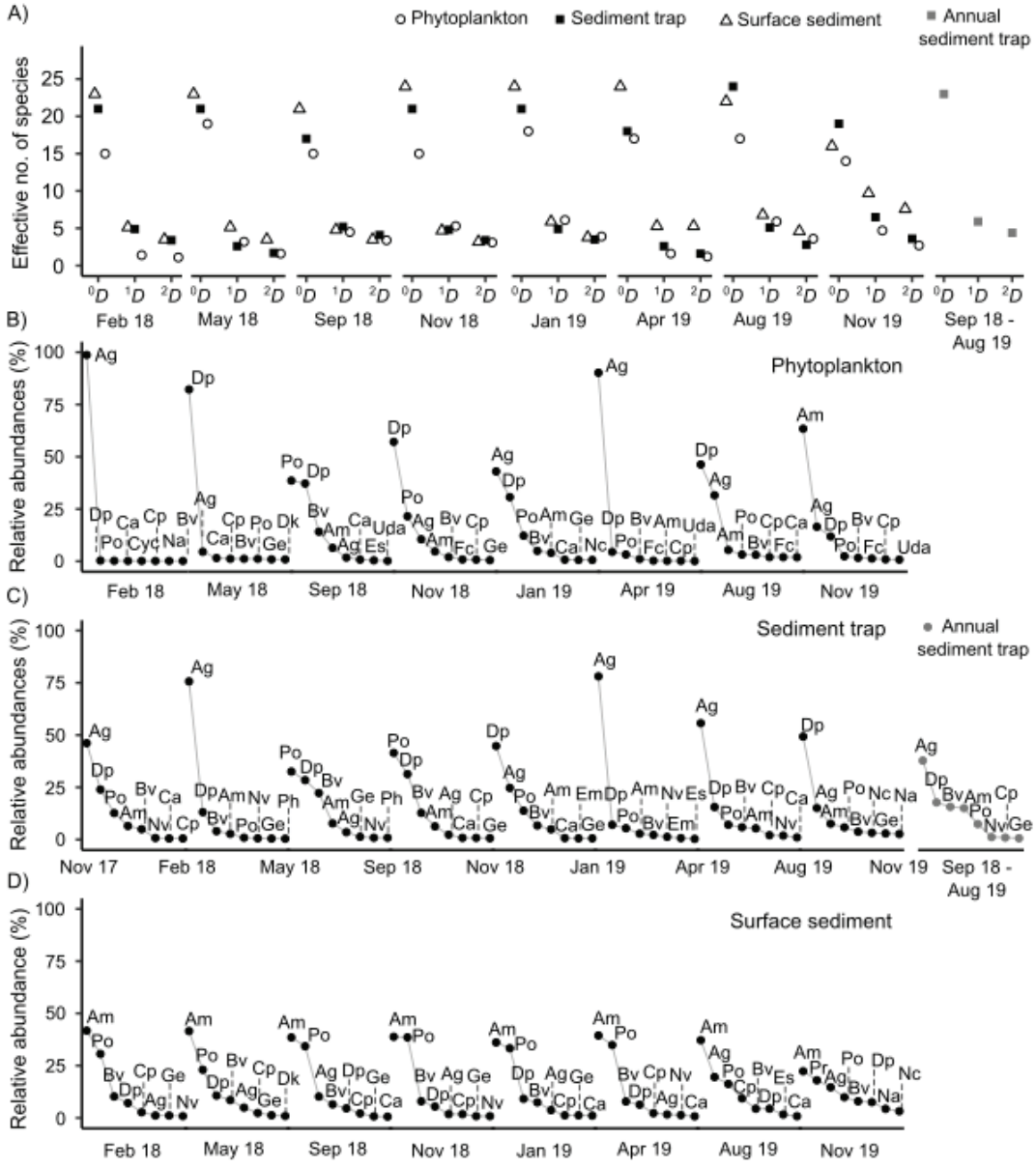

Figure 2 Diatom diversity and species composition in Lake Alberca de Tacámbaro during the period February 2018 – November 2019. A) Diversity measures: species richness (^0D), Shannon's diversity (^1D), and Simpson's diversity (^2D). Dominance-diversity curves for the diatom assemblage in samples from B) phytoplankton, C) sediment trap and D) surface sediments. Ag = *Aulacoseira granulata*; Am = *Achnanthidium minutissimum*; Bv = *Brachysira vitrea*; Ca = *Cymbella affiniformis*; Cp = *Cocconeis placentula*; Cyc = *Cyclotella* sp.; Dk = *Denticula kuetzingii*; Dp = *Discostella pseudostelligera*; Es = *Encyonema silesiacum*; Em = *Encyonopsis microcephala*; Fc = *Fragilaria crotonensis*; Ge = *Gogorevia exilis;* Na = *Nitzschia amphibioides*; Nc = *Navicula cryptotenella*; Nv = *Navicula veneta*; Ph = *Psammothidium helveticum*; Po = *Pantocsekiella ocellata*; Pr = *Planothidium rostratum*; Ud = *Ulnaria delicatissima*; Uda = *U. delicatissima* var. *angustissima*.

Table 2 List of sampling dates and characteristics of samples from phytoplankton, sediment traps and surface sediments.

Phytoplankton samples	
Sampling date	Sampling characteristics
02/17/2018	Average of 7 depths along the water column (0, 5, 8, 10, 15, 20 and 25 m).
05/25/2018	
09/01/2018	
11/24/2018	
01/26/2019	
04/27/2019	
08/31/2019	
11/23/2019	

Sediment trap samples	
Sampling date interval	Number of days
11/19/2017 – 02/17/2018	90
02/17/2018 – 05/25/2018	97
05/25/2018 – 09/01/2018	99
09/01/2018 – 11/24/2018	84
11/24/2018 – 01/26/2019	63
01/26/2019 – 04/27/2019	91
04/27/2019 – 08/31/2019	126
08/31/2019 – 11/23/2019	84
09/01/2018 – 08/31/2019	364

Surface sediment samples	
Sampling date	Sampling characteristics
02/17/2018	Collected from the top 1 cm of sediment.
05/25/2018	
09/01/2018	
11/24/2018	
01/26/2019	
04/27/2019	
08/31/2019	
11/23/2019	

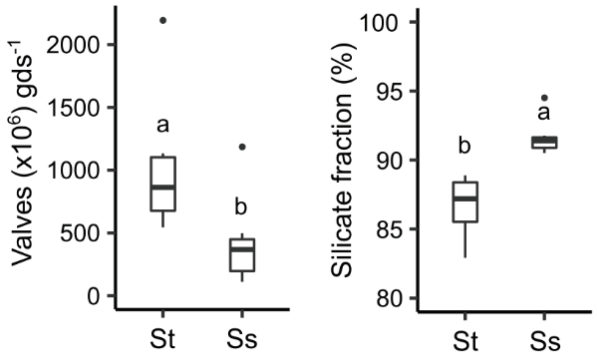

Figure 3 Diatom valves (x106 gds^{-1}), and silicate fraction in trap (St) and surface sediments (Ss). Superscript letters (a, b) indicate significant differences.

Finally, surface sediments had the highest species richness ($p < 0.05$), ranging from 16 to 24 (average 22) (Fig. 2A). Shannon's diversity (^1D) varied from 4 to 7 and these values were statistically similar ($p > 0.05$) to those found in phytoplankton and sediment traps. The number of dominant species was also significantly higher ($p < 0.05$) in surface sediment samples ranging from 3 to 7 (Fig. 2A). A lower beta diversity was observed in these diatom assemblages ($\beta_{SOR} = 0.45$), where species replacement accounted for 77 % of the overall dissimilarity. Whittaker's beta diversity was also the lowest (1.5). The diatom assemblage was dominated by *A. minutissimum* closely followed by *P. ocellata*, which together contributed to 50 – 70% of the total valve counts (Fig. 2D). *Aulacoseira granulata* was always present in the surface sediment assemblage, but it reached higher abundances in the late stratification periods, following the early stratification blooms recorded in the phytoplankton and trap sediments. *Discostella stelligera/pseudostelligera* and *B. vitrea* were also constantly present in the surface sediment assemblages, and less frequently, *Cocconeis placentula* and *Planothidium rostratum*. The diatom assemblages in surface sediment samples were characterized by a higher proportion of benthic species (57% on average), but with an important presence of planktonic taxa (42% on average).

Trap and surface sediment characteristics. The average diatom valve concentration in trap sediments was 1000 ± 530 x10^6 v gds^{-1} and the silicate fraction was 88% (Fig. 3). The valve concentration of the most abundant diatoms in the sediment samples is shown in figure 4 (diatoms with valve concentration ≥ 5% of total valve concentration in at least one sample). It can be observed that the sediment traps evidenced the seasonal dynamics of *A. granulata, D. stelligera/pseudostelligera,* and *P. ocellata* recorded in the phytoplankton samples. As mentioned above, the annual sediment trap was a compilation of the seasonal trap samples between September 2018 and August 2019, *A. granulata* was the most abundant diatom, followed by *D. pseudostelligera, B. vitrea, A. minutissimum,* and *P. ocellata*.

Surface sediments presented lower ($p < 0.05$) average diatom valve concentration (415 ± 340 x106 v gds^{-1}) than trap sediments but higher ($p < 0.05$) silicate fraction (92%) (Fig. 3). In contrast to trap sediments, high concentrations of *A. granulata* and *D. stelligera/pseudostelligera* were not observed in the surface sediment samples. Instead, *A. minutissimum* showed the highest valve concentration followed by *P. ocellata*. Other taxa such as *B. vitrea*, *C. placentula*, and *P. rostratum* had similar valve concentrations in trap and surface sediments (Fig. 4).

Diatom assemblage comparison. The cluster analysis based on diatoms relative abundance displayed four groups constituted by the combination of samples from phytoplankton and sediment traps, and one with a large majority of samples from surface sediments (Fig. 5). The first group was comprised by phytoplankton and sediment trap samples in which *A. granulata* was the dominant species. The second group included all the surface sediment samples and one phytoplankton sample, characterized by the high relative abundance of *A. minutissimum* and *P. ocellata*. The third group was represented by phytoplankton and trap samples (including the annual trap) in which *A. granulata*, and *D. stelligera/pseudostelligera,* were the main components of the diatom assemblage. Finally, the fourth group was formed by phytoplankton and sediment trap samples with dissimilarities ranging from 15 to 40 %, where *D. stelligera/pseudostelligera* was the main component of the diatom assemblage, with an important presence of *P. ocellata* and *B. vitrea.*

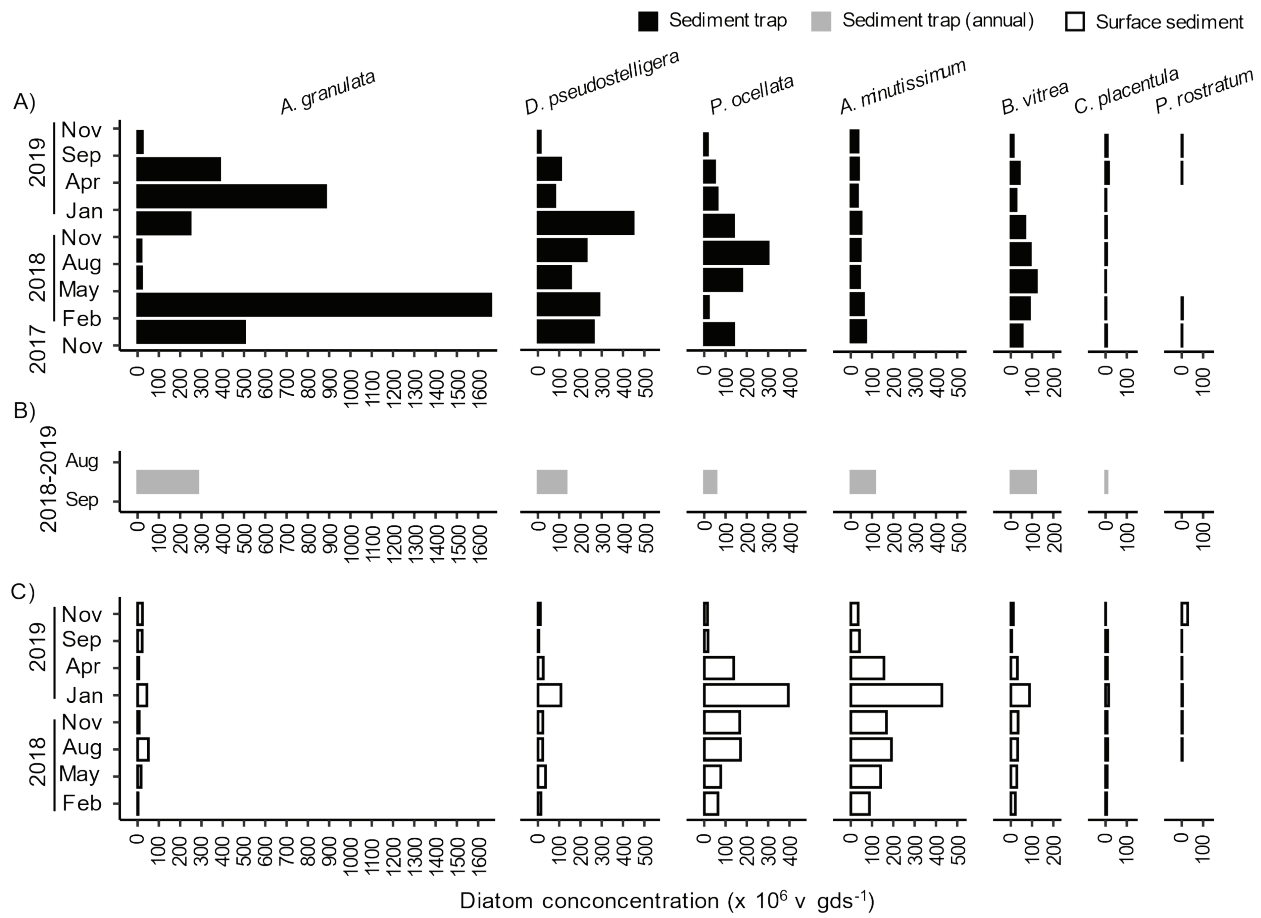

Figure 4 Diatom concentration (x 106 v gds^{-1}) through time of the most abundant diatoms (abundance ≥ 5 % in at least one sample) in sediment samples. Diatom concentration in sediments from seasonal trap (A), annual trap (B) and surface sediments (C).

DISCUSSION

The comparative assessment of the diatom assemblages in Lake Alberca de Tacámbaro allowed to determine that phytoplankton samples, sediment traps, and surface sediments samples provided a consistent spectrum of the diatom species composition and diversity in the study site. During the studied interval (2018 – 2019), 80% of the diatom species were present in the samples collected by the three sampling methods. Furthermore, the same five main taxa (*A. granulata, D. stelligera/pseudostelligera, B. vitrea, P. ocellata,* and *A. minutissimum*) were present in the three kinds of samples. These results showed that despite the intrinsic differences between the three sampling methods, these will not provide antagonistic results on the ecological condition of the lake.

Furthermore, the diatom assemblages obtained by the three sampling methods did not show significant differences in their Shannon's diversity, even though, as was hypothesized, the species richness (^0D) and Simpson´s diversity (^2D) were significantly higher in the surface sediment samples. Relating to the habit of the diatom taxa in each kind of samples, according to what was expected, the surface sediment samples had a relatively equitable split between planktonic (42%) and benthic (57%) forms, however, it was an unexpected result to find a relatively high proportion of benthic taxa (of nearly 20%) in the plankton and sediment trap samples. These included taxa such as *A. minutissimum* (particularly in Nov 2019) and *B. vitrea* (particularly in Sep 2018). The presence of these species in phytoplankton samples has been documented previously in this lake (Caballero *et al.*, 2016) and in other deep, warm-monomictic lakes in Mexico (Vázquez & Caballero, 2013). It is possible that these species are re-suspended from the bottom sediments where they live, but in this lake the photosynthetically active radiation penetrates to a maximum of about 12 m (Montero *et al.*, 2021). Therefore, the vast majority of the lake bottom (>70%) is inhospitable for diatoms, and strongly suggests that in spite of being raphe bearing taxa generally considered to have a benthic habit, these species can adopt a tychoplanktonic form of live, with floating strategies that allow them to survive in the plankton (Cantonati & Lowe, 2014; Cvetkoska *et al.*, 2018).

In addition, some studies have pointed out that benthic species usually dominate diatom assemblages from surface sediments because the concentration of planktonic species is frequently diluted by the horizontal transport of sediments (Buchaca & Catalan, 2007; Hofmann *et al.*, 2020). Considering that diatom valve concentration was higher in the trap sediments than in surface sediments, it is reasonable to assume that terrigenous materials account mostly for this difference

pointing to the existence of lateral sediment transport and sediment focusing on Lake Alberca de Tacámbaro, bringing terrigenous and organic sediments from the perimeter of the lake to the central regions. Given that *A. minutissimum* and *P. ocellata* are the dominant diatoms in the sediment of this lake, it is reasonable to expect that this material could include a high number of diatom valves of *A. minutissimum* and *P. ocellata*, which may contribute to a decrease in the proportion of more recent planktonic diatoms. The loss of frustules by fragmentation or dissolution in the surface sediments was discarded in this study because there was no evidence in our material to support this assumption, as we did not find redissolved valves or abundant fragments that might suggest preferential breaking or dissolution of *A. granulata* or *D. stelligera/pseudostelligera*.

The beta diversity of the plankton and sediment trap samples as well as the results of the cluster analysis showed species replacement related with water column mixing and stratification which resulted in at least two main seasonal diatom assemblages in this lake. *Aulacoseira granulata* was favored by the winter mixing processes when there was a higher turbulence and higher total phosphorus availability in the lake (Montero *et al.*, 2021). In contrast, *P. ocellata* and *D. stelligera/pseudostelligera* flourished during times of warmer temperatures, a stratified water column, and lower nutrients concentration associated with the stratification process, even though in at least one sampling this "stratification" assemblage was dominated by *A. minutissimum*. In addition, phytoplankton and sediment trap samplings often displayed a coupled temporal variability in their diatom assemblages; for example, phytoplankton blooms of *A. granulata* were visible in the trap sediments collected at the same time or in the following sampling date. In relation to the annual sediment trap, it accurately reflected the average species abundances in the phytoplankton.

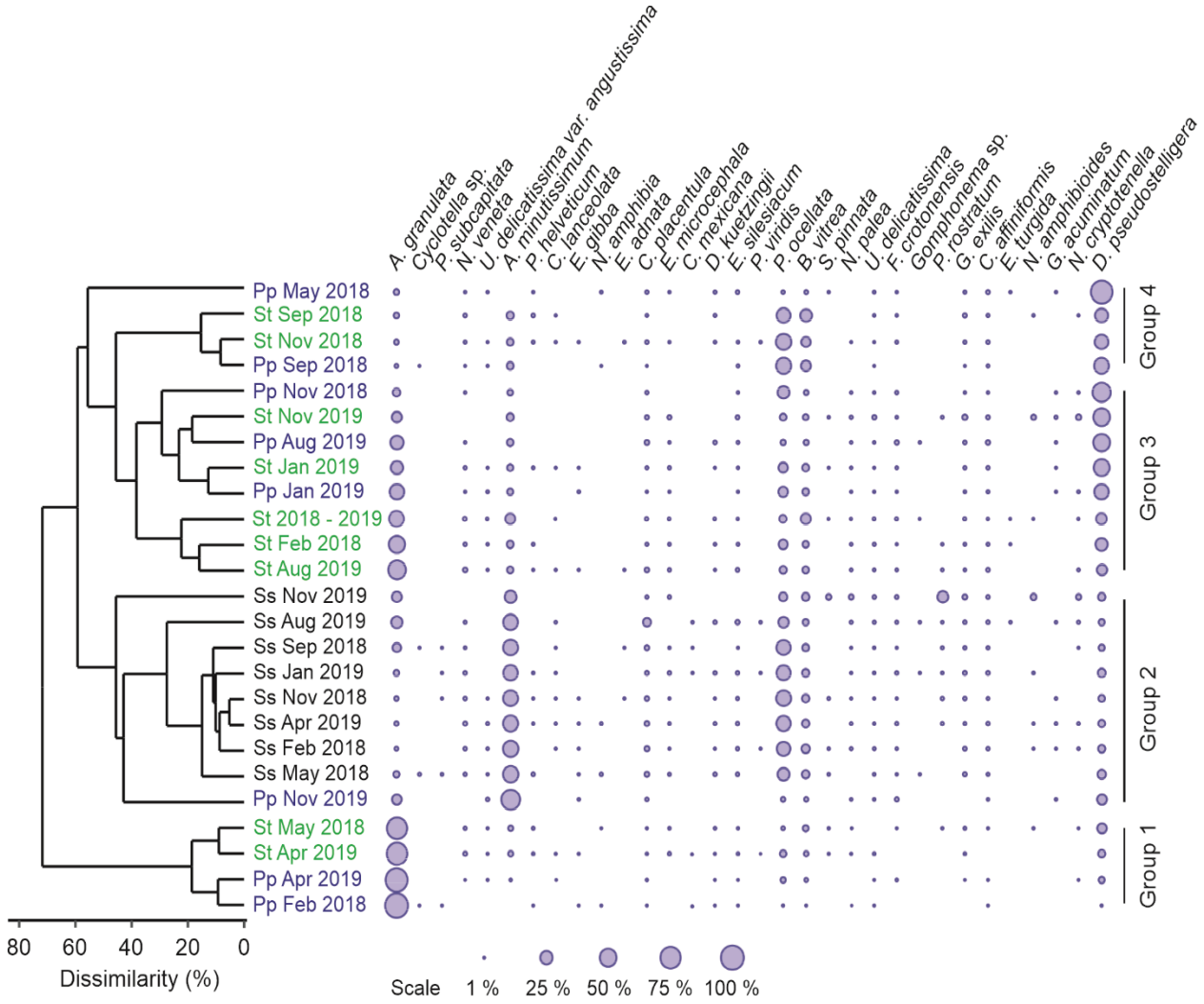

Figure 5. Dendrogram from the cluster analysis based on quantitative data (percentage similarity, UPGMA) of the diatom community observed in samples from phytoplankton (Pp in blue), sediment trap (St in green), and surface sediment (Ss in black). Bubble plot represents the relative abundance of diatom species in the different samples.

Table 3 List of diatom species observed in samples from phytoplankton, sediment trap, and surface sediments in Lake Alberca de Tacámbaro. Diatom species are classified by their habit into planktonic, benthic, and tychoplanktonic species. Question marks indicate that the taxon identification is still uncertain.

	Phytoplankton	Sediment trap	Surface sediments
Planktonic species			
Aulacoseira granulata (Ehrenberg) Simonsen	✓	✓	✓
Aulacoseira granulata var. *angustissima* (O. Müller) Simonsen	✓	✓	✓
Cyclotella sp.	✓	✓	✓
Cyclotella meneghiniana Kützing	✓	✓	✓
Discostella stelligera (Cleve & Grunow) Houk & Klee	✓	✓	✓
Discostella pseudostelligera (Hustedt) Houk & Klee	✓	✓	✓
Fragilaria crotonensis Kitton	✓	✓	✓
Pantocsekiella ocellata (Pantocsek) K. T. Kiss & Ács	✓	✓	✓
Ulnaria delicatissima (W. Smith) Aboal & P. C. Silva	✓	✓	✓
Ulnaria delicatissima var. *angustissima* ? (Grunow) Aboal & P. C. Silva	✓	✓	✓
Ulnaria ulna (Nitzsch) Compère		✓	✓
Benthic species			
Achnanthidium minutissimum (Kützing) Czarnecki	✓	✓	✓
Amphipleura pellucida (Kützing) Kützing	✓	✓	
Brachysira vitrea (Grunow) R. Ross	✓	✓	✓
Cocconeis placentula Ehrenberg	✓	✓	✓
Cymbella affiniformis Krammer	✓	✓	✓
Cymbella lanceolata C. Agardh	✓	✓	✓
Cymbella mexicana (Ehrenberg) Cleve	✓		✓
Denticula kuetzingii Grunow	✓	✓	✓
Encyonema silesiacum (Bleisch) D. G. Mann	✓	✓	✓
Encyonopsis microcephala (Grunow) Krammer	✓	✓	✓
Epithemia adnata (Kützing) Brébisson	✓	✓	✓
Epithemia turgida (Ehrenberg) Kützing	✓	✓	✓
Gogorevia exilis (Kützing) Kulikovskiy & Kociolek	✓	✓	✓
Gomphonema sp.			✓
Gomphonema acuminatum Ehrenberg	✓	✓	✓
Gomphonema lagenula Kützing		✓	✓
Iconella linearis ? (W. Smith) Ruck & Nakov	✓	✓	
Navicula cryptocephala ? Kützing	✓	✓	✓
Navicula veneta Kützing	✓	✓	✓
Nitzschia amphibia Grunow	✓	✓	✓
Nitzschia amphibioides Hustedt		✓	✓
Pinnularia subcapitata ? W. Gregory	✓		✓
Pinnularia viridis (Nitzsch) Ehrenberg	✓	✓	✓
Planothidium rostratum (Østrup) Lange-Bertalot		✓	✓
Psammothidium helveticum (Hustedt) Bukhtiyarova & Round	✓	✓	✓
Rhopalodia gibba (Ehrenberg) O. Müller	✓	✓	✓
Sellaphora pupula (Kützing) Mereschkovsky		✓	
Surirella elegans Ehrenberg	✓		
Tychoplanktonic species			
Staurosirella pinnata (Ehrenberg) D. M. Williams & Round	✓	✓	✓

The pattern of seasonal species replacement along the year was attenuated in the diatom assemblage from surface sediments, in agreement with results from other studies comparing planktonic and sedimentary diatom assemblages (Kato *et al*., 2003; Ryves *et al*., 2003; Pla-Rabés & Catalan, 2018). Surface sediment samples showed a richer, more equitable and more homogeneous diatom assemblage throughout the year. This assemblage was dominated by *A. minutissimum* and *P. ocellata* in association with *B. vitrea*, *D. stelligera/pseudostelligera* and *A. granulata;* small peaks of *A. granulata* were recorded during late stratification, following the winter and early stratification blooms recorded in the phytoplankton and trap sediments. These samples showed a much smaller temporal variability in their diatom assemblages, and species replacement occurred only among diatoms with low abundance (e.g., *C. placentula, Cymbella affiniformis, Gogorevia exilis, Encyonema silesiacum, Navicula veneta* and *P. rostratum*). These diversity patterns are common in surface sediments because these samples often represent a time-average window larger than the trap or the phytoplankton samples (Dong *et al*., 2008), which may explain why the high abundances of *A. granulata* and *D. stelligera/pseudostelligera* observed in phytoplankton and trap samples were attenuated in the surface sediment samples. This time window depends on the sediment accumulation rate in the lake, and even though this is a variable parameter, in a previous study in this lake it was established to be around 7 mm/year (Caballero *et al*., 2016). In this way, the surface layer of sediments (~1cm) in Lake Alberca de Tacámbaro is likely to represent around two years of sedimentation (Caballero *et al*., 2016) and could therefore have a memory of a recent past where *A. minutissimum* and *P. ocellata* were more abundant in the phytoplankton. In fact, these species were the dominant taxa in the annual sediment trap deployed in this lake from April 2015 to April 2016 (Caballero, unpublished data).

From previous work in this lake (Caballero *et al*., 2016) we also know that a diatom assemblage dominated by *A. minutissimum* and *P. ocellata* has been present in the surface sediments since the 2010 "El Niño" event when *P. ocellata* became abundant in the phytoplankton. Previously, from 1988 to 2010, the surface sediments were dominated by *A. minutissimum* in association with needle-shaped diatoms such as *Ulnaria delicatissima, Fragilaria neotropica* or *F. crotonensis*, but since then, these planktonic diatoms have become increasingly rare in the phytoplankton and sediments of Lake Alberca de Tacámbaro (Caballero *et al*., 2016). In this way, the discrepancies between the phytoplankton and surface sediment diatom assemblages are an indication of recent changes in the diatom flora of this lake, with a trend towards a reduction in needle shaped planktonic taxa and increasing abundance of centric planktonic taxa such as *A. granulata* and *D. stelligera/pseudostelligera*. These changes could be related to a recent increase in nutrient levels in this lake, particularly nitrogen (Montero *et al*., 2021).

The comparison of the living diatom community with the diatom assemblage in sediments provided valuable information to understand the sedimentary diatom assemblage in Lake Alberca de Tacámbaro, its association with the ecological features of some species and complex processes occurring in the sediments. The surface layer of sediments of this lake is likely to represent a diatom assemblage integrated by diatoms from several zones of the lake and different habits, with an average-time window of about two years. The diatom assemblage in these samples would take a slightly longer time to show the recent changes in the structure of the water column diatom community. The higher silicate fraction in surface sediment samples indicates that horizontal transport and sediment focusing may also have an important influence on the diatom assemblage found in the surface layer of sediments. Even though our results also suggest that in tropical deep lakes some species traditionally considered to have a benthic habit (*A. minutissimum* and *B. vitrea*) can be at least temporarily incorporated into the plankton, functioning as tychoplanktonic taxa.

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Research Article
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Estructura poblacional del ostión americano Crassostrea virginica (Gmelin,1791) (Mollusca: Bivalvia: Ostreidae) en Tamaulipas, México

Population structure of the *Eastern oyster* Crassostrea virginica (Gmelin,1791) (Mollusca: Bivalvia: Ostreidae) in Tamaulipas, Mexico

Ulises de Jesus Balderas-Mancilla[1], Ausencio Azuara-Domínguez[1], Luis Antonio Vázquez-Ochoa[1], Edgar Pérez-Arriaga[1] & Jorge Homero Rodríguez-Castro[1*]

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RESUMEN

Antecedentes. En Tamaulipas, México, el ostión *Crassostrea virginica* es una de las especies de mayor importancia comercial. **Objetivos**. Se determinó la estructura poblacional de *C. virginica* en el Río Tigre y en la Laguna de San Andrés del Estado de Tamaulipas, México. **Métodos**. Se recolectaron ostiones en tres bancos de enero a diciembre del 2022, mediante el método basado en cuadrantes. Se determinó la estructura de tallas (largo, ancho y peso), distribución de estadios, relación peso-longitud, la abundancia y biomasa de *C. virginica*. **Resultados**.En total se recolectaron 2,277 organismos. La talla representativa, en los tres bancos en conjunto se presentó en un intervalo de 43-65 mm de largo. Se registraron diferencias significativas en el ancho, largo y peso de los ostiones recolectados, siendo los organismos del Banco 1 y 2 de mayor longitud y peso. Los tres bancos presentaron un crecimiento alométrico negativo al establecerse un valor b <3. En el número de reclutas entre los bancos no se registró diferencia estadística significativa. Se observó una diferencia estadística significativa entre los bancos en el número promedio de juveniles-preadultos, adultos y adultos con más de una reproducción. La abundancia en los bancos está por debajo del número mínimo establecido (> 200 ostras/m^2). Se presenta inadecuado manejo del recurso en los tres bancos, donde la mayor parte capturada de ostiones se encuentra por debajo de la talla permitida para su extracción. **Conclusión.** La estructura de tallas muestra que la mayor parte de la población capturada de *C. virginica* se encuentra por debajo de la talla permitida para su extracción, por lo que se sugiere el desarrollo de estudios enfocados al manejo sustentable de esta especie.

Palabras clave: *Crassostrea virginica*, estructura poblacional, Tamaulipas.

ABSTRACT

Background. In Tamaulipas, Mexico, the Eastern oyster, *Crassostrea virginica*, is one of the most commercially important species. **Goals.** The population structure of *C. virginica* in the Tigre River and Laguna de San Andres in the State of Tamaulipas, Mexico, was determined. **Methods.** Oysters were collected from three banks from January to December 2022 using the quadrat-based method. The size structure (length, width, and weight), instar distribution, weight-length relationship, abundance, and biomass of *C. virginica* were determined. **Results.** In total, 2,277 organisms were collected. The representative size, in the three banks, occurred in a range of 43-65 mm in length. Significant differences were recorded in the width, length, and weight of the oysters collected, with Bank 1 and 2 organisms having the greatest length and weight. The three banks presented negative allometric growth when establishing a b value <3. No significant statistical difference was recorded in the number of recruits between the banks. A statistically significant difference was observed between banks in the average number of juveniles-preadults, adults, and adults with more than one reproductive. The abundance on the banks is below the established minimum number (> 200 oysters/m2). There is inadequate management of the resource on the three banks, where most oysters captured are below the size allowed for extraction. **Conclusions.** The structure shows that most of the *C. virginica* population is below the size allowed for its extraction, which is why the development of studies focused on the sustainable management of this species is suggested.

Key words: *Crassostrea virginica*, population structure, Tamaulipas.

[1] Tecnológico Nacional de México/Instituto Tecnológico de Ciudad Victoria. Boulevard Emilio Portes Gil No. 1301, Ciudad Victoria, Tamaulipas, C.P. 87010 México

***Corresponding author:**
Jorge Homero Rodríguez-Castro: e-mail: jorge.rc@cdvictoria.tecnm.mx

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INTRODUCCIÓN

La extracción de ostión americano *Crassostrea virginica* (Mollusca: Bivalvia: Ostreidae) se ha incrementado en E.E.U.U y en México por su importancia comercial (Kennedy, 2018; CONAPESCA-SAGARPA, 2018). Debido a esto, los bancos de ostión han disminuido en número y la sobreexplotación de estos es más frecuente (Beck *et al*., 2011; Wilberg *et al*., 2011; Baggett *et al*., 2014).

En México, la extracción del ostión se realiza en los sistemas lagunares y estuarinos de Tamaulipas, Veracruz, Tabasco y Campeche (SAGARPA-CONAPESCA, 2012). Con el fin de regular la extracción en dichos estados, las poblaciones silvestres *de C. virginica* poseen el estatus de "recurso aprovechado al máximo sustentable" (Diario Oficial de la Federación, 2012). No obstante, sólo para el estado de Tabasco, se formuló la norma NOM-015-SAG/PESC-1994 con la finalidad de regular la extracción de ostión silvestre y establecer programas de aprovechamiento y manejo. Con base en esta norma, los ostiones son categorizados de acuerdo con la longitud de su concha como sub-adultos (50-69 mm) y adultos (≥70 mm). Considerando lo anterior, la norma establece que solo los ostiones de talla de ≥70 mm de longitud de concha se deben de extraer para consumo.

En el estado de Tamaulipas, el ostión *C. virginica* se encuentra en Matamoros, San Fernando, Soto la Marina, Aldama y Altamira (INEGI, 2011). En estos municipios, los bancos de ostión se localizan en la Playa Bagdad, Laguna Madre, Laguna Almagre, Barra del Tordo y laguna de San Andrés (Arias-De León, 2014). En el caso de la Laguna Madre, a pesar de que el ostión tiene una pesquería establecida y regulada, los bancos de ostión han sido sobreexplotados (CONANP, 2015). Esto se refleja en la disminución de la producción del ostión, por ejemplo, en el 2012, el estado de Tamaulipas ocupó el tercer lugar con una producción de 3,990 toneladas. En la actualidad, el estado ocupa el sexto lugar con 2,528 toneladas de ostión producidas (FAO, 2018).

La sobreexplotación de los bancos de ostión se debe en parte a la falta de una norma oficial que regule la pesquería del ostión en Tamaulipas. Actualmente, es urgente contar con la información biológica-pesquera del ostión con el fin de elaborar un Plan de Manejo del ostión en Tamaulipas y Veracruz, tomando como base las acciones realizadas para el aprovechamiento sustentable del ostión en el estado de Tabasco (INAPESCA, 2018).

En la actualidad, información de la estructura poblacional del ostión *C. virginica* es muy escasa para el Golfo de México (Vidal-Briseño *et al*., 2015). Al respecto, George-Zamora & Aranda-Aldana (2000) evaluaron la producción de *C. virginica* e *Ischadium recurvum* (Bivalvia) en Mecoacán, Tabasco, México. Mientras que, Vidal-Briseño *et al*. (2015) evaluaron la estructura de tallas de la captura del ostión *C. virginica* en la Laguna de Tamiahua y Tampamachoco en Veracruz. Por su parte, Mayorga-Cruz (2021) realizó una caracterización poblacional de *C. virginica* existente en tres lagunas costeras de Tabasco. De la misma manera, Díaz-Jiménez *et al*. (2022) evaluaron aspectos de la dinámica poblacional de *C. virginica* en la laguna de Mecoacán, Tabasco.

Con base en la información mínima disponible es necesario orientar investigaciones hacia aspectos poblacionales en los sitios donde diariamente se realiza la extracción de *C. virginica*, con el fin garantizar un recurso sustentable y recuperar los bancos silvestres sobreexplotados en el estado de Tamaulipas. Debido a lo anterior, en el presente trabajo se determina la estructura poblacional del ostión americano *C. virginica* en el estado de Tamaulipas, México.

MATERIALES Y MÉTODOS

Área de estudio. Los ostiones fueron recolectados en los bancos ostrícolas localizados en el Río Tigre y en la Laguna de San Andrés (Figura 1). Ambos sitios se ubican en el litoral del Golfo de México, entre los municipios de Aldama y Altamira, Tamaulipas, en las coordenadas: 22° 47' y 22° 32' de latitud norte y 97°54› y 97° 41› de longitud oeste. El agua dulce de estos sistemas la proveen el Río Tigre y el Río Barberena. La laguna es muy somera, con poca vegetación alrededor, cuenta con bancos de ostiones (*C. virginica)* y alta actividad pesquera de camarón, jaiba y algunas especies de peces.

Recolecta de los ostiones. *Los ostiones fueron recolectados en el Río Tigre (Banco 1), la desembocadura del Río Tigre (Banco 2) y en la laguna de San Andrés (Banco 3) de enero - diciembre del 2022.*

En cada banco los ostiones fueron recolectados en 2,500 m^2, a una profundidad de 43±12 cm en el Río Tigre, 67±12 cm en la Desembocadura y 90±12 cm en la Laguna de San Andrés. En esta área, el muestreo de los ostiones fue mediante el método basado en cuadrantes (Garrido et al., 2007; Betanzos-Vega et al., 2018). Para ello, cada banco fue dividido en cinco cuadrantes, en cada cuadrante se lanzó aleatoriamente un marco cuadrado de acero (1 m^2) cinco veces con el fin de determinar la densidad poblacional (número de ostiones/m^2).

La recolección de los ostiones fue de forma manual. Los ostiones recolectados se limpiaron y lavaron con agua para retirar el exceso de materiales adheridos a la concha (pequeños bivalvos, algas y sedimento). Una vez limpios, los ostiones fueron medidos (largo y ancho), con un vernier con precisión de 0.1 mm, y pesados con una balanza digital (Ohaus Compass ™ CX) con precisión de 0.1 g. Después, los ostiones fueron clasificados por reclutas (0 a 20 mm), juveniles-pre-adultos (20 a 40 mm) y adultos (40 a 60 mm) y adultos con más de una reproducción (≥ 60 mm) (Rodríguez-De La Cruz, 1988; Galtsoff, 1964). Para estimar el porcentaje de población pescable, en el presente estudio se tomó como referencia la talla mínima de extracción (70 mm de longitud concha) establecida en la Norma Oficial Mexicana NOM-015-SAG/PESC-2016; norma utilizada para regular el aprovechamiento del ostión *C. Virginica* en los sistemas lagunar estuarinos del Estado de Tabasco.

Análisis de datos. El análisis de distribución de la frecuencia de tallas de los ostiones recolectados se efectuó mensualmente por banco, siendo 4 mm el tamaño de intervalo de clase para cada banco. El intervalo fue calculado con la fórmula de Freedman & Diaconis (1981a, b): $\mathbf{h = 2\ (RIC)\ n^{-1/3}}$

Dado que en el presente estudio la talla no presentó distribución normal (test Kolmogorov-Smirnov: $p<0.05$), se utilizó la prueba de Kruskal Wallis, que permitió comparar las medianas de diferentes muestras (Zar, 2010) para ese factor.

Se determinó la relación longitud peso mediante la aplicación de un modelo potencial $\mathbf{Y = a\ X^{b}}$, donde: **"Y"** representó el peso total y **"X"** representó la longitud total de la concha. Bajo el supuesto de que si $b< 3$ existe un crecimiento alométrico negativo, si $b = 3$ el crecimiento es isométrico y si $b> 3$ el crecimiento que presenta la especie es un crecimiento alométrico positivo (Ibáñez & Fernández, 2006)

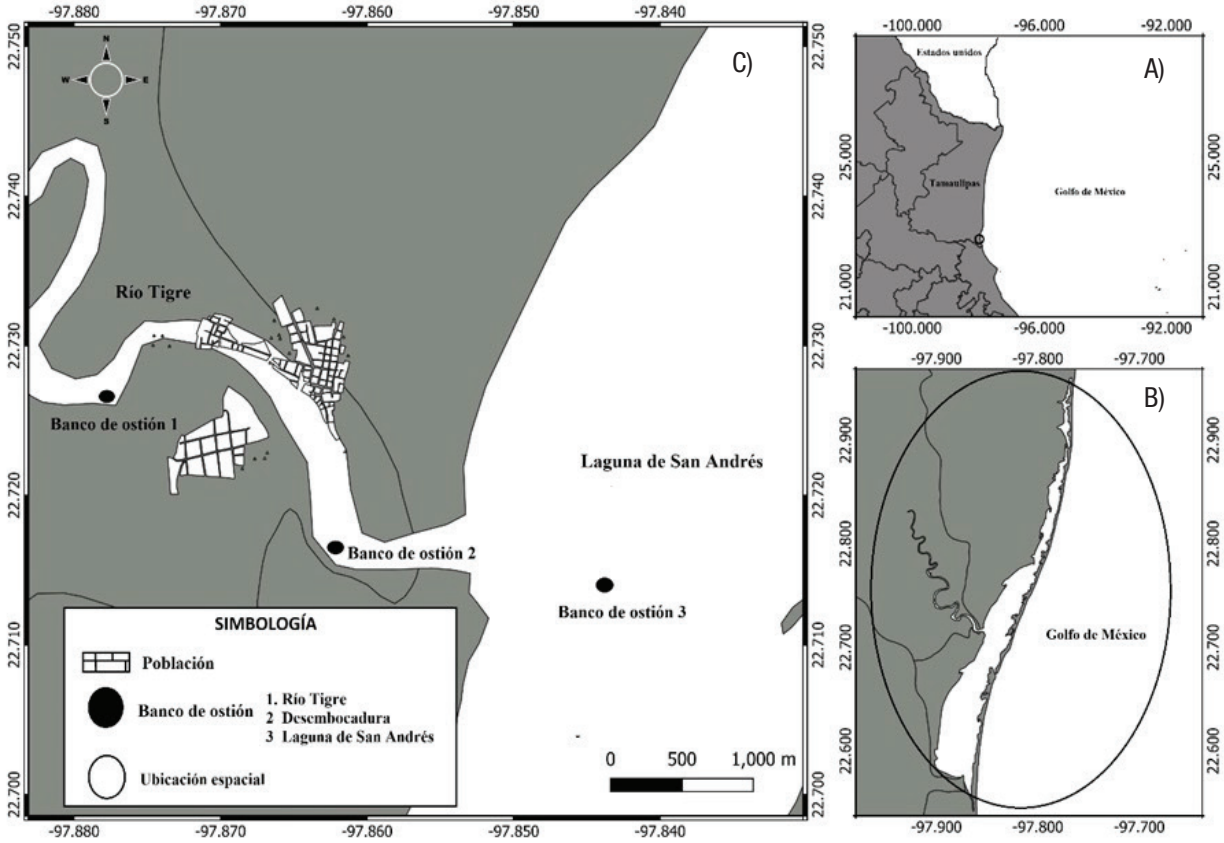

Figura 1. Ubicación geográfica de los bancos ostrícolas en el Río Tigre y en la laguna de San Andrés en el municipio de Aldama, Tamaulipas. Faltan las coordenadas geográficas para los mapas de la derecha, se sugiere poner letras a cada mapa.

Se determinó la abundancia en número y peso (N/m2 y kg/m^2) de cada réplica, de cada zona evaluada, y la abundancia total del banco realizando las conversiones necesarias para obtener la abundancia total en kg/km^2 (productividad por área).

A partir de la productividad por área o densidad (D) en peso (kg/km^2), estimada para todo el banco y del área total del banco (A) en km^2, se estimó la biomasa (B) total de la población en toneladas métricas (t) con la fórmula **B = A*D** (Cadima, 2003).

Finalmente, antes del análisis, se comprobó la ausencia de normalidad y homogeneidad de la varianza con las pruebas de Kolmogorov-Smirnov y Leven en el número promedio de largo (mm), ancho (mm) y peso (g), número promedio de los reclutas, jóvenes, adultos y adultos con más de una reproducción. Cuando no se cumplieron los supuestos estadísticos mencionados anteriormente, los resultados fueron analizados con la prueba noparamétrica de Kruskal-Wallis. Una vez que se rechazó la hipótesis nula con la prueba de Kruskal-Wallis, se procedió a comparar las medias de rangos con la prueba de Nemenyi (Alfa= 0.05).

RESULTADOS

La prueba de Kruskal-wallis (KW) presentó diferencias significativas en tallas para cada sitio ($p<0.05$).

Se recolectaron 2,277 ostiones de *C. virginica*, en el Banco 1 (n=532), Banco 2 (n=488) y Banco 3 (n=1,257).

En el Banco 1, la estructura de tallas de *C. virginica* estuvo constituida por organismos de 17 a 118 mm largo total (Lt). No obstante, hubo una mayor representación de individuos con tallas comprendidas entre 45 a 58 mm. La mayor proporción de ostiones (82.21%) se ubicó en longitudes valvares mayores a 42 mm (Fig. 2a). La talla media de los ostiones muestreados fue de 57.86 mm Lt. En este banco se recolectó un ejemplar en la etapa de recluta (0.18 %), 20 en la etapa juvenil-pre-adulto (3.75 %), 306 adultos (57.51 %) y 205 adultos con más de una reproducción (38.56 %).

En el banco 2, la estructura de tallas de *C. virginica* estuvo constituida por organismos de 13 a 93 mm largo total (Lt). Hubo una mayor frecuencia de individuos con tallas comprendidas entre 54 a 65 mm de

longitud total. La mayor proporción de ostiones (86.58%) se ubicó en longitudes valvares mayores a 37 mm (Fig. 2b). La talla media general de los ostiones muestreados fue de 57.84 mm Lt. En este banco se recolectaron tres ejemplares en etapa de recluta (0.61 %), 36 en etapa juvenil-pre-adulto (7.37%), 237 adultos (48.56%) y 212 adultos con más de una reproducción (43.46 %; Fig. 2b).

En el banco 3, la estructura de tallas de *C. virginica* estuvo constituida por organismos 12 a 106 mm largo total (Lt), con una mayor concentración de individuos de 43 a 57 mm de longitud total. La mayor proporción de ostiones se ubicó en longitudes valvares mayores a 37 mm (75.17%; Fig. 2c). La talla media general de los ostiones muestreados fue de 51.56 mm Lt. En este banco se recolectaron 15 ejemplares de la etapa recluta (1.19 %), 269 de la etapa juvenil-pre-adulto (21.40%), 625 adultos (49.75%) y 348 adultos con más de una reproducción (27.66 %).

Mediante la aplicación del modelo de regresión potencial del peso total (PT)-longitud total (Lt) para el ostión *C. virginica* en el banco 1 se obtuvo un crecimiento alométrico negativo en los ejemplares muestreados para esta zona, al estimar que el valor de b= 2.179 y se determinó la ecuación: ($PT = 0.005 * Lt^{2.179}$; $r = 0.774$), la cual indicó que existe una relación entre el peso y la longitud total de los organismos (Fig.3a).

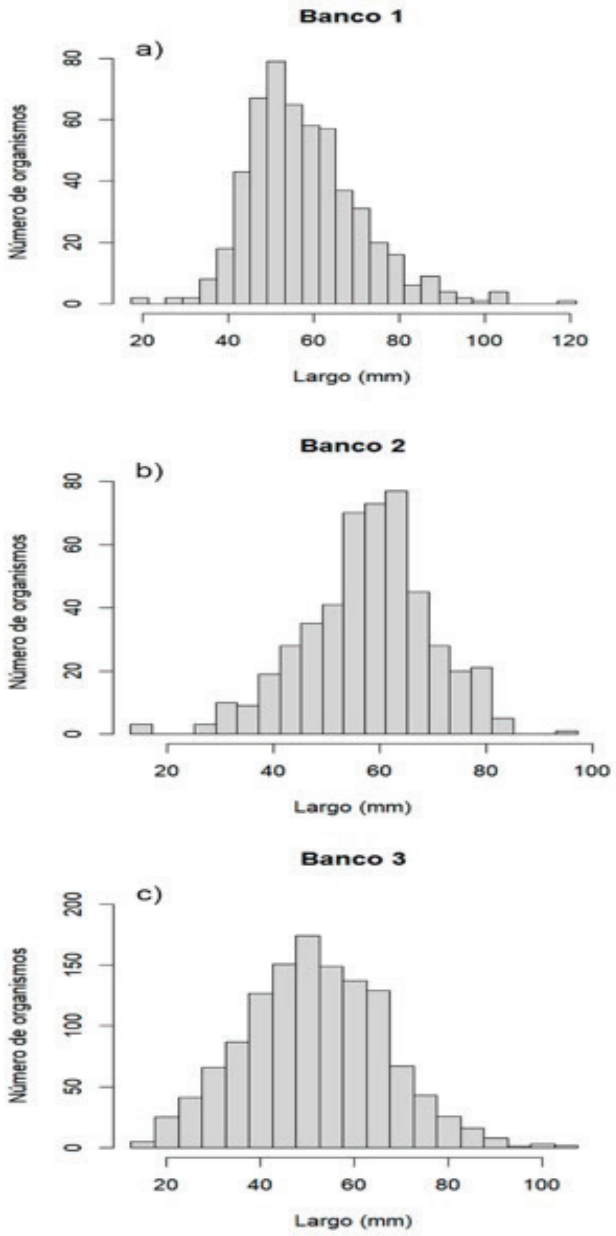

Figura 2. Distribución de tallas de *C. virginica* a) Río Tigre, b) desembocadura y c) laguna de San Andrés, Aldama, Tamaulipas, México en el periodo enero-octubre 2022.

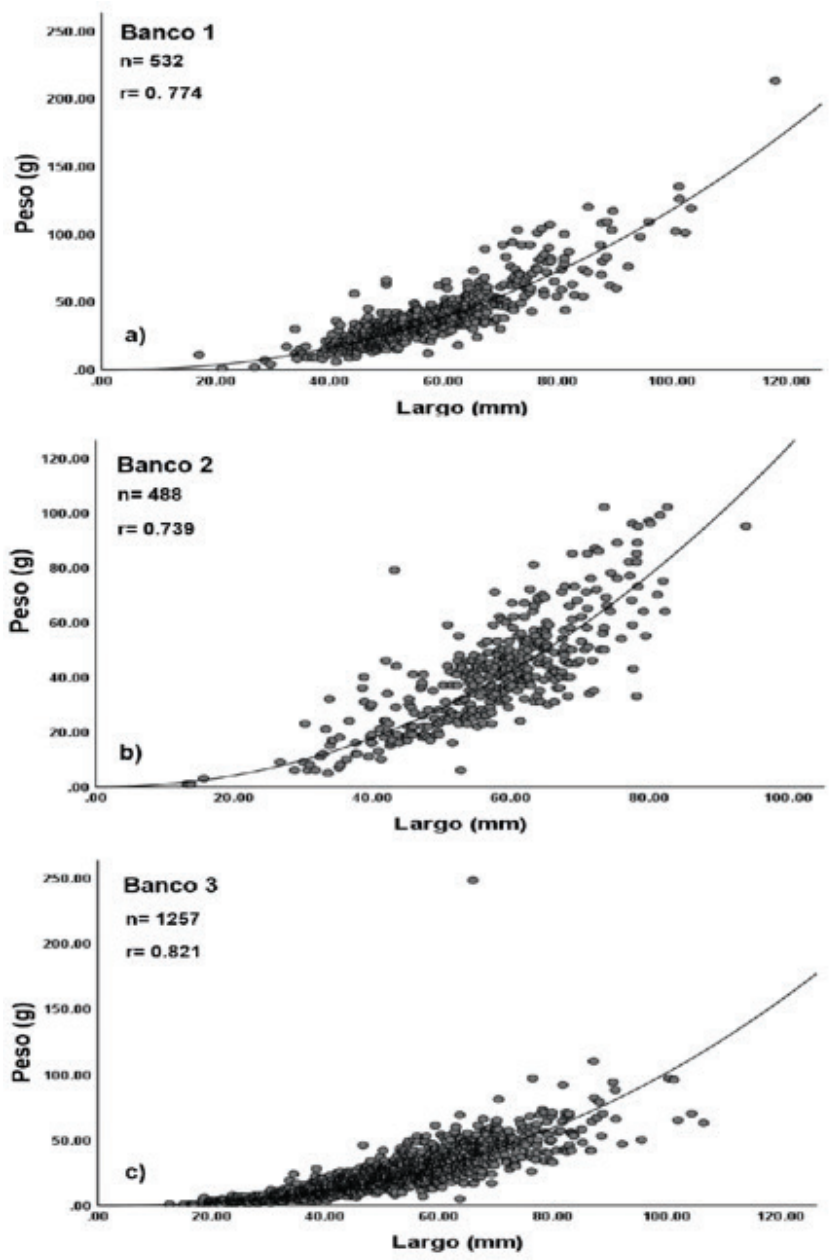

Figura 3. Relación entre las variables longitud-peso de *C. virginica* a) Río Tigre, b) desembocadura y c) laguna de San Andrés.

Con respecto a la relación longitud-peso de *C. virginica* del banco 2 determinó un valor b = 2.110, lo que evidenció que los ejemplares muestreados para el presente estudio presentaron un crecimiento alométrico negativo. Así mismo, se estableció la ecuación: (PT = 0.007 *Lt $^{2.110}$ r = 0.739) (Fig.3b).

Con respecto a la relación longitud-peso de *C. virginica* para el banco 3, se determinó un valor b = 2.458, lo que evidenció que los ejemplares muestreados para el presente estudio presentaron un crecimiento alométrico negativo. Así mismo, se estableció la ecuación: (PT = 0.001 *Lt$^{2.458}$ r = 0.821), la cual demostró que existe una relación entre las variables analizadas (Fig. 3c).

A través de la prueba de Kruskal- Wallis, el ancho, largo y peso de los ostiones recolectados en cada banco se registró diferencia estadística significativa ($p<0.05$; Figs. 3 a, b y c). En el acho del cuerpo de los ostiones del banco 1 y banco 2 no hubo diferencia estadística significativa (P=0.180), en contraste con el banco 3 (P=0.001). No obstante, el ancho del cuerpo de los ostiones recolectados en ambos bancos fue mayor al ancho del cuerpo de los ostiones recolectados en el banco 3 (Fig. 4a).

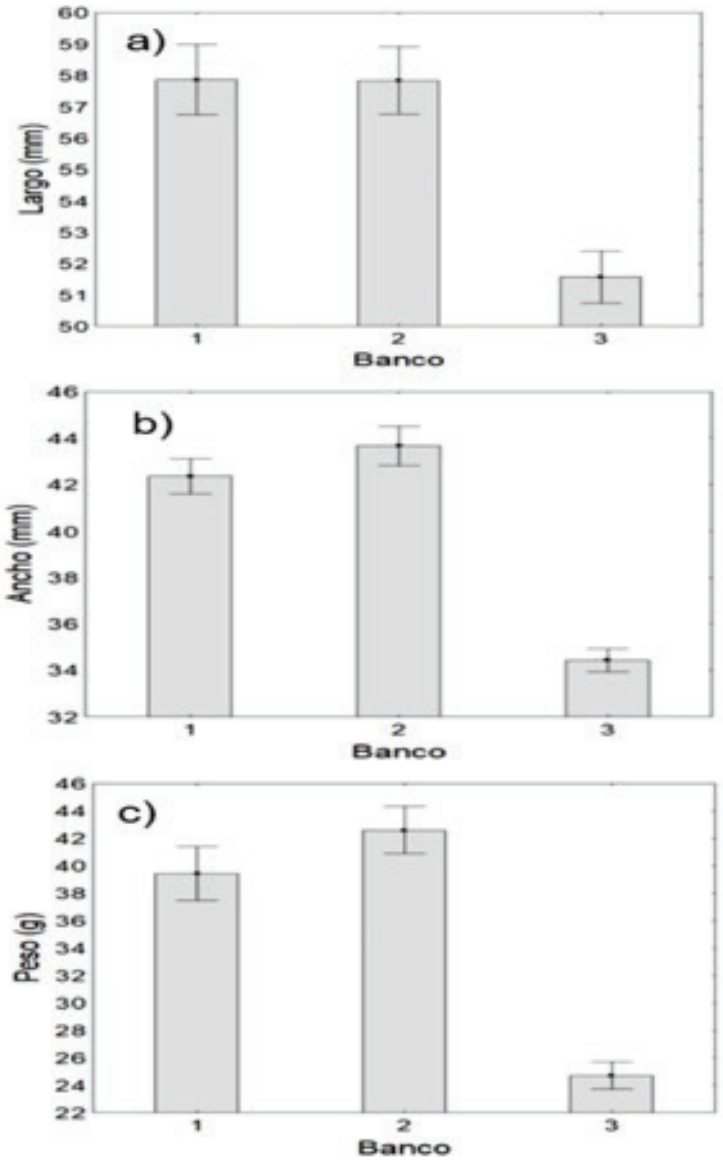

Figura 4. Análisis estadístico de los parámetros poblaciones (largo, ancho y peso) de *C. virginica*. T1= Banco 1, T2= Banco 2 y T3= Banco 3.

Por otro lado, en el banco 1 y en el banco 2 no hubo diferencia estadística significativa en el largo del cuerpo de los ostiones (P=0.128), en contraste con el banco 3 (P=0.001). No obstante, el largo de los ostiones recolectados en ambos bancos fue mayor al largo del cuerpo de los ostiones recolectados en el banco 3 (Fig. 4b).

De forma similar, en el banco 1 y en el banco 2 no hubo diferencia estadística significativa en el peso del cuerpo de los ostiones (P=0.110), en contraste con el banco 3 (P=0.001). No obstante, el peso de los ostiones recolectados en ambos bancos (1 y 2) fue mayor al peso los ostiones recolectados en el banco 3 (Fig. 4c).

En el número de reclutas de los ostiones recolectados en cada banco no se registró diferencia estadística significativa (P=0.05; Fig. 5a).

La prueba de Kruskal- Wallis para juveniles mostró diferencia estadística significativa ($p<0.05$), sin embargo, el número promedio de juveniles de los ostiones, en el banco 1 y en el banco 2 no hubo diferencia estadística significativa en la cantidad de juveniles recolectados (P=0.115) en contraste con el banco 3 (P=0.001). En ambos bancos el número promedio de juveniles fue menor a los registrados en el banco 3 (Fig. 5b).

La prueba de Kruskal- Wallis para adultos registró diferencia estadística significativa ($p<0.05$). En el número promedio de adultos de los ostiones, en el banco 1 (P=0.251), banco 2 (P=0.251) y en el banco 3 (P=0.251) no hubo diferencia estadística significativa en la cantidad de adultos recolectados. El número promedio de adultos fue mayor registrado en el banco 1 que el banco 2 y 3 (Fig. 5c).

La prueba de Kruskal- Wallis para adultos con más de una reproducción registró diferencia estadística significativa ($p<0.05$). En el número promedio de adultos con más de una reproducción, en el banco 1 y banco 2 no hubo diferencias significativas (P=0.131) en contraste con el banco 3 (P=0.011). En ambos bancos el número promedio de adultos con más de una reproducción fue mayor a los registrados en el banco 3 (Fig. 5d).

De enero a abril, el número de ostras por metro cuadrado (N/m^2) fue mayor en los tres bancos. No obstante, en el banco 2 y 3 se registró el número mayor de kilogramos por metro cuadrado (kg/m^2) (Tabla 1).

En cuanto a la productividad, para organismos adultos, el banco 1 y 3 registraron más biomasa que el banco 2. El banco 3 registro más biomasa para organismos adultos con más de una reproducción. La productividad en organismos en etapa de recluta registró valores menores a 1t para los tres bancos. De igual manera, para jóvenes pre-adultos se registraron valores menores a 1t para el banco 1 y 2. En contraste, el banco 3 produjo mayor productividad (Tabla 2). La biomasa con talla de captura comercial (≥ 60 mm) fue más del 40% para los tres bancos

DISCUSIÓN

En la presente investigación la talla promedio de los ejemplares de la población capturada de la Laguna de San Andrés fue menor estadísticamente a la estimada en el Río Tigre y en su desembocadura. Por otro lado, se corroboró que las tallas más representativas fueron similares para todos los sitios. Este resultado es similar al reportado por Vera (2012) quien registró tallas de 45-53 mm y 53-60 mm como las más representativas. Así mismo, con lo reportado por Vidal-Briseño *et al*. (2015) quienes reportaron tallas de 52-56 mm y 56-60 mm.

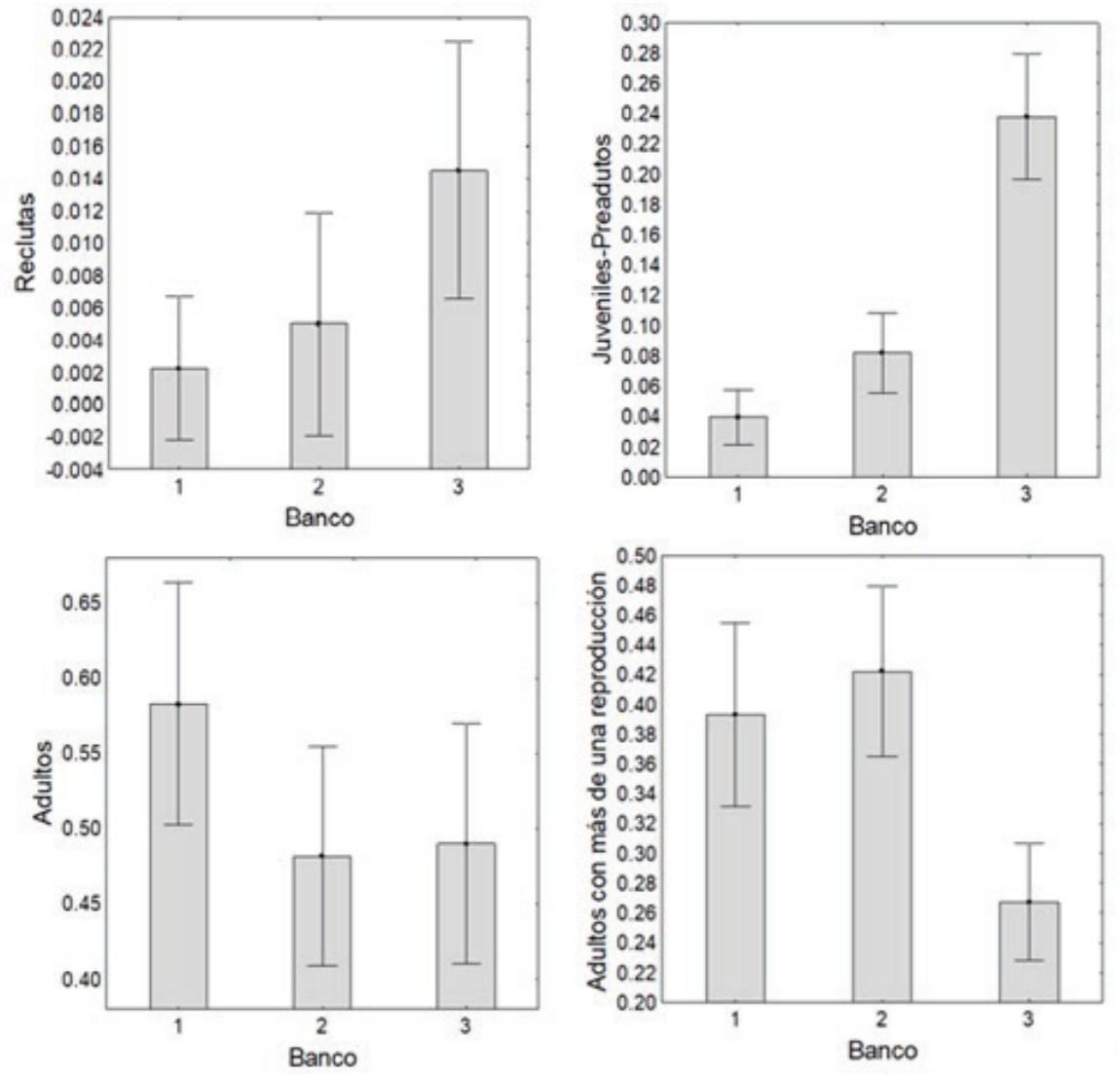

Figura 5. Análisis de varianza de los parámetros poblaciones (recluta, joven-preadulto, adulto, adultos con más de una reproducción) de *C. virginica*. T1=Banco 1, T2= Banco 2 y T3= Banco 3.

Con respecto a la relación entre el peso total (PT) y longitud total (Lt) de los organismos, los resultados del presente trabajo señalan que los organismos de los tres bancos presentaron una relación alométrica negativa al registrar valores de la pendiente «b» significativamente menores a 3 (Banco1 b = 2.179; Banco 2 b = 2.110 y Banco 3 b= 2.458). Estos valores son menores a los reportados por Fuentes (2012), quien calculó un valor b = 2.62 para *C. virginica* de julio a diciembre en el 2010 en la Laguna de Tampamachoco, Veracruz, sin embargo, fueron mayores a los reportados por Vidal-Briseño *et al.* (2015), quienes reportaron organismos de la laguna Tamiahua con un valor de b = 1.18 y organismos de la Laguna de Tampamachoco con un valor de b = 1.26. Lo anterior, demuestra que la talla obtuvo mayor incremento en proporción que el peso (Cifuentes *et al.,*2012). Esta relación es requerida en los análisis pesqueros para estimar y determinar el tamaño de la población de una especie, ya que la tasa de incremento en peso y talla refleja la influencia de los factores ecológicos en un hábitat y en los organismos que lo habitan (Vásquez *et al.,* 2015; García-Delgado & Leones-Zambrano, 2016).

Tabla 1. Valores medios mensuales de longitud (mm), abundancia media (número de ostras/m^2 y kg/m^2) y media total ± error estándar de la población de *C. virginica* por banco.

	Periodo del 2022	Banco 1	Banco 2	Banco 3	Media total	Error Estándar
Ene	Tamaño medio (mm)	55.6	55.1	50.5	57.3	2.8
	Ostión / m^2	3	3	17	7.6	8.08
	Kg / m^2	0.047	0.044	0.024	0.038	0.01
Feb	Tamaño medio (mm)	55.3	55.6	52.9	54.6	1.47
	Ostión / m^2	3	2	5	3.3	1.52
	Kg / m^2	0.040	0.047	0.032	0.039	0.007
Mar	Tamaño medio (mm)	57.4	57.0	54.7	56.3	1.45
	Ostión / m^2	2	3	6	3.6	2.08
	Kg / m^2	0.035	0.035	0.027	0.032	0.004
Abr	Tamaño medio (mm)	55.4	56.1	57.0	56.1	0.80
	Ostión / m^2	2	3	4	3.0	1
	Kg / m^2	0.030	0.040	0.028	0.032	0.006
May	Tamaño medio (mm)	57.8	55.9	50.5	54.7	3.7
	Ostión / m^2	1	2	3	4.6	1
	Kg / m^2	0.037	0.049	0.020	0.035	0.014
Jun	Tamaño medio (mm)	59.2	57.9	54.7	57.2	2.3
	Ostión / m^2	1	2	3	2.0	1
	Kg / m^2	0.043	0.040	0.027	0.036	0.008
Jul	Tamaño medio (mm)	56.6	61.2	52.9	56.9	4.1
	Ostión / m^2	1	1	3	1.6	1.4
	Kg / m^2	0.036	0.043	0.023	0.034	0.01
Ago	Tamaño medio (mm)	55.1	60.2	46.5	53.9	6.9
	Ostión / m^2	1	2	3	2.0	1
	Kg / m^2	0.037	0.048	0.020	0.035	0.01
Sep	Tamaño medio (mm)	52.7	54.8	48.8	52.1	3.0
	Ostión / m^2	1	1	2	1.3	0.5
	Kg / m^2	0.038	0.041	0.019	0.033	0.01
Oct	Tamaño medio (mm)	68.8	64.8	53.3	62.3	8.0
	Ostión / m^2	1	1	2	1.3	0.5
	Kg / m^2	0.022	0.049	0.021	0.031	0.01
Nov	Tamaño medio (mm)	60.2	57.1	40.0	52.4	10.0
	Ostión / m^2	1	1	2	1.3	0.5
	Kg / m^2	0.011	0.038	0.011	0.052	0.01
Dic	Tamaño medio (mm)	66.2	63.3	39.4	56.3	14.7
	Ostión / m^2	1	1	2	1.3	0.5
	Kg / m^2	0.011	0.054	0.011	0.025	0.02

Por otro lado, los organismos con mayores tallas (largo y ancho) y pesos se registraron en el Río Tigre y en su desembocadura. Esto puede deberse al tipo de hábitat y las características hidrológicas. Al respecto, Betanzos-Vega *et al.* (2016) reportaron al ostión americano y al ostión de mangle con mayor talla de crecimiento en las zonas interiores del Río Cuyaguateje y Cauto, en contraste con la desembocadura y zona costera. En este estudio la salinidad fue un factor determinante, ya que se registraron valores mayores de salinidad en el hábitat del ostión de mangle (30.20-39.05 ups), en comparación con el hábitat del ostión americano (15.42-34.01 ups), lo que es coincidente con otros estudios donde sitúan al ostión *C.virginica* en aguas salobres (Castillo-Rodríguez & García-Cubas,1984; Lodeiros & Freites,2008).

En los tres bancos la población estuvo mayormente representada por adultos y adultos con más de una reproducción. El número menor de ostiones en etapa de recluta puede ser atribuido principalmente a que en el Estado de Tamaulipas no hay una norma oficial que establezca una talla mínima permitida de extracción del ostión. En este sentido, si se aplicara la norma NOM-015- SAG/PESC-1994 tal como se lleva a cabo en el Estado de Tabasco, solo el 17.29 % de la población de ostiones del Río tigre, 14.75 % de la población de ostiones de la desembocadura del Río Tigre y el 10.17 % de la población de ostiones de la laguna de San Andrés estaría permitida para su extracción.

Adicional a lo anterior, durante el año, en el presente estudio, se registró en todas las zonas evaluadas una densidad por debajo de los 200 ostiones/m^2, esto indica que los bancos carecen de la abundancia mínima aceptable en una población natural para garantizar una pesquería sustentable de *C. virginica* (Palacios-Fest *et al.*, 1988). Como antecedente de lo observado anteriormente, a principios de 1980 Solano (1995) registro 394 ostiones/m^2 en la Laguna Mecoacán del estado de Tabasco, México. Años más tarde, en el 2007, en el mismo lugar, Garrido *et al.* (2007) reportaron una disminución del 64% de la producción del ostión americano. La disminución se debió a una gestión deficiente de la pesquería por no aplicar criterios de manejo sustentable (FAO, 1997) más que a problemas bio-ecológicos.

Por otro lado, la biomasa total de *C. virginica* en el Río Tigre, desembocadura del Río Tigre y laguna de San Andrés estuvo en concordancia con la disminución anual en la abundancia total de ostras/m^2, observando abundancia muy por debajo de **<** 200 ostras/m^2, y la biomasa con talla de captura comercial para los tres bancos de ≥ 60 mm. De acuerdo con Palacios-Fest *et al.* (1988), una condición adicional para lograr una captura sostenible es que la pesquería se realice en los bancos naturales con más de 200 ostras/m^2 y que estos muestren más del 40 % de ostras con una talla ≥ 60 mm.

En los tres bancos las mayores colectas se registraron de enero a abril. En este periodo, en el área de estudio por la ocurrencia de frentes fríos, tormentas y altas precipitaciones se reducen la población de organismos depredadores (Palma, 2008), esto puede estar permitiendo que los ostiones alcancen a vivir más tiempo. Lo anterior mencionado coincide con lo reportado por Cordero (2000), donde observó que en el periodo de febrero-abril existe una mayor producción en la Laguna de Tamiahua, Veracruz. Mientras que, Vidal-Briseño *et al.* (2015) reporta el número mayor de ejemplares en los meses febrero y marzo en la Laguna de Tampamachoco, Veracruz.

Tabla 2. Biomasa (t) anual estimada de *C. virginica* en el Río Tigre, desembocadura y laguna de San Andrés.

Variables	Banco 1	Banco 2	Banco 3
Reclutas (0-20 mm)	0.015	0.005	0.042
Jovenes- Preadultos (20-40 mm)	0.437	0.665	2.2
Adultos (40-60 mm)	10.7	8.6	14.6
Adultos con más de una reproducción (≥ 60 mm)	11.3	11.9	14.5
Biomasa total	22.45	21.17	31.34

Por otro lado, la escasez de la talla permitida para su extracción en los bancos del Río Tigre y desembocadura es posible que se deba a la contaminación generada por las constantes descargas de aguas residuales por parte de la Comisión de Agua Potable y Alcantarillado, ya que ambos bancos se localizan cerca al ejido "Las Flores" y "Morón", donde es conocido el impacto de la urbanización en los estuarios afectando la calidad del agua, con efectos subsiguientes de estos cambios ambientales en la vida silvestre marina (Paerl, 2014; Lemley *et al.,* 2018). Otra causa probable puede ser la sobreexplotación del ostión en esta zona, ya que en Tamaulipas se ha disminuido la producción pesquera ostrícola (FAO, 2018) debido a la sobreexplotación (CONANP, 2015). Otra posible causa es que en el ejido Morón y las Flores se encuentra el comercio restaurantero donde se consume el ostión por los turistas, lo que implica una fuerte demanda de este recurso. Lo antes mencionado no afecta a la población del banco localizado en la Laguna San Andrés, ya que este está fuera del alcance de los pescadores.

Escasos estudios se han realizado en el Río Tigre y laguna de San Andrés en cuanto al impacto en los ostiones por contaminantes persistentes como son los metales pesados. Uno de estos estudios es el reportado por Goldaracena- Islas (2007), donde se demostró la bioacumulación de cobre y niquel en *C. virginica* en la laguna de San Andrés. Por otro lado, Vázquez-Sauceda (2005) encontró una concentración de metales pesados (cobre, cadmio, fierro, manganeso, plomo, níquel, y zinc) en agua, sedimentos y en el tejido en la laguna de San Andrés y en la desembocadura del Río Tigre. Esto sugiere que una de las posibles fuentes de entrada de estos metales hacia la laguna es a través de este río. Debido a esto, es pertinente realizar estudios de contaminación en los sitios muestreados en sedimentos, agua y organismos. Aunado a lo anterior, en Aldama Tamaulipas la producción agrícola, acuícola y ganadera se lleva a cabo de manera intensiva, donde el uso de plaguicidas es recurrente. Así mismo, la ubicación de un puerto industrial situado a 20 kilómetros al sur del Río Tigre y laguna de San Andrés podría ser un impacto significante en la macrofauna de los cuerpos de agua del sur de Tamaulipas.

En conclusión, los resultados muestran un manejo inadecuado del recurso al observar que en los tres bancos la mayor parte de la población ostiones capturada se encuentra por debajo de la talla permitida para su extracción. Con base en la distribución de tallas se puede definir estudios enfocados a proponer planes de manejo o estrategias de explotación y manejo sustentable del recurso en el Río Tigre y en la laguna de San Andrés.

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Differential effects on the toxicity and bioconcentration of hexavalent and trivalent chromium on the rotifer *Lecane papuana* (Murray, 1913) (Monogononta: Lecanidae)

Efectos diferenciales en la toxicidad y bioconcentración de Cromo hexavalente y trivalente en el rotífero *Lecane papuana* (Murray, 1913) (Monogononta: Lecanidae)

Carlos Vicente Garza-León*[1], Cecilia Alejandra Fernández-Flores*[2], Mario Alberto Arzate-Cárdenas[3], Isidoro Rubio-Franchini[4], and Roberto Rico-Martínez[2‡]

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[1] Laboratorio de Química Solar, Centro de Investigaciones en Óptica, A.C., Prolongación Constitución 607, Fraccionamiento Reserva Loma Bonita. Aguascalientes, Ags., 20200, México.

[2] Departamento de Química, Centro de Ciencias Básicas, Benemérita Universidad Autónoma de Aguascalientes, Avenida Universidad 940, Aguascalientes, Ags., 20131, Mexico.

[3] Investigadoras e Investigadores por México - CONAHCYT. Benemérita Universidad Autónoma de Aguascalientes, Avenida Universidad 940, Aguascalientes, Ags, 20131, México.

[4] Laboratorio Estatal de Salud Pública, Instituto de Salud del Estado de Aguascalientes, Avenida siglo XXI 105, Morelos, Aguascalientes, Ags., 20270, México.

***Corresponding author:**
Roberto Rico-Martínez: e-mail: rrico@correo.uaa.mx; robertoricomartinez@gmail.com

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ABSTRACT

Background: While naturally occurring, heavy metals such as chromium, lead, and mercury also reach aquatic environments via anthropogenic activities, sometimes at alarming concentrations thereby altering the dynamics of the communities. Chromium, which is present in the discharge from automotive and tannery industries, occurs in two stable forms: trivalent (Cr III) and hexavalent (Cr VI). Because these forms differ in their chemical properties, their bioavailability differs and, as a result, so does their effects on organisms. **Goals:** The aim of our study was to assess effects of both Cr III and Cr VI on the rotifer *Lecane papuana* (Murray, 1913) by determining how these forms affect the demographic parameters of survival (l_x) and fecundity (m_x). **Methods:** we performed 48-h acute and 5-d chronic toxicity tests on both forms of chromium. In addition, we determined the bioconcentration factor and metal body burden after 24-h exposure to Cr III and Cr VI. According to their respective LC50 values our results indicate that Cr III was less toxic than Cr VI (Cr III = 2.613 mg/L; Cr VI = 0.177 mg/L). **Results**: Intrinsic growth rate was significantly affected by Cr III, while Cr VI caused no significant changes, but only at 0.0885 mg/L, a concentration representing 0.5 times of its LC_{50} value. Although Cr III was not as toxic as Cr VI, our bioconcentration experiments demonstrated that *L. papuana* accumulated more Cr III than Cr VI and did so at concentrations of environmental concern.

Keywords: alternative test organisms, bioconcentration factor (BCF), intrinsic growth rate, lethal median concentration (LC_{50}), metals.

RESUMEN

Antecedentes: Si bien, los metales pesados como el cromo, el plomo y el mercurio se encuentran de forma natural, también llegan a los ambientes acuáticos a través de actividades antropogénicas, a veces en concentraciones alarmantes, alterando así la dinámica de las comunidades. El cromo, que está presente en los vertidos de las industrias automotrices y curtiduría, se presenta en dos formas estables: trivalente (Cr III) y hexavalente (Cr VI). Debido a que éstas formas difieren en sus propiedades químicas, su biodisponibilidad es distinta, y como resultado, también los efectos sobre los organismos. **Objetivos:** El objetivo de nuestro estudio fue evaluar los efectos de Cr III y Cr VI en el rotífero *Lecane papuana* (Murray, 1913) determinando cómo estas formas afectan los parámetros demográficos de supervivencia (l_x) y fecundidad (m_x). **Métodos:** Realizamos pruebas de toxicidad aguda de 48-h y crónicas de 5-d en ambas formas de cromo. Además, se obtuvo el factor de bioconcentración y la carga corporal de metal después de 24 h de exposición a Cr III y Cr VI. **Resultados:** Respecto a los valores de CL50, nuestros resultados indican que Cr III fue menos tóxico que Cr VI (Cr III = 2.613 mg/L; Cr VI = 0.177 mg/L). La tasa intrínseca de crecimiento se vio significativamente afectada por Cr III, mientras que Cr VI no mostró cambios significativos, solo a 0.0885 mg/L cuya concentración representa 0.5X de su CL50. Aunque Cr III no fue tan tóxico como Cr VI, nuestros experimentos de bioconcentración demostraron que *L. papuana* acumuló más Cr III que Cr VI, y lo hizo en concentraciones ambientales relevantes.

Palabras clave: concentración letal media (CL50), factor de bioconcentración (BCF), metales, organismos de prueba alternativos, tasa intrínseca de crecimiento.

INTRODUCTION

Heavy metal pollution has developed in many countries due to unregulated or illegal discharges to the soil or water systems (He *et al.,* 2015). The sources of these include byproducts from chemical manufacturing (alloys, ornamental plating, green color glass, aluminum anodization, and oxidant agent in diverse chemical reactions), metallurgy (metal fabrication and industrial mining), and production of refractory materials (Jacobs and Testa, 2004). In the environment, chromium occurs in two stable redox states, chromium (III) (Cr III) and chromium (VI) (Cr VI). However, they differ in their bioavailability and mobility (Aharchaou *et al.,* 2017). While Cr III is present in the soil where it is available as a micronutrient for plants, Cr VI is more toxic and considered a human carcinogen (ATSDR, 2012).

Cr III has a low solubility and low mobility, forms complexes with organic matter in soils and waters, and is considered relatively innocuous (Fendorf, 1995)this paper describes surface reactions that influence Cr chemistry in soils. Specifically, retention reactions of Cr(III. Nonetheless, its presence in water results from the release of Cr VI in wastewater and its subsequent biotransformation by both bacteria and eukaryotic cells (Norseth, 1986). Thereafter, in Cr VI-contaminated waters the presence of organic-Cr III complexes must be assessed because such complexes represent the main source for Cr III mobility and thus, the intoxication of exposed biota. Currently, information about the fate and toxicity of Cr III and its organic complexes is limited (Chatterjee & Luo 2010). Nevertheless, while Cr III plays an important role as an essential trace metal in plant metabolism (Bielicka *et al.,* 2005), its role in animals remains controversial (Di Bona *et al.,* 2011; Vincent, 2017).

These two forms of Cr differ in their solubility and mobility. In comparison to Cr III, Cr VI exhibits higher solubility and greater mobility and it because of the active sulfate transporter it through pass through biological membranes (Pereira *et al.,* 2008). Besides Cr VI uptake via sulfate transporter, recent references highlight Cr VI-induced over-expression of several ABC transporters (Feng *et al.,* 2018). Cr VI is considered the most toxic form of chromium due to its strong oxidant power; once it has passed through the cell's membrane Cr VI initiates complex mechanisms involving different biochemical pathways and multiple targets (Rudolf & Červinka, 2006). Althought Cr VI is reduced by enzymatic and non-enzymatic reactions to produce less reactive forms like Cr III and CrV, such reduction reactions produce reactive oxygen species (ROS) that alter the redox environment within the organism, thereby inducing oxidative stress (Arzate-Cárdenas & Martínez-Jerónimo, 2011).

With at least 2000 species rotifers are a very diverse group of micrometazoans that play significant roles in aquatic ecosystems in both the microbial loop and classic food webs (Segers, 2007; Wallace *et al.,* 2015). Rotifers have been used for ecotoxicological studies because of their ease to culture and maintenance, their parthenogenetic mode of reproduction, their short life cycle and generation time, their relatively high growth rates, and their wide geographical distribution (Sarma *et al.,* 2006; Segers, 1996; Pérez-Legaspi & Rico-Martínez, 2001).

The genus *Lecane* (Family Lecanidae) is found in shallow waters, littoral areas, and eutrophic environments (Keppeler *et al.,* 2010). While many species of *Lecane* are cosmopolitan, depending on the zoogeographical region between 6.5 to 22% are endemic (De Manuel, 1994; Segers, 1996). Besides their geographic distribution some *Lecane* spp. are used in activated sludge systems, to improve the quality of the process and removal of organic contaminants (Fiałkowska & Pajdak-Stós, 2008). Pérez-Legaspi & Rico-Martínez, (2001) used three *Lecane* species: *L. hamata* (Stokes, 1896), *L. luna* (Müller, 1776), and *L. quadridentata* (Ehrenberg, 1832), to assess the effect of several chemical compounds and suggested these rotifers as alternatives test species. Klimek *et al.,* (2013) have suggested that *Lecane inermis* (Bryce, 1892) could represent a better option in toxicological evaluations because is more sensitive than species used in the more commonly used *Brachionus* test.

The process of bioconcentration begins when a chemical is absorbed by an organism from the environment through its respiratory or dermal surfaces or through ingestion; concentration of the substance increases with subsequent uptake. The degree to which bioconcentration occurs over the concentration in the environment is expressed as the bioconcentration factor (BCF) and can only be measured under controlled conditions in which dietary intake of the chemical is not included (Arnot & Gobas, 2011). Acute and chronic toxicity tests are helpful to assess the impact of environmental pollutants. In the last few years, bioconcentration has been used to allow better assessment of toxicity together with toxicological tests (Gagneten *et al.,* 2009). Bioconcentration tests using rotifers as model organisms is advantageous because these invertebrates are easily cultured, their life cycles of are short, and their life history parameters are easily determined. Moreover, the stationary stage of analytes (like pesticides and semiochemicals) are reached in a few days (Rivera-Dávila *et al.,* 2021). Lethal Body Burden (LBB) is defined as the body burden in micrograms (µg) per body weight or mmol/kg at which a specific toxic effect is detected: e.g., mortality, reduction in reproduction, enzymatic inhibition. Some authors have applied LBB to evaluate toxicity of compounds and metals in rotifers (Hernández-Flores *et al.,* 2020; van Wezel *et al.,* 1995).

The maximum limits of chromium in water for the protection of aquatic biota and drinking water quality are set in specific guidelines. However, the Mexican regulations are set higher than those of several other countries (DOF, 2022). For instance, the U.S. Environmental protection agency has a standard of 0.1 mg/L (USEPA, 2008); in Canada and Europe drinking water guideline for chromium is a maximum acceptable concentration of 0.05 mg/L (Government of Canada, 2018; HBM4EU, 2020). The Australian Government (2011) has established 0.003 mg/L (for Cr III) and 0.001 mg/L (for Cr VI).

Given the many variations in international governmental norms and legislation dealing with Cr III and Cr VI in water, and taking into consideration the role of both Cr species affecting different trophic levels in freshwater ecosystems and the quality of drinking water in several regions of Mexico; this contribution set a goal of analyzing how Cr III and Cr VI affect the demographic response of *Lecane papuana* (a zooplanktonic native species of Mexico), using acute and chronic tests, and determined the Bioconcentration Factor (BCF) for both Cr chemical speciations.

MATERIALS AND METHODS

Culture of test organisms. *Lecane papuana* was originally collected at El Ocote, Aguascalientes (21.464'N, 102.313'W), Mexico (Saucedo-Ríos *et al.,* 2017), and cultured in the laboratory for more than five years prior to the beginning of the experiments. To start experiments we placed 60 to 80 parthenogenetic females in Petri dishes (~ 70 mm

diameter) filled with ~ 50 mLs of moderately hard, reconstituted water (MHRW) (USEPA, 2002). Stock cultures were maintained in a bioclimatic chamber (Revco Scientific, Inc.) at 25 ± 2 ºC. Rotifers to be used in the toxicity tests were obtained by removing amictic embryos from the stock culture and placing them into Petri dishes with MHRW, without food supplementation, and kept in a bioclimatic chamber at 25 ± 2 ºC.

Acute toxicity tests. ACS grade ≥98% potassium dichromate ($K_2Cr_2O_7$) and chromium (III) potassium sulfate dodecahydrate ($KCr(SO_4)_2 \cdot 12H_2O$) (Fisher Chemical, Hampton NH) were used as Cr VI and Cr III species, respectively. A standard solution (1000 mg/L) of each was made with deionized water; later these standard solutions were used in our experiments. pH was monitored throughout the experiments to ensure that Cr III and Cr VI species were maintained during the experiment.

To do these experiments we placed, 10 neonates (<24 h old) into the wells of a 24-well polystyrene plate (Corning Inc. New York) prefilled with the appropriate solution of MHRW. Each well had a total volume of 1 mL. All treatments were replicated four times. These plates were incubated in a bioclimatic chamber (Revco Scientific, Inc.), without food, for 48 h with a photoperiod of 16:8 h (light: dark), at 25 ± 2ºC. Following exposure, the number of dead or immobilized animals was recorded. We only accepted experimental runs where mortality in the control plates was less than 10%. The LC_{50} values were estimated with the *dcr* package in R.

Chronic toxicity tests. Briefly, eight replicates at six concentrations (0.0, 3.125, 6.25, 12.5, 25, and 50% of the corresponding acute LC_{50}) were used to assess chronic toxicity. For each replicate, five neonates of < 24-h old were placed in 2 mL of EPA medium. All experiments were conducted in 24-well polystyrene plates (Costar Co., USA). After five days, the number of individuals per well was counted and used to estimate the intrinsic rate of population increase, *r*, as follows:

$$r = \frac{ln\left(\frac{N_t}{N_o}\right)}{t},$$

where N_t is the final number of individuals, N_0 is the initial number of rotifers, *Ln* is the natural logarithm, and *t* is the exposure period (5 d). Statistical analyses were performed using GraphPad Prism version 6.0.0

Biomass determination. Ten thousand non-ovigerous, adult females of *L. papuana* were separated (3X), rinsed with deionized water, and dried at 60°C (Fisher Scientific, Isotemp® 500 Series; Waltham, MA) in microtubes until we recorded a constant mass using an analytical balance (Chyo, model JK-200, Japan, ± 0.001 g). The difference in the dry weight of the empty microtube with the microtube with the rotifer biomass is the dry weight expressed as nanograms per individual.

Bioconcentration determinations. We placed 600 rotifers in small Petri dishes (1 mm diameter) with 2 mL of either Cr III or Cr VI solution without food. The concentrations tested correspond to the LC_{50}, LC_{10} and LC_1 for every metal species (n = 4). Rotifers cultured in MHRW without metals served as a negative control. These Petri dishes were placed into a bioclimatic chamber (Revco Scientific, Inc.) at 25 ± 2ºC for 24 h with a photoperiod of 16:8 h (light: dark). After this exposure, rotifers were collected in Petri dishes and carefully rinsed with deionized water to eliminate excess chromium. Then the rotifers were placed in an microtube with 1 mL of deionized water and 500 µL of nitric acid (65%), at 4°C, until analysis.

We quantified chromium in rotifers exposed to all metal concentrations tested in our experiments. Exposure concentrations of the acute toxicity tests were also analyzed with three replicates to obtain actual concentrations instead of nominal concentrations. We performed atomic absorption spectrophotometry with an Analyst 800 Spectrometer (Perkin Elmer, Norwalk, CT) at three settings: (1) Transversely heated graphite furnace, (2) longitudinal Zeeman-effect background correction, and (3) AS-60 autosampler. Chromium was quantified according to the Mexican normative NOM-117-SSA1-1994 (DOF, 1995). To do this we performed Cr analysis methodology with 5 points of a calibration curve, with a minimum r^2 of 0.995. The percentage variability was less than 5% (% RSD) and the maximum standard deviation was 5%, analyzing reagent blanks and fortified and replicated samples, with a variation less than 20 % of the analyte. The detection limit of this method was 0.372 µg/L. In all cases blanks were below detection limits.

The accumulated amount (q) of chromium (µg/g dry weight) was calculated according to Hernández-Flores *et al.,* (2020):

$$q = \frac{(C_o - C_t) * V}{W}$$

Where: C_0, initial chromium concentration in the medium (µg/L); C_t, metal concentration at time t (µg/L); *V*, total volume of sample in liters (L) and *W* dry weight of rotifers in grams (g).

The Bioconcentration Factor (BCF) (dimensionless) was determined according to the following formulas:

$$BCF = \frac{q}{C_0},$$

$$MBB = \frac{q}{n},$$

$$LBB = (LC_{50}) \cdot (BCF).$$

RESULTS

Acute and chronic toxicity tests. Results of the acute toxicity tests showed that *L. papuana* is more tolerant to Cr III than it is to Cr VI; the LC_{50} for Cr III was 15x higher than the respective value for Cr VI (Table 1). Actual exposure concentrations are similar to nominal concentrations (98.75% of similarity, n = 4 for three concentrations tested at beginning of acute exposure). Table 2 shows LC_{50} of several invertebrate species.

Chronic toxicity exposure concentrations were based on the corresponding LC_{50} values for both chromium species (3.125, 6.25, 12.5, 25, and 50% of each LC_{50}) and we obtained their respective intrinsic growth rates. Cr III affects *L. papuana* significantly at 0.163 mg/L (6.25% of its respective LC_{50}); Cr VI affects significantly at 0.0885 mg/L (50% of its respective LC_{50}) (Figure 1).

Bioconcentation determinations. In metal-exposed rotifers the content of Cr III was significantly higher (LC_{10}, 0.1028 mg/L) when compared to controls (Figure 2). Exposure to 0.163 mg/L (6.25% of the LC_{50}) of Cr III negatively affected the intrinsic growth rate of *L. papuana* (Figure 1). Rotifers exposed to Cr VI bioconcentrated this metal at 0.177 mg/L (the respective LC_{50}) (Figure 2). Fecundity was significantly affected when Cr VI reached 0.0885 mg/L (Figure 1), which corresponds to the 50% of the respective LC_{50}.

Table 3 presents results of Cr content in rotifers exposed to Cr, determining the respective BCF and LBB for both redox forms. These data show that *L. papuana* accumulated higher amounts of Cr III and that the LBB for Cr III was higher (1.6X) than that of Cr VI. The BCF for Cr III was 9.4-fold lower than that of Cr VI.

DISCUSSION

This contribution assessed the acute and chronic effects of Cr III and Cr VI in the rotifer *L. papuana* including acute and chronic body burdens (LBB and CBB) and bioconcentration factors (BFC) to make progress in the understanding on the fate and potential environmental effects of chromium in non-target freshwater organisms.

We found *L. papuana* to be a good model to assess the toxicity of Cr III and Cr VI and as a monitor of chromium bioaccumulation. Our results in the acute and chronic toxicity tests indicate that *L. papuana* presents similar sensitivity to toxicants as Cladoceras but offers the advantage to assess the effect of contaminants in the sediment-water interphase (Garza-León *et al.,* 2017).

Acute toxicity tests. As expected from published research, Cr VI caused toxicity at a lower concentration than Cr III (Table 1). This is because Cr VI passes through cell membranes and can be actively transported into the cell by the sulfate transporter. Once inside of a cell, Cr VI generates reduced intermediates of chromium that with hydrogen peroxide (H_2O_2) generate reactive oxygen species (ROS) via Fenton and Haber-Weiss reactions. The result of that metabolism is substantial oxidative damage (Ercal *et al.,* 2001). Moreover, Cr VI can be reduced by enzymatic and non-enzymatic reactions and then interact with some endogenous reductants, such as glutathione (GSH), cysteine, or nucleotides (Arzate-Cárdenas & Martínez-Jerónimo, 2011). However, cells are not very permeable to Cr III and these mechanisms do not occur in the presence of Cr III. Cr III is accumulated at cation-binding sites of the cell membrane. While Cr III is toxic due to its capacity to form complexes with proteins and organic compounds, it is not as toxic as Cr VI (Albert, 1997; Dayan & Paine, 2001; Gagneten & Imhof, 2009).

Table 1. Toxicity values obtained from the acute toxicity test with *Lecane papuana*

Chromium species	LC_{50}, mg/L (CI 95%)	LC_{10}, mg/L (CI 95%)	LC_1, mg/L (CI 95%)	r^2
Cr III	2.613 (2.13-3.10)	0.1028 (0.061-0.143)	0.0117 (0.0005- 0.0004)	0.92
Cr VI	0.177 (0.13-0.23)	0.0788 (0.041-0.116)	0.0132 (0.0044- 0.0264)	0.96

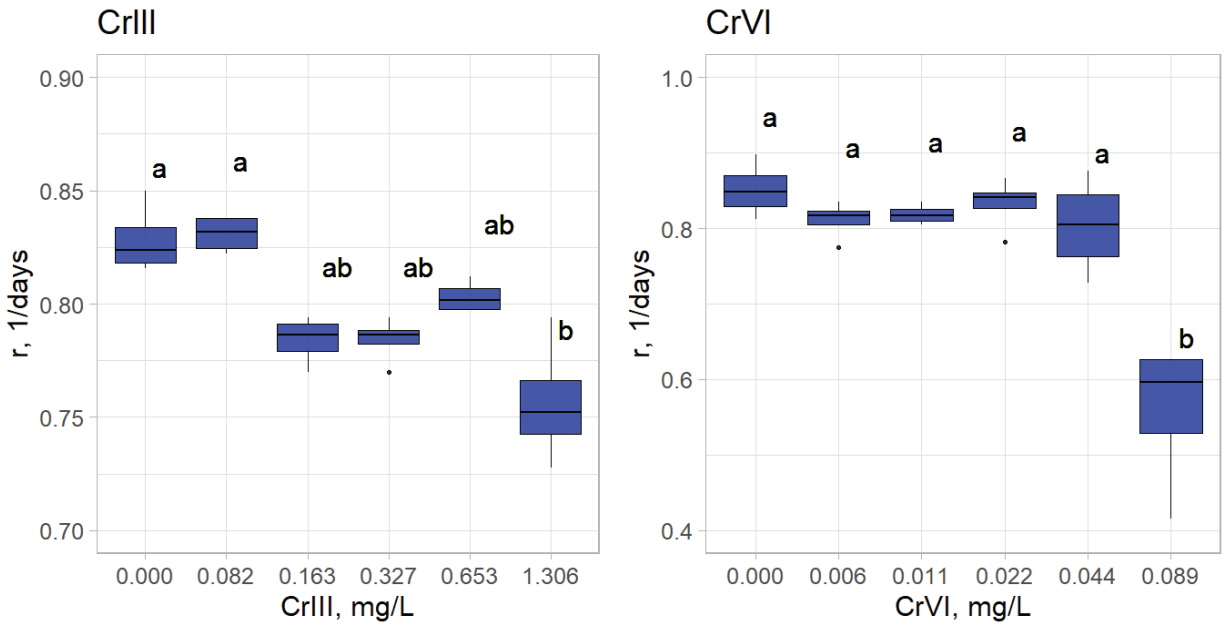

Figure 1. Intrinsic growth rates (r) of *Lecane papuana* exposed to five different concentrations of Cr III and Cr VI. Significant differences were established through one-way ANOVA and Bonferroni's multiple comparison tests. Different letters above the boxes indicate significant differences ($P<0.05$). (N = 4). Statistical analyses were performed with the packages *agricolae* and *ggplot2* in R.

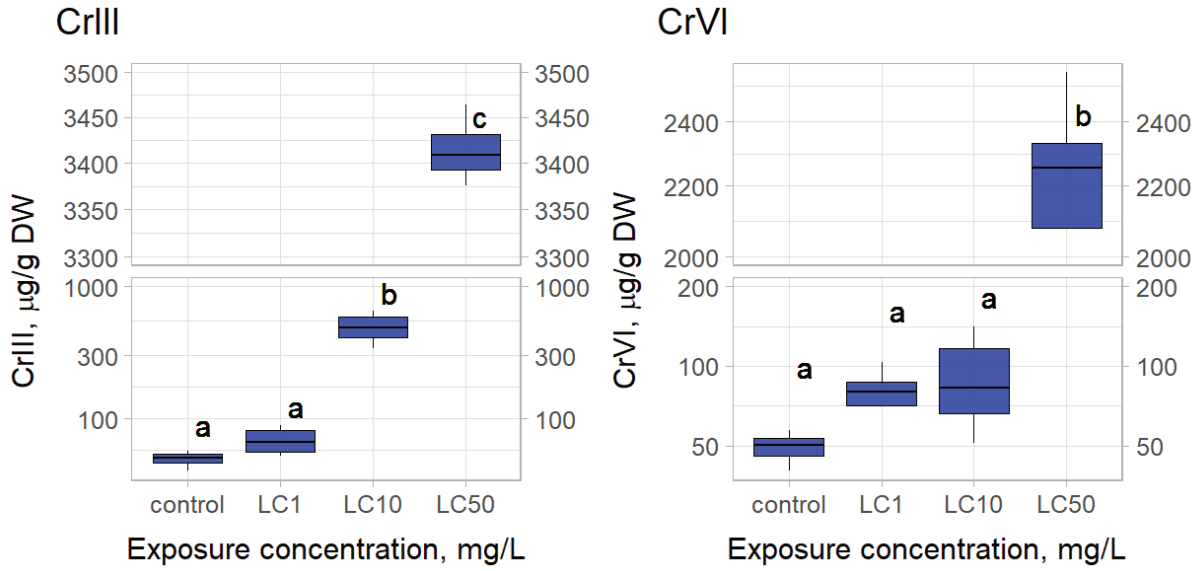

Figure 2. Results of bioconcentration on *Lecane papuana* exposed to three different concentrations of chromium. For Cr III: LC1, LC10 and LC50 correspond to 0.012, 0.103, and 2.613 mg/L, respectively. For Cr VI: LC1, LC10 and LC50 correspond to 0.013, 0.0.079, and 0.177 mg/L, respectively. Significant differences were established through one-way ANOVA and Bonferroni's multiple comparison tests. Different letters above the boxes indicate significant differences ($P<0.05$). (N = 4). Statistical analyses were performed with the packages *agricolae* and *ggplot2* in R.

As observed in Table 2, Cr VI is more toxic for *L. papuana* than for *Brachionus calyciflorus* (Pallas, 1766) (a 48h). However, Cr VI is more toxic for *L. quadridentata* than for *L. papuana*. Cr III is more toxic to *B. calyciflorus* and *L. quadridentata*, in comparison to the effects elicited on *L. papuana* (Table 2). As observed, Cr III promoted lower toxicity than Cr VI.

Several authors have found differences in chromium susceptibility within the family Lecanidae (Saucedo-Ríos *et al.,* 2017) and in comparison to Brachionidae (Sarma *et al.,* 2006).

In addition, cladocerans as *Daphnia exilis* Herrick 1895*, Daphnia magna* Straus 1820*,* and *Daphnia pulex* (Leydig, 1860), exhibited LC_{50} values for Cr VI very close to those ones found for *L. papuana* (Tabla 2). All these tests were carried out in similar conditions to the ones employed in this work (except for the variation in temperature); which it might suggest that *L. papuana* is as sensitive as the freshwater model organisms used worldwide such as *Daphnia magna* (Martínez-Jerónimo *et al.,* 2008). Some authors have suggested that Neoartic cladocerans like *D. magna* are not good model species to predict the effects of contaminants in neotropical ecosystems (Gutierrez *et al.,* 2010)a representative calanoid copepod, we carried out two (acute and chronic. Moreover, it has been described that this rotifer species is also susceptible to the effects of pesticides at environmental concerning concentrations (Garza-León *et al.,* 2017).

For protection of aquatic biota, the maximum allowable limit of chromium is 0.5 mg/L. However, that level is at least 3 times higher than the LC_{50} values recorded for *L. papuana*. Moreover, 0.5 mg/L is even higher for some other native species that are more sensitive to this metal. International guidelines report lower limits for chromium concentration in comparison to the Mexican normative. Nevertheless, these limits are higher than concentrations that cause deleterious effects on rotifers. Thus, while substantial variation exists for acceptable chromium limits among countries, Mexican law is less protective. Our results add information to the need to establish lower protective values for Cr to better protect native freshwater species from acute toxicity.

Chronic toxicity test. The intrinsic population growth rate of *L. papuana* was significantly affected by exposure to Cr (Figure 1). *Lecane papuana* was affected significantly at 0.163 and 0.0885 mg/L, which correspond to LOEC values for Cr III and Cr VI, respectively. Snell & Moffat (1992) carried out a study with similar conditions as ours, in which *Brachionus calyciflorus* was exposed to Cr VI and obtained a LOEC of 3.2 mg/L; representing lower toxicity to *Brachionus* than for *L. papuana.*

Hermens *et al.,* (1984), found a chronic Cr VI EC50 value of 0.27 mg/L for *D. magna*. Wong & Pak, (2004) reported a EC50 of at least 0.268 mg/L for larval development inhibition in the copepod *Mesocyclops pehpeiensis* Hu 1943. Our value for Cr VI lethal effects was lower that both chronic values (Table 1). Gutierrez *et al.,* (2010) found EC50 values from 0.170 to 0.599 mg/L in the copepod *Notodiaptomus conifer* (Kiefer, 1936), for 48 and 24 h respectively. Planktonic organisms are more tolerant to Cr VI than *L. papuana*.

The effects of metals in lifetable parameters have been analyzed in many zooplanktonic species of rotifers and cladocerans (Sarma *et al.,* 2006). A decrease in peak population of a species due to stress may mainly result in (a) poor filtration, consumption or assimilation of the food and (b) reduced neonate production or higher rate of mortality (Sarma *et al.,* 2006).

Bioconcentration determinations. The criterion to classify a chemical substance as "bioaccumulative" requires the BCF to be comprised between 1000 and 5000 (Arnot & Gobas, 2011). Thus, Cr VI in *L. papua-*

na can be classified as "very bioaccumulative" because its BCF was higher than 5000. In contrast, Cr III has a BCF of 1,308.84, which is about 10X lower than one obtained for Cr VI (BCF = 12,299.44). Thus, there is a significant difference in the accumulation rate between Cr III and Cr VI. These results suggest that the patterns of accumulation of the two chromium chemical species and the mechanisms of toxicity are different (Rainbow, 2007)all of which have the potential to cause toxic effects. Subsequent tissue and body concentrations of accumulated trace metals show enormous variability across metals and invertebrate taxa. Accumulated metal concentrations are interpreted in terms of different trace metal accumulation patterns, dividing accumulated metals into two components — metabolically available metal and stored detoxified metal. Examples of different accumulation patterns are described from crustaceans but have a general applicability to all aquatic invertebrates. Toxicity does not depend on total accumulated metal concentration but is related to a threshold concentration of internal metabolically available metal. Toxicity ensues when the rate of metal uptake from all sources exceeds the combined rates of detoxification and excretion (if present. These differences are clear when we compare the concentrations used for each chemical species; while for Cr III we used 2.613 mg/L, for Cr VI we used a 15-fold lesser concentration (0.177 mg/L). Even so, the Cr VI BCF was an order of magnitude higher (10-fold). This behavior of chromium chemical species was already reported for other freshwater rotifer species like *L. quadridentata* and *B. calyciflorus*, where Cr VI was more efficiently bioconcentrated than Cr III (Hernández-Ruiz *et al.,* 2016). A comparison of bioconcentration studies shows that *L. papuana* bioconcentrated more Cr VI than freshwater fishes (Table 4).

The results of Bioconcentration of *L. papuana*, show that even at low concentrations Cr III is bioconcentrated in the organism (Figure 2). However, adverse effects are detected at a slightly higher concentration (see LOEC value in Figure 1). Regarding Cr VI, bioconcentration starts at LC_{50} value, but chronic effects are observed at very low concentrations at ½ of the LC_{50} value (see figures 1 and 2). However, we have to consider that acute tests only last 24h, while chronic tests last 5 days.

Due to the scarcity of data on Cr VI accumulation in invertebrates, we decided to compare our data with freshwater invertebrates and freshwater/coastal fishes (Table 4). In the fish *Puntius sarana* (Hamilton, 1822) exposed to 2.19 mg/L (12-fold higher than the exposure concentration for *L. papuana*) with supplemental food; the concentration of Cr VI found in the fish indicates that *L. papuana* bioconcentrated 21-fold more Cr VI than the fish. Bioacummulation of Cr VI in the fish *Catla catla* (Hamilton, 1822) (exposed to 2.19 mg/L) was 2.7-fold lower than *L. papuana* (Table 4). The fish *Mugil cephalus* (Linnaeus, 1758), bioaccumulated at least 3-fold less Cr VI with an exposure concentration at least 56-fold higher (160 mg/L) than *L. papuana*. Therefore, we can infer that the rotifer *L. papuana* bioconcentrates more Cr VI than these freshwater fishes. These differences can be explained by the presence of more sophisticated mechanisms to excrete Cr VI in fishes and the fact that the metabolism of small organisms is more accelerated with respect to larger organisms (Gutierrez *et al.,* 2010).

Table 2. Comparisons of values for chromium in other invertebrates

Species	values mg/ L	Source
Brachionus calyciflorus (Monogononta: Brachionidae)	0.64 - 1.051 (24 h) Cr III	Hernández-Ruiz *et al.* (2016)
Lecane quadridentata (Monogononta: Lecanidae)	$4x10^{-6}$ (24 h) Cr VI	
	1.279 (24 h) Cr III	
	$4.7 \times 10_{-5}$ (24 h) Cr VI	
Lecane papuana (Monogononta: Lecanidae)	2.613 (48 h) Cr III	This study
	0.177 (48 h) Cr VI	
Brachionus calyciflorus (Monogononta: Brachionidae)	8.3 (48 h) Cr VI	Snell and Moffat (1992)
Daphnia exilis (Anomopoda: Daphniidae)	0.1170 (48 h) Cr VI	Martínez-Jerónimo *et al.* (2008)
Daphnia magna (Anomopoda: Daphniidae)	0.2076 (48 h) Cr VI	Martínez-Jerónimo *et al. (*2006)
Daphnia pulex (Anomopoda: Daphniidae)	0.13 (48 h) Cr VI	Velandia and Montañez (2010)
Lecane hamata (Monogononta: Lecanidae)	4.41 (48 h) total Cr	Pérez-Legaspi and Rico-Martínez (2001)
Lecane luna (Monogononta: Lecanidae)	3.26 (48 h) total Cr	
Lecane quadridentata (Monogononta: Lecanidae)	4.50 (48 h) total Cr	
Daphnia magna (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	CCME (1999)
Procambarus clarkia (Decapoda: Cambaridae)	500 (96 h) Cr VI	
Simocephalus vetulus (Anomopoda: Daphniidae)	0.015 (24 h) Cr VI	

Table 3. Bioconcentration, Body burdens, and chronic toxicity ("r" inhibition) of chromium in *Lecane papuana* after 24-h exposure to Cr III or Cr VI. n = 4.

Chromium species	(µg of Cr/rotifer)	Accumulated Cr concentration in *L. papuana* (µg/g)	BCF	LBB mmol/kg DW	EC_{50} "r"	$LOEC_{chronic}$ mg/L	$NOEC_{chronic}$ mg/L	MATC	ACRr
III	$1.71x10^{-4} \pm 3.15x10^{-6}$	3420 ± 62.93	1308.84	65.76	0.1181	0.163	0.0816	0.115	22.66
VI	$1.089x10^{-4} \pm 8.97x10^{-6}$	2177 ± 179.38	12299.44	41.86	0.1127	0.0885	0.04425	0.062	2.83

Hyalella azteca (Smith, 1874), bioaccumulated 16-fold lower concentration of Cr VI than what we found in *L. papuana* (Table 4), but this study was carried out in a period of 4 weeks of exposure (ours was 24 h) and the exposure concentration was 0.176 mg/L, almost the same concentration that we used (0.177 mg/L). The bioconcentration of chomium in *Daphnia magna* and *Argyrodiaptomus falcifer* (Daday, 1905) was approximately 20 and 10-fold lower (respectively) than that found in *L. papuana* (Table 4). In addition, the concentration to which *L. papuana* was exposed (0.177mg/L) was about the 50% of that used with those two species (0.350 mg/L); however, our study was carried out in 24 h and those mentioned above in two days. The crab *Zilchiopsis collastinensis* (Pretzmann, 1968), showed a bioconcentration 10-fold lower than the one found in *L. papuana*, which were exposed 14 and 5 days respectively (Table 4). Moreover, the crab was exposed to a concentration 28-fold times higher (5 mg/L) than what was used in *L. papuana*. Thus, comparison of bioconcentration and adverse effects of Cr among aquatic species suggests that some species had a strong ability to resist the Cr VI exposure and/or to remove it from the body, involving Glutathione S-transferases and metallothionein proteins in the detoxification process; antioxidant enzyme systems have been reported in cases of recovery from Cr VI damage (Yuan *et al.,* 2017). Similarly, previous studies with organisms of the Lecanidae family mention that these rotifers have a thick lorica that acts as a barrier against metal ions, which can be deposited in the lorica of *Lecane* rotifers; thus, bioconcentrating more chromium than other species (Table 4). The ecological niche occupied by species might have played a significant role in the accumulation; *Lecane* as a surface feeder and planktivorous species, can accumulate chromium from water, mud, debris, and detritus in addition to macrophytes and algae (Joadder, 2014). Binding of chromium in the sediment soil, depends on oxidation status, which is altered by pH, and microbial processes (Sanyal *et al.,* 2017). *Lecane papuana* due to its high sensitivity to Cr VI can be considered a biomonitor species; biomonitor species can indicate the presence of contaminants even when they are not detected in a specific environment (Gagneten &

Table 4. Bioconcentration Factor for Chromium (VI) in different taxa

Species	Accumulated Cr concentration (µg/g)	BCF	Exposure concentration (mg/L)	LC_{50}	Source
Argyrodiaptomus falcifer (Calanoida: Diaptomidae)	50 (2 d) Cr VI	231	0.350	NA	Gagneten *et al.* (2009)
	80 (2 d) Cr VI	281			
Daphnia magna (Anomopoda: Daphniidae)					
Catla catla (Cypriniformes: Cyprinidae)	800 (7 d) Cr VI	NA	2.19	105.87(24h); 51.41 (48h)	Sanyal *et al. (*2017)
	400 (7d) Cr VI			24.53(24h); 17.13(48h)	
Labeo bata (Cypriniformes: Cyprinidae)	100 (21 d) Cr VI			59.31(24h); 29.44(48h)	
Puntius sarana (Cypriniformes: Cyprinidae)					
Hyalella Azteca (Amphipoda: Hyalellidae)	134.59 (4 weeks) Cr VI	200	NA	NA	Norwood *et al.* (2006)
Lecane papuana (Monogononta: Lecanidae)	2,177 (24 h) Cr VI	12,299.44	0.177	0.177	This study
Mugil Cephalus (Mugiliformes: Mugilidae)	700 (96 h) Cr VI	NA	160	65.01 (96h)	Rajkumar and Tennyson (2013)
Zilchiopsis collastinensis (Decapoda: Trichodactylidae)	200 (14 d) Cr VI	766.9	2 and 5	NA	Gagneten and Imhof (2009)

Imhof, 2009). The sensitivity of an organism depends on several toxicokinetic mechanism; bioaccumulation of a substance is the process that confers the organism with more resistance to adverse effects (Muggelberg *et al.,* 2017). In the case of *L. papuana* it has been documented elevated BCF´s (which explain its tolerance) when compared with other species and contaminants (Rivera-Dávila *et al.,* 2021).

The Maximum permissible levels of chromium in water of diverse uses in Mexico is at least 0.5 mg/L, and the values that we obtained for chronic LOEC and NOEC for Cr III were 0.163 and 0.0816 respectively, which are around 3 and 6 times lower than 0.5 mg/L in Mexican Regulations. The chronic LOEC for Cr VI was 0.0885 mg/L and the NOEC was 0.0443 mg/L, which are 5.6 and 11.3 times lower than the maximum concentration allowed in the Mexican Regulation. These findings mean that *L. papuana* could be harmed in chronic exposures to chromium, in spite of compliance with the current environmental legislation.

There are few studies of chromium carried out with aquatic invertebrates (including cladocerans and rotifers), and the differences in the ranges for acute and chronic values (such as LC_{50}) is great. There is a need to analyze these values and the protocols employed by different researchers. A first step is to use actual values instead of nominal ones (or at least indicate the percentage of similarity between actual and nominal values). The use of acute tests were acute values are obtain in absence of food somehow reduces the differences in ranges. The differences among species exposed to chromium could be due that this metal can act in different ways, in different species. Chromium has a role in glucose, fat and protein metabolism, participating in the insulin action. It links directly to macromolecules; fragments the DNA chains and acts in the peroxidation of lipids, generating free radicals and modifying the routes of cell signals. All these processes can contribute to the toxicity and carcinogenicity of chromium compounds (Gagneten & Imhof, 2009). Moreover, depuration of accumulated chromium in aquatic organisms differ markedly among the different taxa and the process generally has a complex pattern of elimination, showing large differences among metals and invertebrate groups (Rainbow, 2007).

CONCLUSION

In this research, acute, chronic, and bioconcentration toxicity tests were evaluated with the rotifer *L. papuana*, which is a sensitive organism to effects of both chromium species. We evaluated the susceptibility of this organism at concentrations that are of environmental concern; we discussed the acute rotifer sensitivity comparing our results with those of other aquatic invertebrate species. In general, *L. papuana* is one of the most sensitive species for both Cr III and Cr VI. In general, both chromium species showed high BFCs values. Our results support the idea that *L. papuana* is a suitable organism for toxicity assessment in tropical and subtropical environments, in acute, chronic, and bioconcentration tests. Maximum allowable chromium concentration in Mexican Regulations are higher than LC_{50} values obtained for this rotifer; and mostly above international values for protection of aquatic biota.

Acute and chronic effects on *L. papuana*, including acute and lethal body burdens and bioconcentrations factors aid answering questions on the fate, mechanisms of toxicity, and potential environmental effects of chromium in organisms.

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ARTÍCULO DE REVISIÓN

Diversidad y flexibilidad metabólica de consorcios nitrificantes y desnitrificantes usados en el tratamiento de aguas residuales

Diversity and metabolic flexibility of nitrifying and denitrifying consortia used in wastewater treatment

José Juan Ramírez-Muñoz, Omar Oltehua-López, Flor de María Cuervo-López, Anne-Claire Texier*

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Laboratorio de Fisiología Microbiana, Departamento de Biotecnología, División CBS, Universidad Autónoma Metropolitana-Iztapalapa. Av. Ferrocarril San Rafael Atlixco 186, Leyes de Reforma 1A Sección, Iztapalapa, Ciudad de México, 09310. México

***Corresponding author:**
Anne-Claire Texier: e-mail: actx@xanum.uam.mx

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RESUMEN

Antecedentes. Los procesos de la nitrificación y desnitrificación forman parte del ciclo biogeoquímico del nitrógeno. Los microorganismos que los llevan a cabo son empleados en los sistemas dedicados al tratamiento de aguas residuales para eliminar un contaminante muy común; el amonio (NH_4^+), y liberar nitrógeno molecular (N_2). **Objetivo**. Mostrar la diversidad y flexibilidad metabólica de consorcios nitrificantes y desnitrificantes usados en la eliminación de nitrógeno de aguas residuales. **Resultados**. En estos microorganismos taxonómicamente diversos, las bacterias son las mejor estudiadas. Se las divide y nombra según el proceso principal que realizan. Aunque en realidad gracias a los genes que comparten, pueden presentar una diversidad y flexibilidad metabólica, que las capacita para sobrevivir en condiciones cambiantes y con funciones distintas del proceso que canónicamente se les atribuye. Los genes característicos de estos procesos son empleados como marcadores moleculares en estudios de comunidades. Sin embargo, taxones conocidos canónicamente como nitrificantes pueden tener genes funcionales propios del proceso desnitrificante. Microorganismos catalogados como típicamente desnitrificantes pueden tener genes funcionales del proceso nitrificante. Los consorcios (flóculos, gránulos y biopelículas) empleados en la eliminación de NH_4^+ son un ejemplo de comunidades que pueden tener capacidades superiores o distintas de las que tienen sus integrantes individualmente. **Conclusiones**. La presente revisión conjunta información fisiológica, genética y ecológica que contribuye a entender mejor la gran diversidad y flexibilidad metabólica de los consorcios nitrificantes y desnitrificantes. Se destaca que, en los sistemas artificiales, un mayor conocimiento de los taxones participantes, así como de sus relaciones tróficas, metabólicas y de comunicación posibilitaría un mejor control de los procesos nitrificante y desnitrificante para que estos sean más eficientes y estables.

Palabras clave: Ciclo del nitrógeno, consorcio, diversidad y flexibilidad metabólica, proceso desnitrificante, proceso nitrificante.

ABSTRACT

Background. Nitrification and denitrification processes are part of the biogeochemical nitrogen cycle. The microorganisms that carry them out are used in wastewater treatment systems to remove a very common pollutant; ammonium (NH_4^+) and release molecular nitrogen (N_2). **Objective**. Show the diversity and metabolic flexibility of nitrifying and denitrifying consortia used in the elimination of nitrogen from wastewater. **Results**. Among these taxonomically diverse microorganisms, bacteria are the best studied. They are divided and named according to the main process they carry out. Although thanks to the genes they share, their diversity and metabolic flexibility can enable them to survive under changing conditions and through functions different from the process that is canonically attributed to them. The characteristic genes of these processes are used as molecular markers in community studies. However, taxa known canonically as nitrifying may have functional genes of the denitrifying process. Microorganisms classified as typically denitrifying may have functional genes of the nitrifying process. The consortia (flocules, granules and biofilms) used in the elimination of NH_4^+ are an example of communities that can have superior or different capacities than those of their individual

members. **Conclusions**. This review compiles physiological, genetical, and ecological information that contributes to a better understanding of the great diversity and metabolic flexibility of nitrifying and denitrifying consortia. It stands out that in artificial systems, a better knowledge of the participating taxa and their trophic, metabolic and communication relationships, would allow a better control of the nitrifying and denitrifying processes for making them more efficient and stable.

Keywords: Consortium, denitrifying process, diversity and metabolic flexibility, nitrifying process, nitrogen cycle.

INTRODUCCIÓN

El nitrógeno es el elemento más abundante en la atmósfera del planeta Tierra (78%) y uno de los principales elementos de la célula como integrante de ácidos nucleicos, aminoácidos. Sin embargo, este elemento no es fácilmente accesible para la mayoría de los organismos, debido a que el triple enlace del nitrógeno molecular (N_2) resulta en una molécula poco reactiva. Al nitrógeno elemental que se une a un átomo distinto y que forma compuestos orgánicos o iones como el amonio (NH_4^+) o nitrato (NO_3^-), se le conoce como nitrógeno reactivo o biológicamente disponible (Elser, 2011; Boyle, 2017). En la naturaleza, la mayor parte del nitrógeno reactivo se origina mediante procariotas fijadores de nitrógeno atmosférico (diazótrofos) mientras que una pequeña parte es generada por rayos. Sin embargo, actualmente las fuentes antropogénicas de nitrógeno reactivo (combustión de hidrocarburos, desechos de crianza de animales, fertilizantes nitrogenados) exceden las contribuciones naturales, liberando gases de efecto invernadero (óxido nitroso: N_2O, óxido nítrico: NO, dióxido de nitrógeno: NO_2), deteriorando la calidad del agua, la salud y alterando el ciclo global del nitrógeno (Ren *et al*., 2017; Beeckman *et al*., 2018; Scheer *et al*., 2020).

En los cuerpos de agua, la presencia de compuestos nitrogenados puede llegar a ser tóxica, conducir a la eutrofización, hipoxia y pérdida del hábitat y de la biodiversidad (Ahmed *et al*., 2017; Stevens, 2019). El principal contaminante nitrogenado en los cuerpos de agua es el NH_4^+ y sus compuestos asociados como son NO_3^- y nitrito (NO_2^-). Para eliminar estos y otros contaminantes, así como el carbono de la materia orgánica, se ha recurrido a procesos físicos, químicos o biológicos, siendo estos últimos los que se emplean preferentemente en las plantas de tratamiento de aguas residuales (Karri *et al*., 2018). La eliminación biológica de nitrógeno se basa en dos procesos microbiológicos que forman parte del ciclo del nitrógeno: la nitrificación y la desnitrificación. Son llevados a cabo por las bacterias y arqueas, así como por otros grupos microbianos, como algas y hongos desnitrificantes, siendo el grupo de las bacterias el mejor estudiado (Ferrera & Sánchez, 2016; Yin *et al*., 2018). A pesar de la amplia diversidad taxonómica y capacidad metabólica de los microorganismos transformadores del nitrógeno (MTN), tradicionalmente se les ha agrupado, por ejemplo, como "nitrificantes" o "desnitrificantes" según el proceso respiratorio principal que realicen para obtener energía, sin tener en cuenta otros procesos metabólicos que puedan efectuar (Kuypers *et al*., 2018). Para que la eliminación biológica de nitrógeno y otros contaminantes se lleve a cabo a pesar de las variaciones en la composición del influente y otras alteraciones medio ambientales, los microorganismos, de forma individual o en comunidades, deben ser resistentes y resilientes. Deben contar, por ejemplo, con vías metabólicas alternativas que les permitan seguir cumpliendo un proceso o sobrevivir, es decir, deben ser metabólicamente flexibles (Kuypers *et al*., 2018). La comprensión de la estabilidad funcional de los ecosistemas microbianos y su respuesta ante cambios son temas fundamentales en el estudio y el control de estos sistemas (Isobe & Ohte, 2014). Al conjunto de poblaciones microbianas con sus distintas especies interactuando para realizar una función o un proceso de nuestro interés y que a las poblaciones por separado se les dificulta o no son capaces de realizar, se le llama consorcio microbiano (Congestri *et al*., 2020; Shahab *et al*., 2020). Gracias al avance en la ciencia y en particular al desarrollo de la biología molecular y las ciencias ómicas, como la transcriptómica y genómica, es posible conocer qué tipo de microorganismos y en qué proporción están presentes en un sistema natural o artificial (Qin *et al*., 2018), en otras palabras, conocer la estructura de las comunidades y asociar un cambio en las comunidades con el cambio en algún parámetro medio ambiental a través del tiempo (Ferrera & Sánchez, 2016; Dangi *et al*., 2019). No está de más recordar la sinergia positiva que puede darse entre la ecología, fisiología microbiana y biología molecular, por un lado, para dar respuestas a interrogantes ecológicas, metabólicas, genéticas y evolutivas, y por otro lado, para la ingeniería de los sistemas diseñados y lograr que las comunidades de microorganismos realicen un proceso eficiente, seguro y estable (McMahon *et al*., 2007; Sharma & Shukla, 2020; Biswas *et al*., 2022).

El objetivo de este trabajo es mostrar evidencia de la diversidad y flexibilidad metabólica de las bacterias y consorcios involucrados en la eliminación de NH_4^+ y sus compuestos asociados de aguas residuales por los procesos de nitrificación y desnitrificación en ecosistemas artificiales. La información recopilada fue analizada principalmente a través de tres enfoques: ecología, fisiología microbiana y biología molecular, abordando aspectos de formación de comunidades, estabilidad de estas ante cambios del medio, diversidad metabólica y genética de los microorganismos relacionados con los procesos de la nitrificación y desnitrificación.

DISCUSIÓN

Procesos biológicos en el ciclo del nitrógeno. El ciclo del nitrógeno (Figura 1) se compone de los siguientes procesos biológicos: la fijación de N_2, la amonificación, la reducción desasimilatoria de NO_3^- a NH_4^+ (por sus siglas en inglés DNRA: dissimilatory nitrate reduction to ammonium), la oxidación anaerobia de NH_4^+ (en inglés anammox: anaerobic ammonium oxidation), la oxidación completa del amoníaco (NH_3) (en inglés comammox: complete ammonia oxidation), la nitrificación y la desnitrificación. La formación de NH_3/NH_4^+ puede realizarse por la fijación o reducción biológica del N_2, por la mineralización de la materia orgánica, por la DNRA o en menor proporción de manera abiótica a través de rayos (Stein & Klotz, 2016; Takai, 2019). La fijación biológica del nitrógeno ($N_2 \rightarrow NH_3$) es un proceso fundamental en el ciclo del nitrógeno porque genera nitrógeno bioaccesible a partir de una molécula muy poco reactiva. Para combinar N_2 con H_2 y obtener NH_3 se necesitan de 300-400 °C y una presión de ~300 atm, mientras que las bacterias y arqueas diazótrofas con sus nitrogenasas catalizan esta reacción a temperatura y presión ambiental (Lee *et al*., 2014; Soumare *et al*., 2020; Zehr & Capone, 2020). La amonificación es parte de la mineralización de la materia orgánica que comienza con la ruptura de proteínas a aminoácidos (aminación) y la posterior conversión de los grupos amino (NH_2) a NH_3 (Stein & Klotz, 2016). El proceso de DNRA sirve de vínculo entre la oxidación de compuestos nitrogenados y su reducción, y es un proceso reportado en diversos ambientes anaerobios como suelos, sedimentos de humedales marinos o de estuario y re-

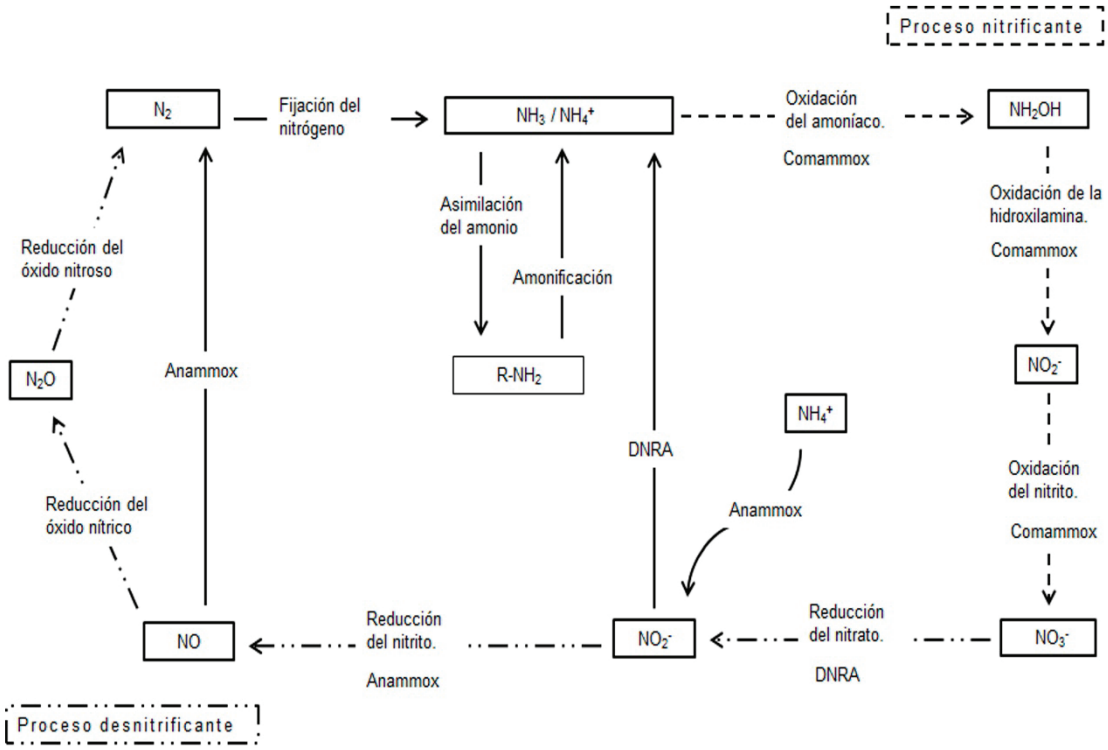

Figura 1. Ciclo del nitrógeno. N_2: nitrógeno molecular, NH_3: amoníaco, NH_4^+: amonio, R-NH_2: amina, NH_2OH: hidroxilamina, NO_3^-: nitrato, NO_2^-: nitrito, NO: óxido nítrico, N_2O: óxido nitroso. Comammox: oxidación completa del amoníaco. DNRA: Reducción desasimilatoria del nitrato a amonio. Anammox: oxidación anaerobia del amonio.

cientemente en la interfaz óxica-anóxica de zona ribereña, así como en sistemas artificiales (Wang *et al*., 2020a, b). El proceso anammox utiliza el NH_4^+ como donador de electrones y al NO_2^- como aceptor final, con los intermediarios NO e hidrazina (N_2H_4) para formar N_2 (Kuenen, 2020; Weralupitiya *et al*., 2021). En el proceso comammox, la nitrificación u oxidación del NH_3 hasta NO_3^-, es efectuada por un solo microorganismo (*Nitrospira* spp.), a diferencia del proceso nitrificante en el que participan dos grupos bacterianos distintos (Daims *et al*., 2016; Mehrani *et al*., 2020; Zheng *et al*., 2022). La nitrificación (Figura 2) es un proceso respiratorio aerobio donde el NH_4^+ y el NO_2^- sirven como donadores de electrones y el oxígeno (O_2) se emplea como aceptor final de electrones, y canónicamente se le ha atribuido principalmente a dos grupos de bacterias quimiolitoautótrofas filogenéticamente no relacionadas entre sí, donde la parte de la nitritación ($NH_4^+ \rightarrow$ hidroxilamina (NH_2OH) $\rightarrow$ NO_2^-) la llevan a cabo las bacterias amonio oxidantes (AOB) así como arqueas amonio oxidantes (AOA), y la parte de la nitratación ($NO_2^- \rightarrow$ NO_3^-) las bacterias nitrito oxidantes (NOB) (Stein & Klotz, 2016; Takai, 2019). El proceso desnitrificante (Figura 2) por su parte comprende una serie de reducciones enzimáticas donde se emplean compuestos orgánicos o inorgánicos como donadores de electrones para reducir el NO_3^- o NO_2^- hasta N_2: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, y ocurre generalmente cuando la concentración de O_2 disuelto es menor que 0.2 mg O_2/L (Zumft, 1997; Seitzinger *et al*., 2006; Takai, 2019).

Bacterias nitrificantes. Taxonómicamente las bacterias nitrificantes AOB y NOB se agrupan en el phylum Proteobacteria (clases *Alfaproteobacteria*, *Betaproteobacteria* y *Gamaproteobacteria*), en cuatro phyla de Deltaproteobacteria y en el caso del género *Nitrospira*, pertenece al phylum Nitrospirae (Waite *et al*., 2020). Varios de estos microorganismos especializados poseen una alta diversidad filogenética y metabólica (Garrity & Holt, 2015; Spieck & Bock, 2015). En plantas de tratamiento de aguas residuales, además de bacterias nitrificantes, se han encontrado AOA, la mayoría de la clase *Nitrososphaeria*, phylum Thaumarchaeota (Kerou *et al*., 2016; Xu *et al*., 2021) o Thermoproteota (Rinke *et al*., 2021). Las condiciones ambientales (temperatura, concentración de O_2 disuelto) y características de las aguas residuales (por ejemplo, concentración de NH_4^+ y materia orgánica) influyen en determinar cuáles grupos son los dominantes tanto en las AOB como en las AOA (Lang *et al*., 2018; Yin *et al*., 2018). La composición en cuanto a microorganismos participantes de una comunidad y la función de esta pueden no estar relacionadas, microorganismos filogenéticamente cercanos pueden presentar diferentes características metabólicas y, por el contrario, la capacidad de oxidar el NH_4^+ o de reducir NO_3^- a N_2 puede presentarse en microorganismos filogenéticamente distantes. Es por esto que, al abordar el estudio de un grupo de microorganismos en cuanto a caracterización y cuantificación, es preferible hacerlo desde un punto de vista funcional a través de genes marcadores funcionales, cuyas secuencias puedan ser utilizadas para crear cebadores o sondas génicas que puedan presentar una alta especificidad y poder de resolución. De esta forma, sería posible relacionar la presencia de estos genes con características del entorno o función de las poblaciones en una comunidad (Petersen *et al*., 2012; Ma *et al*., 2019; Zheng *et al*., 2021). Es posible que los genes de los principales ciclos biogeoquímicos hayan surgido y diseminado por transferencia horizontal o lateral, mientras que las presiones de selección de tipo nutricional o energético

hayan promovido su permanencia en los MTN en diferentes entornos (Alvarez *et al.*, 2011; Khadka *et al.*, 2018; Parsons *et al.*, 2021).

Enzimas amonio monooxigenasa (AMO, gen *amoA*) e hidroxilamina oxidorreductasa (HAO, gen *hao*). En el primer paso del proceso nitrificante (Figura 2), la oxidación de $NH_3 \rightarrow NH_2OH$ es catalizada por la metaloenzima amonio monooxigenasa (AMO) de 283 kDa (Gilch *et al.*, 2009; Lehnert *et al.*, 2018). La enzima AMO está ligada a la membrana plasmática y contiene cobre, hierro y zinc, siendo el cobre el elemento clave en la oxidación del NH_3. La AMO bacteriana está formada por las subunidades AmoA, AmoB y AmoC, codificadas respectivamente por los genes *amoA*, *amoB* y *amoC*. Estos genes se presentan en una o múltiples copias, la mayoría agrupados en operones *amoCAB* (Norton *et al.*, 2002, 2008). Otros genes que forman parte del *amoCAB* son el *amoD* y el *amoE*, con proteínas cuya función está aún por determinarse (Musiani *et al.*, 2020). La enzima AMO además de ser clave en el proceso nitrificante es importante porque es capaz de participar en la eliminación de muchos otros compuestos (Sayavedra-Soto *et al.*, 2010; Su *et al.*, 2021). El sitio activo de la enzima AMO se sitúa en el polipéptido producido por el gen *amoA*, donde el acetileno, un inhibidor de la AMO, se une de manera irreversible (Gilch *et al.*, 2009). El gen *amoA* está altamente conservado y puede presentarse en las AOB en varias copias por célula (Norton *et al.*, 2002; Musiani *et al.*, 2020). Cabe señalar que la enzima AMO es poco conocida a nivel estructural y de procesos (Lehnert *et al.*, 2018). La enzima bacteriana periplásmica hidroxilamina oxidorreductasa (HAO) interviene en el paso $NH_2OH \rightarrow NO_2^-$ y su gen *hao* puede ser utilizado también como un marcador molecular alternativo para las AOB (Caranto & Lancaster, 2017). Se debe considerar que el gen *hao* también está presente en los organismos no amonio oxidantes, incluidas las bacterias oxidantes de metano y otros microorganismos (Junier *et al.*, 2010; Lehnert *et al.*, 2018). Las AOA codifican distintos genes homólogos a las subunidades de la AMO y se han propuesto genes (*amoX*, *amoY*, *amoZ*) que podrían codificar las subunidades AmoX, AmoY, AmoZ (Tolar *et al.*, 2017; Hodgskiss *et al.*, 2023). Al no encontrarse un gen homólogo de la hidroxilamina oxidorreductasa (pero sí la presencia de NH_2OH como intermediario), el paso de oxidación del NH_3 a NO_2^- en las AOA no está aún definido (Vajrala *et al.*, 2013; Yin *et al.*, 2018; Wu *et al.*, 2020). Las AOA serían un grupo dominante en ambientes con una baja concentración de NH_3, de O_2 disuelto, baja carga orgánica, temperaturas altas o bajas y en aguas residuales salinas (Yin *et al.*, 2018).

Enzima nitrito oxidorreductasa (NXR, genes *nxrA, nxrB*). El paso de nitratación (Figura 2): $NO_2^- \rightarrow NO_3^-$, es llevado a cabo por la enzima nitrito oxidorreductasa (NXR). La enzima NXR consta de tres subunidades codificadas por el gen *nxr*: alfa (*nxrA*), beta (*nxrB*) y gamma (*nxrC*). La subunidad alfa (NxrA) que se une al sustrato puede encontrarse orientada en dos formas, hacia el citoplasma o al periplasma. La forma orientada hacia el citoplasma se presenta por ejemplo en *Nitrobacter* y la forma orientada al periplasma en *Nitrospira* (Lücker *et al.*, 2010). Las NXR citoplásmicas están afiliadas filogenéticamente con las enzimas nitrato reductasas y las formas periplásmicas con la familia de enzimas de unión a cofactores de molibdopterina de tipo II (Pester *et al.*, 2014). Los genes *nxrA*, y en particular el *nxrB*, son usados como marcadores genéticos para las NOB, así como para las bacterias de *Nitrospira* que llevan a cabo la oxidación completa del NH_3 (comammox). El gen *nxrB* también se presenta en múltiples copias. Por ejemplo, *Nitrospira marina* Watson *et al.* 1986 (Schoch *et al.*, 2020) puede contener hasta seis copias. *Nitrospira* y *Nitrobacter* son NOB comunes en las plantas

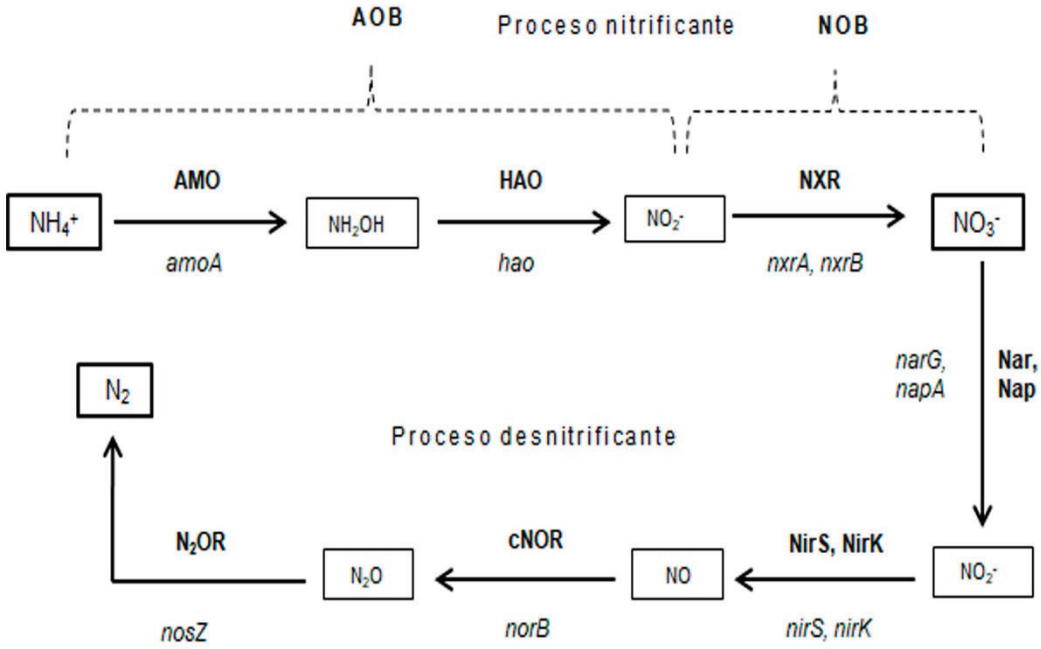

Figura 2. Procesos nitrificante y desnitrificante. **AOB**: bacterias amonio oxidantes. **NOB**: bacterias nitrito oxidantes. **AMO**: amonio monooxigenasa, gen *amoA*. **HAO**: hidroxilamina oxidorreductasa, gen *hao*. **NXR**: nitrito oxidorreductasa, genes *nxrA*, *nxrB*. **Nar**, **Nap**: nitrato reductasa, genes *narG, napA*. **NirS**, **NirK**: nitrito reductasa, genes *nirS, nirK*, **cNOR**: óxido nítrico reductasa, gen *norB*. **N_2OR**: óxido nitroso reductasa, gen *nosZ*.

de tratamiento de aguas residuales, pero *Nitrospira* es el género más diverso y abundante en este y muchos otros ambientes (Mehrani *et al*., 2020; Park *et al*., 2020). *Nitrospira* puede ser dominante en medios con bajas concentraciones de NO_2^- y *Nitrobacter* en ambientes con altas concentraciones de NO_2^-. Por su importancia ecológica, biotecnológica y su diversidad filogenética y metabólica, es necesario continuar avanzando en el estudio de las NOB (Daims *et al*., 2016; Vijayan *et al*., 2021).

Bacterias desnitrificantes. El proceso desnitrificante (Figura 2) es llevado a cabo por grupos taxonómicos tan diversos como bacterias (proteobacterias, α, β, γ, δ), arqueas (Euryarchaeota, Crenarchaeota) y hongos (Lu *et al*., 2014; Buratti *et al*., 2022). Algunas especies de haloarqueas han mostrado capacidades de desnitrificación, lo que puede indicar un papel determinante en la reducción del NO_3^- a N_2 en suelos o cuerpos de agua salinos tanto naturales como artificiales (Torregrosa-Crespo *et al*., 2017). Si bien es común la distinción entre "sistemas naturales" y "sistemas artificiales", esto es sólo por precisión de tema ya que los microorganismos no hacen distinción de un entorno u otro, y así, por ejemplo, pueden hallarse organismos propios del suelo en la composición de las comunidades de los llamados sistemas artificiales (Pholchan *et al*., 2013; Lang *et al*., 2018). En las plantas de tratamiento de aguas residuales, los genes marcadores de la desnitrificación se usan ampliamente en la caracterización de la estructura y dinámica de las comunidades microbianas participantes (Lu *et al*., 2014; Castellano-Hinojosa *et al*., 2020).

Enzima nitrato reductasa (Nar, Nap, genes *narG, napA*). Con la reducción del NO_3^- inicia el proceso de desnitrificación y la enzima que cataliza la reacción $NO_3^- \rightarrow NO_2^-$ es la nitrato reductasa (Figura 2). En bacterias, hay dos enzimas reductasas que difieren en localización y propiedades, una orientada hacia el citoplasma (Nar) y la situada del lado del periplasma (Nap). La enzima Nar es una enzima heterotrimérica (NarGHI) que contiene el sitio activo en la subunidad NarG (gen *narG*) ubicada hacia el citoplasma, la subunidad NarH con la función de transferencia de electrones y la subunidad NarI que sirve de unión a la membrana (González *et al*., 2006; Kraft *et al*., 2011). La enzima Nap es una enzima soluble formada por una subunidad mayor NapA y una menor NapB, donde NapA (gen *napA*) contiene la actividad catalítica, mientras que NapB es esencial en la transferencia de electrones a NapA. Además, puede presentarse la proteína NapC integrada en la membrana, la cual libera electrones a NapB. El orden y presencia de los genes del operón *nap* es variable dependiendo de la especie, así por ejemplo puede haber expresión de NapABCGH entre otras combinaciones. Un microorganismo puede presentar el gen *narG* o *napA* (y sus enzimas correspondientes), o los dos genes. Estas y otras nitrato reductasas son molibdeno dependientes (González *et al*., 2006; Asamoto *et al*., 2021). Es probable que en ambientes con alto contenido en NO_3^- la enzima Nar sea más eficiente, pero con una baja afinidad, mientras que la enzima Nap con mayor afinidad, pero menos eficiente, se presentaría preferentemente en entornos donde el NO_3^- es limitado (Pérez-Rodríguez *et al*., 2013; Asamoto *et al*., 2021). El O_2 puede inhibir a la enzima Nar (sensible a este gas), o bien inhibir la actividad de los transportadores de NO_3^- al interior de la célula. La enzima Nap en cambio se considera que no es afectada por el O_2 y no requiere un transporte de NO_3^- ya que se sitúa en el espacio periplásmico. Ambos genes, *narG* y *napA*, pueden emplearse como marcadores genéticos (Ma *et al*., 2019), aunque el gen *napA* puede ser particularmente útil en identificar desnitrificantes aerobio tolerantes (Zhao *et al*., 2018).

Enzima nitrito reductasa (NirS, NirK, genes *nirS*, *nirK*). En el proceso desnitrificante (Figura 2), la reducción de NO_2^- a NO puede catalizarse por dos enzimas periplásmicas que tienen funciones homólogas, pero distinta estructura, la enzima NirK que contiene cobre y es codificada por el gen *nirK*, y la enzima NirS (citocromo cd_1) que contiene hierro y tiene por origen al gen *nirS*. Generalmente, en un microorganismo, se presenta el gen *nirK* o el *nirS*, aunque hay excepciones, es decir, especies desnitrificantes que pueden presentar ambos genes (Wittorf *et al*., 2018). La enzima NirK tiene dos centros Cu, uno perteneciente a la subclase de proteínas con Cu de tipo I y el otro de tipo II, siendo en este último donde se produce la unión del sustrato (Rinaldo & Cutruzzolà, 2007). La presencia de Cu puede ser un elemento clave en determinar los niveles de expresión del gen *nirK*. En el caso de NirS, el sitio activo es d_1 y la síntesis de la enzima puede depender de la presencia de NO_3^- (Wittorf *et al*., 2018). Ambos genes se han utilizado como marcadores moleculares y se han encontrado en diversos grupos bacterianos (Proteobacteria, Nitrospirae, Actinobacteria, entre otros filos) así como en Archaea (Wei *et al*., 2015; Ma *et al*., 2019; Miralles-Robledillo *et al*., 2021).

Enzima óxido nítrico reductasa (cNOR, gen *norB*). La enzima óxido nítrico reductasa (NOR) que cataliza la reducción del NO a N_2O, es una enzima oxidasa con hierro, localizada en la membrana. En bacterias desnitrificantes comúnmente se encuentra en la forma cNOR ("complejo NorBC"), un dímero formado por las subunidades NorB que contiene el sitio activo y NorC, que son codificadas por los genes *norB* y *norC*, respectivamente (Matsumoto *et al*., 2012; Shiro, 2012). Esta forma acepta electrones del citocromo c o de la azurina. La enzima qNOR de subunidad única (gen *qnor*, *qnorB* o *norZ*) es otra forma de la enzima NOR presente en bacterias, arqueas y microorganismos no desnitrificantes en los cuales la función de NOR se relaciona con la detoxificación del citotóxico NO. Otra forma de la enzima NOR reportada en el género *Bacillus* es la Cu_ANor, con el citocromo c551 como donador de electrones (Torregrosa-Crespo *et al*., 2017). El NO es un gas que puede contribuir a generar o ser sumidero de ozono y la oxidación del NO resulta en ácido nítrico, componente de la lluvia ácida (Pilegaard, 2013). Los genes *norB* y *qnor* se han empleado para detectar desnitrificantes óxido nítrico reductores en diferentes ambientes (Torregrosa-Crespo *et al*., 2017; Ma *et al*., 2019; Castellano-Hinojosa *et al*., 2020).

Enzima óxido nitroso reductasa (N_2OR, gen *nosZ*). El último paso en el proceso desnitrificante corresponde a la reducción del N_2O a N_2 (Figura 2) y es catalizado por la enzima óxido nitroso reductasa (N_2OR) (Jones *et al*., 2013). El gen *nosZ* codifica la subunidad catalítica. Se han identificado dos tipos de enzimas que realizan este paso. La llamada N_2OR clado I es una metaloenzima periplásmica que contiene múltiples átomos de cobre, con dos dominios, un centro de transferencia de electrones (CuA) y un centro catalítico (CuZ). El gen *nosZ* se presenta en el grupo de genes *nos* comúnmente en el arreglo *nosRZDFYL* (el gen *nosX* puede presentarse antes o después). Los otros genes de este conjunto están implicados por ejemplo en la regulación de la expresión y maduración de la enzima N_2OR (Carreira *et al*., 2017). La enzima N_2OR clado II difiere en que está asociada probablemente a la membrana y tiene un dominio adicional hemo C en el extremo C terminal. El gen *nosZ* forma parte del arreglo nosCZ-ORF-nosDYF-ORF, y los genes *nosX* y *nosR* no se presentan. Aparte del dominio bacteria, las arqueas también pueden presentar estos tipos de enzimas (Ma *et al*., 2019; Keeley *et al*., 2020). Debido a la modularidad del proceso desnitrificante, puede no presentarse el gen *nosZ* en una población microbiana, de forma

que se libere el gas N_2O de efecto invernadero, o por el contrario una población puede expresar la enzima N_2OR, y la comunidad de la cual forma parte puede tener la capacidad de eliminar el N_2O del entorno. El gen *nosZ* se ha empleado como marcador en diversos estudios a fin de identificar los grupos capaces de reducir el N_2O a N_2 bajo diferentes condiciones ambientales (Conthe *et al*., 2019; Castellano-Hinojosa *et al*., 2020; Bernabeu *et al*., 2021). Cabe mencionar que es posible que se presente más de una copia de algunos de los genes desnitrificantes mencionados en un mismo microorganismo o cepa. Se han encontrado copias múltiples del *nirS* en el genoma de especies del género *Thauera* (Wang *et al*., 2020c).

Diversidad y flexibilidad metabólica de bacterias nitrificantes y desnitrificantes. La ubicuidad, abundancia, diversidad taxonómica y metabólica del dominio bacteria es enorme. Se ha estimado que pueden existir en nuestro planeta de millones a miles de millones de especies del dominio bacteria y arqueas (Pallen *et al*., 2021), de los cuales solo 150000 genomas han sido secuenciados y almacenados en Genome Taxonomy Database. Sin embargo, de ellos el 40% carece de una asignación taxonómica específica (Parks *et al*., 2020). La cantidad de secuencias que esperan ser clasificadas continúa en aumento día a día (Overmann *et al*., 2019; Pallen *et al*., 2021). Otro problema asociado con esta diversidad es que no existe un concepto de especie para procariotas que sea ampliamente aceptado. Siendo microorganismos asexuales y con un habitual intercambio horizontal de material genético, puede resultar en una imprecisión al tratar de distinguir una especie de otra. Se ha optado por una solución pragmática y más bien dirigida a especies cultivables, con hibridación DNA–DNA del 70% como límite entre especies (Gevers *et al*., 2005; Doolittle & Rapke, 2006) o por la propuesta enfocada a los microorganismos no cultivables con el promedio de similitudes de nucleótidos (ANI: average nucleotide similarities) entre genomas, donde un ANI < 90% es un criterio para distinguir poblaciones coexistentes (Doolittle & Rapke, 2006; Overmann *et al*., 2019; Parks *et al*., 2020). Teniendo en cuenta la búsqueda de orden mediante la categorización de la gran diversidad microbiana, se comprende que la clasificación de los microorganismos de acuerdo con un solo proceso ("fijador de nitrógeno", "nitrificante", "desnitrificante") haya sido desde un inicio artificial y no refleje completamente la diversidad y versatilidad metabólica de los MTN (Stein & Klotz, 2016; Kuypers *et al*., 2018).

La Tabla 1 presenta ejemplos de la diversidad y versatilidad metabólica de algunas bacterias nitrificantes y desnitrificantes clasificadas tradicionalmente de acuerdo con un proceso respiratorio único. Por ejemplo, la bacteria *Nitrosomonas europaea* Winogradsky 1892 (Schoch *et al*., 2020) se ha clasificado como un quimiolitoautótrofo obligado que obtiene energía para su crecimiento de la oxidación del NH_3 a NO_2^- en presencia de O_2 y que cubre su necesidad de carbono mediante la fijación de dióxido de carbono (CO_2). Sin embargo, *N. europaea* puede consumir y crecer con sustratos orgánicos como fructosa y piruvato usados como únicas fuentes de carbono, si bien sigue necesitando el NH_3 como fuente de energía (Chain *et al*., 2003; Hommes *et al*., 2003). Otras AOB también han sido reportadas como capaces de utilizar la vía heterotrófica utilizando sustratos orgánicos como fuentes de carbono para su crecimiento (Kjeldal *et al*., 2014). Además, en *N. europaea*, una representante típica de las bacterias amonio oxidantes, se han encontrado secuencias homólogas de los genes *nirK* y *norB* y sus enzimas correspondientes. Esto le permite realizar parte del proceso desnitrificante en ambientes con bajas concentraciones de O_2 y altas en NO_2^-. Puede decirse que *N. europaea* es también una bacteria desnitrificante (Schmidt, 2004; Yu & Chandran, 2010). *Nitrobacter hamburgensis* Bock *et al*. 2001 cepa X14 (Schoch *et al*., 2020) (Tabla 1) es una bacteria que obtiene su energía de la oxidación del NO_2^- y el carbono del CO_2, pero también es capaz de tener un crecimiento mixotrófico con NO_2^- y D-lactato como fuentes de energía, nitrógeno y carbono, o un crecimiento organotrófico sin NO_2^-, pero con NH_3/NH_4^+ como fuente de nitrógeno y con D-lactato como fuente de energía y carbono (Starkenburg *et al*., 2008a, b). Si bien el lactato se asimila y metaboliza hasta CO_2, se requiere la exposición al CO_2 atmosférico o la adición de carbonato de sodio tanto en el crecimiento mixotrófico como en el organotrófico, en este último caso a fin de obtener un crecimiento óptimo. Otros *Nitrobacter* como *N. agilis* (actualmente *N. winogradsky* Winslow *et al*. 1917) y *N. vulgaris* Bock *et al*. 2001 también tienen la capacidad de crecimiento quimioorganotrófico (Bock, 1976; Bock *et al*., 1990; Schoch *et al*., 2020). Es posible que la utilización de compuestos orgánicos sea una estrategia empleada para complementar requerimientos energéticos ya que el NO_2^- es un sustrato energéticamente pobre (Starkenburg *et al*., 2008a). La ubicuidad de los nitrificantes en diversos sistemas se podría explicar en parte por la capacidad de emplear como donadores de electrones tanto compuestos específicos como compuestos orgánicos (Bock, 1976). *Acinetobacter* sp. ND7, *Pseudomonas stutzeri* (Lehmann & Neuman 1896) (actualmente *Stutzerimonas stutzeri*) cepa UFV5 y *Vibrio diabolicus* Raguns *et al*. 1997 cepa SF16 (Schoch *et al*., 2020) (Tabla 1) son cepas bacterianas que han sido reportadas como capaces de realizar el proceso de nitrificación heterotrófica y desnitrificación en condiciones aerobias (proceso HN-AD). Es decir, que en condiciones aerobias y utilizando compuestos orgánicos como fuente de carbono y electrones, estas bacterias tienen la capacidad de eliminar $N\text{-}NH_4^+$, donde una parte de estos compuestos puede ser asimilada y otra puede ser liberada como N_2, y como CO_2 respectivamente. *Acinetobacter* sp. ND7 es una cepa que elimina eficientemente NH_4^+, NO_2^- y NO_3^- respectivamente o en combinación: NH_4^+ y NO_3^-, como fuentes de nitrógeno y con acetato como fuente de carbono. Otras fuentes con las que ND7 es capaz de eliminar nitrógeno eficientemente son el succinato o el citrato (Xia *et al*., 2020). Es necesaria la presencia de una fuente de carbono orgánico para que se realice la eliminación de NH_4^+. En el balance de nitrógeno (NH_4^+ y acetato como sustratos iniciales), el 56.5% del $N\text{-}NH_4^+$ inicial se convirtió en N_2, el 40.9% se incorporó en biomasa y 2.1% en intermediarios. Los genes clave que se presentan en esta cepa y cuyas enzimas intervienen en el proceso HN-AD son: *hao*; importante en el proceso nitrificante heterotrófico, *nap;* interviene en la reducción del NO_3^- en condiciones aerobias, y *nirS*; amplificado con frecuencia de bacterias desnitrificantes aerobias (Zhao *et al*., 2018). *P. stutzeri* UFV5 es capaz de eliminar el NH_4^+ de forma eficiente cuando se emplean fuentes de carbono (relación C/N: 6-12, salinidad de 0-3%) como piruvato o citrato entre otros compuestos que forman parte del ciclo de los ácidos tricarboxílicos. A través del balance de nitrógeno empleando citrato, se calcula que 47% del $N\text{-}NH_4^+$ se incorpora en biomasa y 53% puede convertirse en N_2. Los compuestos NH_2OH, NO_2^- y NO_3^- productos de la nitrificación, las enzimas AMO, HAO y sus genes *amoA* y *haoF* no se detectaron en la cepa UFV5, probablemente debido a que participan enzimas y genes distintos a los involucrados en la nitrificación autotrófica (Silva *et al*., 2020). En cambio, las enzimas y los genes de la desnitrificación anaerobia sí se encontraron presentes. En el proceso HN-AD, el NH_4^+ no se emplea como fuente de electrones para generar energía, y se considera que su eliminación posiblemente sea una forma de retirar un compuesto que es percibido como tóxico por *P. stutzeri* UFV5 (Silva *et al*., 2020). *V. diabolicus* SF16 es una bacteria halófila con

la capacidad de eliminar eficientemente NH_4^+ y NO_3^- respectivamente con 1-5% de salinidad y con acetato como fuente de carbono. Puede usar de igual forma otras fuentes de carbono como citrato, glucosa o sacarosa (Duan *et al*., 2015). A través de un balance de nitrógeno (3% de salinidad), se calcula que 35.8% de N-NH_4^+ se incorpora en biomasa, 53.9% puede liberarse como N_2 y 0.2% como NO_2^- y NO_3^-. La cepa SF16 presenta el gen *napA*. En los tres casos mencionados de las bacterias que realizan el proceso HN-AD, la eliminación del NH_4^+ está asociada al crecimiento bacteriano. En el balance de nitrógeno, más de la mitad del N-NH_4^+ se libera como N_2 y menos de la mitad se incorpora en biomasa, lo que resulta conveniente si se considera al bajo volumen de lodos que se pudieran producir. Así también la diversidad en sustratos, vías metabólicas y condiciones que pueden tolerar (por ejemplo, en cuanto a salinidad) vuelve a estos microorganismos un grupo de interés por su posible uso en el tratamiento de aguas residuales (Duan *et al*., 2015; Silva *et al*., 2020; Xia *et al*., 2020).

Otro ejemplo de diversidad metabólica recientemente descubierta en MTN es la capacidad de *Candidatus* Nitrospira inopinata Daims *et al*. 2015 (Oren *et al*., 2020; Schoch *et al*., 2020) (Tabla 1) para realizar el proceso nitrificante de forma completa ($NH_4^+ \rightarrow NO_3^-$) en condiciones aerobias. *Ca*. N. inopinata posee una alta afinidad por NH_4^+, un alto rendimiento de crecimiento en comparación con los nitrificantes canónicos y una tasa máxima de oxidación de NH_4^+ relativamente baja. Lo anterior sugiere que *Ca*. N. inopinata es un organismo propio de ambientes oligotróficos donde presenta un lento crecimiento. *Ca*. N. inopinata posee homólogos de las enzimas AMO y HAO propias de la oxidación del NH_4^+ de las AOB y la enzima NXR que oxida el NO_2^- en las NOB (Daims *et al*., 2015; Kits *et al*., 2017).

Los ejemplos previamente descritos ilustran que existe una amplia gama de combinaciones funcionales que pueden presentarse en los MTN, que tiene explicación en el conjunto de genes y su regulación, las relaciones que se establecen entre los microorganismos de una comunidad, así como la necesidad de supervivencia y desarrollo en una gran variedad de ambientes (Stein & Klotz, 2016; Kuypers *et al*., 2018). Sin embargo, hacen falta más estudios dirigidos a la fisiología, metabolismo, ecología y genética de los MTN para poder entender, controlar y emplear de forma eficiente su actividad a fin de obtener procesos biológicos óptimos o alternativos en la eliminación de nitrógeno en el tratamiento de aguas residuales.

Ecología de comunidades. Una comunidad es un conjunto formado por distintas especies, donde los organismos viven e interactúan entre sí y su entorno en un área específica. Esta organización compleja multiespecífica se presenta en una gran diversidad de ambientes y funciones, estas últimas relacionadas por ejemplo con la agricultura, industria, salud humana y ciclos biogeoquímicos. Al formarse una comunidad, esta puede adquirir comportamientos y características propias (propiedades intrínsecas) que no son identificables en los individuos participantes y que no surgen de la simple adición de las características de sus integrantes (Konopka, 2009; Fierer *et al*., 2012; Madsen *et al*., 2018; Trego *et al*., 2021). En una comunidad microbiana, las diferentes especies se presentan en proporciones relativas y los distintos microorganismos contribuyen con su genoma a dotarla de una diversidad metabólica. Hay poblaciones microbianas que constituyen la parte activa de la comunidad, y el cambio en la riqueza o diversidad de estas poblaciones clave puede alterar la función de esta (Brenner *et al*., 2008; Johnson *et al*., 2015; Goldford *et al*., 2018). Si bien la diversidad de especies no asegura la integridad y permanencia de la comunidad, sí posibilita en general un mejor desempeño ante cambios ambientales y nutricionales (Brenner *et al*., 2008; Awolusi *et al*., 2015; Widder *et al*., 2016). Las comunidades microbianas son sistemas dinámicos con la capacidad inherente de soportar perturbaciones, es decir de ser estables por ejemplo ante eventos repentinos que puedan modificar su función (Arnoldi *et al*., 2019). Las interacciones entre los miembros son fundamentales en la formación, estabilidad y funcionamiento de la comunidad. Estas interacciones son variadas y comprenden: interacciones a nivel trófico; competencia por recursos; metabólicas (sintrofía, bienes públicos); de señalización (quorum sensing); estructurales físicas o metabólicas (biopelículas, flóculos, gránulos) y de intercambio de material genético (transferencia horizontal de genes) (Brockhurst *et al*., 2008; Konopka, 2009; Widder *et al*., 2016; Madsen *et al*., 2018). Los consorcios microbianos en forma de flóculos, gránulos y biopelículas son las comunidades más frecuentes en los sistemas empleados en la eliminación de contaminantes de aguas residuales. Para la formación y permanencia de estas comunidades, se requiere entre sus participantes comunicación, interacción y coordinación (comportamiento colectivo coordinado). La proximidad espacial de los taxones en estos bioagregados multitróficos posibilita una mejor comunicación entre los microorganismos a través de moléculas autoinductoras, así como el montaje de una respuesta por parte de la comunidad. La diversidad y flexibilidad metabólica de los taxones explicaría por qué el consumo de metabolitos, la eliminación de contaminantes y agentes antimicrobianos muestran ser más eficientes en consorcios que en los microorganismos planctónicos o de vida libre (Flemming *et al*., 2016; Maddela *et al*., 2019; Cai, 2020; Trego *et al*., 2021). De esta forma, si en una comunidad algunos microorganismos presentan solo partes de vías metabólicas, los pasos faltantes o vías complementarias pueden estar en organismos próximos, como en el caso de los procesos nitrificante y desnitrificante que se efectúan en gránulos donde las poblaciones pueden localizarse en la estructura de estos de acuerdo con la disponibilidad de nutrientes y O_2 (Cordero *&* Datta, 2016; Di Capua *et al*., 2022).

Conclusiones. Los procesos microbiológicos de nitrificación y desnitrificación forman parte del ciclo del nitrógeno y acoplados tienen la función de regresar el nitrógeno de compuestos como el NH_4^+ a la atmósfera en forma de N_2. Los organismos involucrados en estos procesos se utilizan en los sistemas artificiales de tratamiento de aguas residuales. Lo que se conoce de su metabolismo y fisiología se basa en estudios realizados en unos cuantos microorganismos cultivables. Sin embargo, se debe tener en cuenta que estos microorganismos pueden estar presentes en ambientes muy diversos y que la cantidad de taxones que aún quedan por descubrirse es enorme. En el futuro y con la ayuda de la bioinformática y las ciencias “ómicas”, la extensa diversidad metabólica de bacterias, arqueas y otros MTN será mejor comprendida. La información contenida en esta revisión destaca que un organismo identificado como nitrificante o desnitrificante puede tener la capacidad de acuerdo con su genoma y condiciones del entorno, de efectuar otros procesos y funciones. Los genes funcionales que codifican las principales enzimas de los procesos de la nitrificación y desnitrificación se presentan en diversos taxones y a través de su empleo como genes marcadores, es posible caracterizar y cuantificar un grupo de organismos con una función común. Es conveniente que el análisis de la transcripción y traducción de los genes funcionales sea incluido de manera más amplia en los estudios de los consorcios usados en el tratamiento

Tabla 1. Ejemplos de diversidad y flexibilidad metabólica en bacterias nitrificantes y desnitrificantes. Metabolismo: fuente de energía/aceptor final de electrones/aerobio o anaerobio/fuente de carbono. Coi: compuestos orgánicos e inorgánicos. Co: compuestos orgánicos. BAF: filtro biológico aireado.

Bacteria	Sistema experimental	Metabolismo convencional	Metabolismo alternativo o complementario	Resultados	Información genética	Referencias
Amonio oxidante						
Nitrosomonas europaea Winogradsky 1892	Ensayos en lote	NH_3/O_2/aerobio/CO_2	NH_3/O_2/aerobio/fructosa o piruvato	Crecimiento con fructosa o piruvato como única fuente de carbono	Secuencia del genoma completo	Hommes *et al.*, 2003; Chain *et al.*, 2003
Nitrito oxidante						
Nitrobacter hamburgensis Bock *et al.* 2001, cepa X14	Ensayos en lote	NO_2^-/O_2/aerobio/CO_2	D-lactato/O_2/aerobio/D-lactato, CO_2	Crecimiento organotrófico a partir de D-lactato	Secuencia del genoma completo	Starkenburg *et al.*, 2008a, b
Desnitrificante						
Acinetobacter sp., cepa ND7	Ensayos en lote	Acetato y otros Coi/ NO_3^-/ anaerobio/acetato y otros Co	Con NH_4^+ como única fuente adicionada de N: Acetato y otros Co/NO_3^-/aerobio /acetato y otros Co	Asimilación de N-NH_4^+. Producción de N_2 y CO_2	Genes amplificados *hao*, *napA, nirS*	Xia *et al.*, 2020
Pseudomonas stutzeri (Lehmann & Neuman 1896), cepa UFV5	Ensayos en lote	Piruvato y otros Coi/ NO_3^-/ anaerobio /piruvato y otros Co	Con NH_4^+ como única fuente adicionada de N: Piruvato y otros Co/NO_3^-/aerobio/piruvato y otros Co	Asimilación de N-NH_4^+. Producción de N_2 y CO_2	Genes detectados *nap*, *nir*S, *nor*B, *nos*Z	Silva *et al.*, 2020
Vibrio diabolicus Raguns *et al.* 1997, cepa SF16	BAF en lote. Ensayos en lote	Acetato y otros Coi/ NO_3^-/ anaerobio /acetato y otros Co	Con NH_4^+ como única fuente adicionada de N: Acetato y otros Co/ NO_3^-/aerobio/acetato y otros Co	Asimilación de N-NH_4^+. Producción de N_2 y CO_2	Gen *napA* amplificado de desnitrificación aerobia	Duan *et al.*, 2015
Comammox						
Candidatus Nitrospira inopinata Daims *et al.* 2015	Ensayos en lote	NO_2^-/O_2/aerobio/CO_2	NH_4^+/O_2/aerobio/CO_2	Oxidación de NH_4^+ a NO_3^-. Crecimiento celular	Genes que codifican para enzimas AMO, HAO y NXR	Daims *et al.*, 2015; Kits *et al.*, 2017

de aguas residuales. Esto permitirá relacionar las diferentes condiciones ambientales y nutricionales con los genes y su grado de expresión para la síntesis de sus correspondientes enzimas, ampliando el conocimiento de la capacidad y flexibilidad metabólica de los consorcios. Las poblaciones en general y en este caso las nitrificantes y desnitrificantes pueden reunirse para formar una comunidad o consorcio donde cada componente aporta diversidad genética y taxonómica. Estos consorcios ubicuos en sistemas naturales y artificiales comúnmente presentan capacidades mejores o distintas de las que presentan las especies participantes de manera individual y pueden contribuir a una eliminación más eficiente y estable de contaminantes del agua, como NH_4^+.

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Cyanotoxins bioaccumulation in freshwater ecosystems in Latin America: a review

Bioacumulación de cianotoxinas en ecosistemas dulceacuícolas en América Latina: una revisión

Carlos A. Garita-Alvarado[1], Miriam G. Bojorge-García[1], Enrique A. Cantoral Uriza[1*]

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ABSTRACT

Background: The increasing evidence of risk to the environment and human health by cyanotoxin exposure during cyanobacterial blooms has been reported worldwide. Despite the knowledge of cyanotoxin presence in Latin America, cyanotoxin bioaccumulation from freshwater environments have not been reviewed for the region. **Goals:** To review the current knowledge of cyanotoxin accumulation in tissues of freshwater organisms in field studies in Latin America. **Methods:** An extensive literature search was conducted to construct a database including information on accumulation of cyanotoxins in organisms inhabiting freshwater environments in Latin America (i.e., México to Argentina). **Results:** We found twenty-one studies from 2001 to 2020, including twenty-seven mostly eutrophic water bodies, the majority from Brazil. *Microcystis* was the most reported genus responsible for cyanotoxin production. Fish comprised most of the species accumulating cyanotoxins (20 species). Nile tilapia (*Oreochromis niloticus*) was the most studied species, and 80% of the fish species included have commercial importance, which highlights a potential route of exposure to humans by consumption of contaminated food. Some studies showed the reduction of cyanotoxins in tissues after an experimental depuration time. Also, calculations of the potential human intakes of microcystins by fish consumption exceeded the recommendations of tolerable intakes in most of the cases. **Conclusions:** In Latin America, the geographic extent of studies is narrow, however the summarized information indicates a risk for environment and human health by cyanotoxins bioaccumulation. There is a need for more efforts to generate scientific research on cyanotoxins bioaccumulation, but also for improvement of local level management policies to reduce eutrophication.

Keywords: aquaculture, human health impact, field studies, microcystins, saxitoxins.

RESUMEN

Antecedentes: La creciente evidencia de riesgo para el medio ambiente y la salud humana por la exposición a cianotoxinas durante las floraciones de cianobacterias se ha reportado en todo el mundo. A pesar del conocimiento de la presencia de cianotoxinas en América Latina, la bioacumulación de cianotoxinas en ambientes de agua dulce no ha sido revisada para la región. **Objetivos:** Revisar el conocimiento actual sobre la acumulación de cianotoxinas en tejidos de organismos de agua dulce en estudios de campo en América Latina. **Métodos:** Se realizó una extensa búsqueda bibliográfica para construir una base de datos que incluyera información sobre la acumulación de cianotoxinas en organismos que habitan ambientes de agua dulce en América Latina (de México a Argentina). **Resultados:** Encontramos veintiún estudios de 2001 a 2020, incluidos veintisiete cuerpos de agua en su mayoría eutróficos, la mayoría de Brasil. *Microcystis* fue el género productor de cianotoxinas más reportado. Los peces comprendieron la mayoría de las especies que acumulaban cianotoxinas (20 especies). *Oreochromis niloticus* fue la especie más estudiada, y el 80% de las especies de peces incluidas tienen importancia comercial, lo que destaca una vía potencial de exposición a los humanos. Algunos estudios demostraron la reducción de cianotoxinas en los tejidos después de un tiempo de depuración experimental. Además, los cálculos de las ingestas humanas potenciales de microcistinas por consumo de pescado excedieron las recomendaciones de ingestas tolerables en la mayoría de los casos. **Conclusiones:** En América Latina, los estudios se han realizado en pocos países, sin embargo, estos

[1] Unidad Multidisciplinaria de Docencia e Investigación (UMDI), Facultad de Ciencias, Universidad Nacional Autónoma de México. Boulevard Juriquilla 3001, Juriquilla, Querétaro, 76230, México

***Corresponding author:**
Enrique A. Cantoral Uriza: e-mail: cantoral@ciencias.unam.mx

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trabajos indican un riesgo para el medio ambiente y la salud humana por la bioacumulación de cianotoxinas. Se necesita más investigación científica sobre la bioacumulación de cianotoxinas, pero también esfuerzos para mejorar la gestión a nivel local con la finalidad de reducir la eutrofización.

Palabras clave: acuacultura, estudios de campo, impacto en la salud humana, microcistinas, saxitoxinas.

INTRODUCTION

Cyanobacteria, often referred as blue-green algae, are photoautotrophic microorganisms distributed ubiquitously in the world and are common inhabitants of a wide range of freshwater bodies. Favorable conditions, such as warm water, a stable water column, high phosphorus and nitrogen concentrations, high pH, low CO_2 availability and low herbivory (Zurawell *et al.,* 2005, Glibert & Burkholder 2018, Metcalf *et al.,* 2021), may lead to increased population growth of cyanobacteria generally called "cyanobacterial blooms" (Mowe *et al.,* 2015, Buratti *et al.,* 2017, Moreira *et al.,* 2022). Increases in cyanobacterial cell concentration reduces oxygen concentration and increases ammonia release when cyanobacteria decay. Blooms result in increased turbidity, leading to the decline of other primary producers, and they can enhance the likelihood of production of unpleasant taste and odor compounds (Hudnell & Dortch 2008, Wood 2016). Of major concern is increased levels of cyanotoxins that can be produced in about 40 cyanobacteria genera (Apeldoorn *et al.,* 2007, Singh & Dhar 2013). Cyanotoxins are secondary metabolites of diverse chemical structure and toxicity, including cyclic peptides, alkaloids, non-proteinogenic amino acids, phosphate ester and lipopolysaccharides (Aráoz *et al.,* 2010, Svirčev *et al.,* 2019). Based on their toxic effects in vertebrates, cyanotoxins are classified as hepatotoxins (microcystins, nodularin), neurotoxins (saxitoxins, anatoxins, homoanatoxin-a, B-methylamino-L-alanine), cytotoxins (cylindrospermopsins), dermatoxins (aplysiatoxin, debromoaplysiatoxin, lingbyatoxin) and endotoxins (lipopolysaharides) (Apeldoorn *et al.,* 2007, Svirčev *et al.,* 2019).

The frequency and magnitude of cyanobacterial blooms has received much more scientific attention around the world over the past decades, with blooms generally associated with the increment of eutrophication in freshwaters bodies, watershed modifications and climate change (Paerl & Huisman 2009, Brooks *et al.,* 2016, Glibert 2020, Munoz *et al.,* 2021, Chorus *et al.,* 2021). The toxigenic and adverse effects of cyanotoxins in several groups of organisms have been recognized in field and laboratory settings including bacteria, microalgae, zooplankton, fishes, amphibians, birds, mammals and agricultural plants (Apeldoorn *et al.,* 2007, Valdor & Aboal 2007, Tillmanns *et al.,* 2008, Chen *et al.,* 2016, Banerjee *et al.,* 2021, Zhang *et al.,* 2021). This has led to a growing global consensus of the harmful effects for aquatic organisms and human health due to cyanotoxin exposure (Drobac *et al.,* 2013, Wood 2016, Cantoral Uriza *et al.,* 2017, Scarlett *et al.,* 2020). Specifically for humans, there are well documented events of poisoning that indicate that cyanotoxins were the cause of the symptoms (Humpage and Cunliffe, 2021), however, literature related to human intoxication by cyanotoxins exposure must be taken with caution since, usually, other potential causing agents (e.g., other bacteria and pollutants) have not been simultaneously evaluated (Testai *et al.,* 2016; Humpage and Cunliffe, 2021). In addition, there are other social and economic problems associated to toxic cyanobacterial blooms, including cattle and companion animal deaths, loss of recreational fishing and irrigation value of water bodies, closures of drinking water supplies (Wood 2016, Munoz *et al.,* 2021) and loss of biodiversity (Hudnell & Dortch 2008).

At a global scale, cyanotoxin presence in water bodies (Buratti *et al.,* 2017, Svirčev *et al.,* 2019), accumulation in freshwater organisms (Ettoumi *et al.,* 2011, Testai *et al.,* 2016, Flores *et al.,* 2018, Pham & Utsumi 2018), and their transfer in aquatic food webs (Ferrão-Filho & Kozlowsky-Suzuki 2011, Lance *et al.,* 2014), have been documented and reviewed, highlighting the potential transfer to humans by fish or shellfish consumption (Fig. 1). For example, Sotton *et al.,* (2014) in Lake Hallwil (Switzerland) demonstrated the transfer of microcystins (MCYST's), produced during a bloom of the filamentous cyanobacteria *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek, from filter-feeders herbivorous zooplankton to predator zooplankton. Consequently, this zooplankton contributed to the contamination with MCYST's of the zooplanktivorous whitefish (*Coregonus suidteri* Fatio, 1885), a species with commercial value in that region. The evidence of biomagnification of cyanotoxins (i.e., the increase of concentrations in organisms as the toxin moves up the food chain) has only partial support and is still debated (Kozlowsky-Suzuki *et al.,* 2012, Flores *et al.,* 2018).

In Latin America (i.e., México to Argentina) the presence of cyanobacterial blooms and cyanotoxins in water bodies have been reported in several countries through field research and review papers, including: Argentina, Brazil, Chile, Costa Rica, Colombia, Cuba, Ecuador, Guatemala México, Perú, Uruguay, Venezuela (Avendaño Lopez & Arguedas Villa 2006, Dörr *et al.,* 2010, Vasconcelos *et al.,* 2010, Gomez *et al.,* 2012, Mowe *et al.,* 2015, Pérez-Morales *et al.,* 2016, Rico-Martínez *et al.,* 2017, Moura *et al.,* 2018, León & Peñuela 2019, Munoz *et al.,* 2021, Salomón *et al.,* 2020). MCYST's are the most commonly reported cyanotoxins, followed by cylindrospermopsins (CYN's) and saxitoxin (STX's), and less commonly anatoxins (ATX's) and nodularin (NOD) (Galanti *et al.,* 2013, Sunesen *et al.,* 2021).

Despite the knowledge of cyanobacterial blooms and cyanotoxin presence in Latin America, relevant topics related to cyanotoxin impacts on the environment, including field studies on accumulation of cyanotoxins by freshwater organisms (Fig. 1), have not been reviewed for the region. Bioaccumulation of cyanotoxins is directly correlated with the potential human health risk from ingestion of contaminated tissue from organisms such as fish and shrimp. Moreover, in face of the climate change, population increase, watershed modifications and eutrophication increase, it is expected that cyanobacterial blooms will continue. Thus, there is a need of integrative analyses summarizing the knowledge on cyanotoxins bioaccumulation to highlight the potential impacts on freshwater organisms and on human health in Latin America, which could contribute to define a baseline for future studies, and to show pending topics of research. Based on the later ideas, in this review we aimed to summarize the current knowledge of cyanotoxins accumulation in freshwater organisms in Latin America. We analyzed and summarized major topics shared by studies in the region (i.e., depurations of cyanotoxins by organisms in field studies, potential human intake), highlighting the gaps in current knowledge and principal future directions in the region.

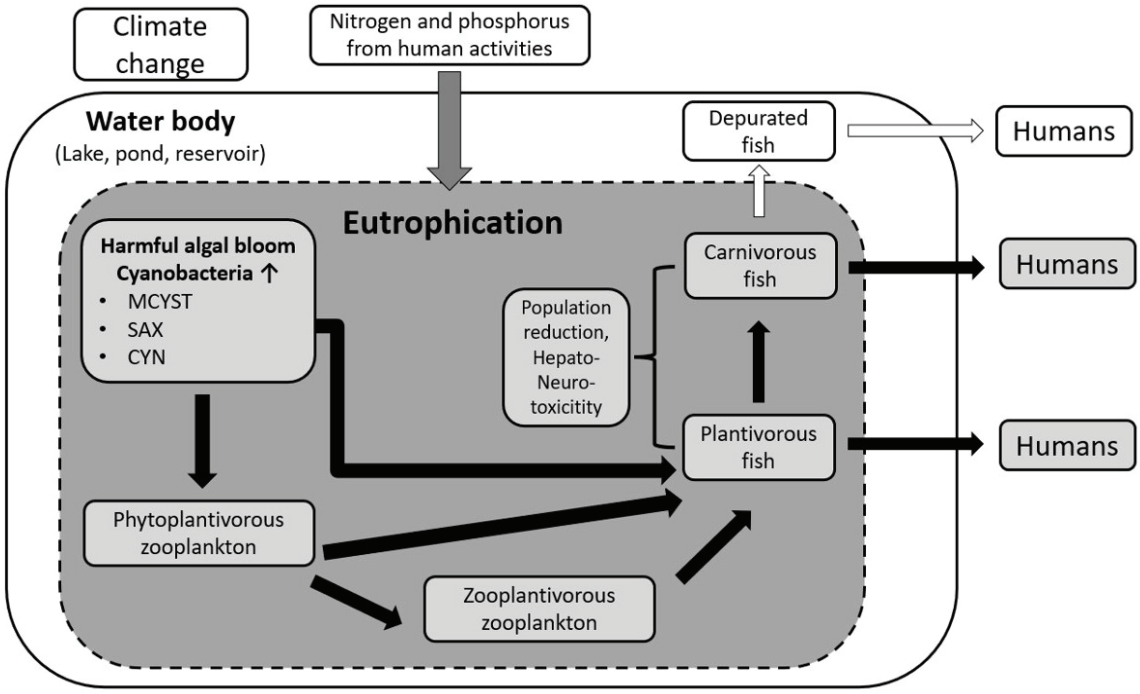

Figure 1. Schematic representation of cyanotoxins production and trophic transfer between several organisms in water bodies. Black arrows represent the cyanotoxins transfer.

MATERIALS AND METHODS

An extensive literature search was conducted to construct a database including information on accumulation of cyanotoxins in organisms inhabiting freshwater environments in Latin America. We collected all published studies to March 2022, using combinations of relevant search terms (i.e., cyanotoxins, Latin America, bioaccumulation, reservoir, aquaculture, cyanobacteria, human health impact) from the Digital Library of Universidad Nacional Autónoma de México, which includes about 170 scientific databases (e.g., Scopus, Web of Science, SciFinder) and Google Scholar. We focused on freshwater field studies in Latin America (from México to Argentina) reporting concentrations of cyanotoxins in organisms and water in natural or artificial/manipulated environments (e.g., ponds for aquaculture, reservoirs for hydroelectric energy). Because analytical methods for determination of concentrations of cyanotoxins in food matrices (e.g., fish and shrimps) is crucial to obtain reliable data, we followed Testai *et al.,* (2016) and Ibelings *et al.,* (2021) on determining the suitability and validation of the analytical methods employed in the studies.

From each study we collected the following information: author, year, country, location, freshwater environment type (e.g., lake, reservoir, pond), uses of the water body and land uses around it, reported nutrient concentrations (e.g., eutrophic), cyanotoxins analyzed and principal cyanobacterial genus/species present known to produce the toxins. For the land uses around the water body, if not reported in the study, we searched for this information in published literature. For the species accumulating cyanotoxins we collected: scientific name, native or introduced, importance in fishing (i.e., aquaculture, commercial/subsistence fishery), tissues and number of samples analyzed and concentrations of cyanotoxin in tissues. When studies reported several species that accumulate cyanotoxins or the same species from several water bodies, information from each species or water body was registered as a different entry in the database. Data about concentrations of cyanotoxins in water was included as reported: diluted, in seston (particulate material in water) and/or total. When not directly reported, cyanotoxin concentration values were extracted from figures. Additionally, we collected information related to temporal fluctuations of cyanotoxins bioaccumulation, cyanotoxins depuration by organisms in field studies and potential human cyanotoxin intakes by consumption of contaminated organisms, which are developed in the next sections.

RESULTS AND DISCUSSION

Information from studies reporting cyanotoxins accumulation in freshwater organisms in Latin America was extracted from journal articles (n=20) and a master thesis (n=1). Cyanotoxins bioaccumulation has been studied only recently in Latin America, since the year of publication of studies found ranged from 2001 to 2020. From these, we gathered a total of 50 entries of several species accumulating cyanotoxins in the database in 27 different locations/water bodies (Table S1). The summary and discussion of the information presented below are referred to the 21 studies and 50 entries.

Considerations of analytical methods employed. In order to evaluate the reliability of the analytical methods used in the determination of cyanotoxins concentration in food matrices, including fish and invertebrates, Testai *et al.,* (2016) developed a 1 to 4 score system, where score 1 constitutes a reliable study without restriction (validated method); score 2, a reliable study (fully characterized method); score 3, a reliable study with restriction (only reporting recovery); and 4 denoted

a not reliable study (no report of recovery). Based on that score system, which does not depend exclusively on the analytical method, the main difference between a reliable and a non-reliable study is to report the % of recovery, often by spiking samples with known concentrations of analyte. It allows to detect the losses of cyanotoxin during sample processing and cleaning previous to the analytical determination method. It is in accordance with Flores *et al.,* (2018), which pointed, in their global study of accumulation of MCYST's in fish, that there is a need to standardize cyanotoxin extractions from tissues and analytical methods for quantification, or to report extraction efficiencies, since the method used is likely important in influencing the observed cyanotoxins concentration in tissues.

The analytical methods of cyanotoxins quantification in the database included high-pressure liquid chromatography (HPLC, 12 studies), enzyme-linked immunosorbent assay (ELISA, 10 studies) and liquid chromatography-mass spectrometry (LC-MS, two studies) (Table S1). However, only 5 studies (25%) reported the % of recovery of the corresponding cyanotoxin analyzed, all of them using HPLC (Cazanve *et al.,* 2005, Ame *et al.,* 2010, Galanti *et al.,* 2013, Morandi 2015, Calado *et al.,* 2019). The other 15 studies would be considered including "a not reliable analytical method" based on Testai *et al.,* (2016) score system. Additionally, 50% of the studies included ELISA as analytical method, which has been considered a semiquantitative method, requiring confirmation of identity (i.e., cyanotoxins variants), and quantity, with strategies to determine extraction efficiency and matrix effects, even more when it is used in a human risk assessment (Testai *et al.,* 2016, Lawton *et al.,* 2021, Ibelings *et al.,* 2021). Unfortunately, it is not the case in studies employing ELISA in Latin America: recoveries were not reported and confirmatory identity of cyanotoxins (and their variants) with a chemico-analytical method were usually not carried out. Exceptions are found in Berry & Lind (2010) and Berry *et al.,* (2011) where CYN's (by HPLC-UV and LC-MS) and MCYST variants (by LC-MS) where confirmed respectively.

Despite the noted pattern of limitations in the analytical methods employed in most of the Latin America studies and differences in cyanotoxins extraction methods used that were similar to the patterns found by Testai *et al.,* (2016) in their comprehensive review, we decided to continue summarizing the information about cyanotoxins bioaccumulation of the region, at least in a screening sense, in order to 1) visualize its geographically reported occurrence, 2) to call attention of the potential transfer of cyanotoxins by contaminated fish to humans and 3) to highlight the need of use reliable analytical methods especially when evaluating potential human risk exposure.

Distribution, water bodies and cyanotoxin types. Despite the global distribution of cyanobacteria and cyanotoxins (Mowe *et al.,* 2015, Flores *et al.,* 2018, Svirčev *et al.,* 2019) and the known potential risk for species that are exposed to cyanotoxins, including humans (Codd *et al.,* 2005, Burrati *et al.,* 2017, Cantoral Uriza *et al.,* 2017, Pham & Utsumi 2018, Banerjee *et al.,* 2021), cyanotoxins accumulation in freshwater organisms in Latin America has been evaluated only in four countries. Brazil is the country having more field studies of cyanotoxins bioaccumulation (13 studies), followed by México (4), Argentina (3) and, finally, Uruguay with only one study. Of the entries in the database, 37% comprise natural lake environments, and the others comprise artificial environments, including reservoirs (49%) and ponds for aquaculture (14%). Of special concern is the use of water in lakes and reservoirs for the potential cyanotoxin transfer to humans. For example, in natural lakes, the uses include mainly fishing and tourism, besides irrigation and aquaculture. Reservoir's water is mainly used for electric power generation and drinking water supply (increasing the risk of direct exposition to cyanotoxins by human). However, recreation, aquaculture and fishing are also important activities (Table S1). Notably, 80% of the water bodies included in our database are considered eutrophic, a nutrient condition (i.e., high phosphorus and nitrogen concentrations in water) widely associated with harmful cyanobacterial blooms worldwide (Glibert 2020, Chorus *et al.,* 2021). Clearly, human activities around the water bodies, and its corresponding watershed basin, alter the nutrients dynamics, indirectly by runoff from agriculture fields, pastures for livestock, vicinity of cities, and directly when sewage is discharged in the water bodies (Friesen, 2015). Most of the locations/water bodies included in the database, where information was available (Table S1), are bordered by agricultural fields and/or grassland, sometimes with a large associated human population. For example, Funil reservoir, constructed on the Paraíba do Sul River located near Rio de Janeiro in southern Brazil, has a catchment area of 12800 km^2 and is one of the most highly industrialized regions in Brazil. Approximately 2 million people live inside the catchment area in 39 cities that depend on the Paraíba do Sul River for their water supply (Deblois *et al.,* 2008, Pacheco *et al.,* 2015).

Almost all studies reported the principal cyanobacterial species/genus present in water bodies responsible of toxins production. *Microcystis* is the most reported genus producing toxins in Latin America, found in 11 studies and 13 different water bodies, followed by *Cylindropermopsis* (8 studies and 10 water bodies) and *Anabaena* (5 studies, 5 water bodies). Other less reported genera included *Planktothrix, Aphanizomenon, Pseudanabaena* and *Dolichospermun,* however, more than

Table 1. Cyanotoxins analyzed in accumulation studies in freshwater environments in Latin America. Some studies included more than one cyanotoxin or waterbody.

Cyanotoxin	Countries	Number of studies	Number of waterbodies	Group analyzed
MCYST's	Argentina, Brazil, México, Uruguay	15	25	fish, snail, zooplankton
STX's	Brazil, Mexico	6	3	fish, snail, zooplankton
CYN's	Mexico	2	1	fish, snail, zooplankton
NOD	Argentina	1	1	shrimp

one genus was also detected within a water body (Table S1). Studies of cyanotoxins accumulation in Latin America have focused on four toxins with MCYST's being the most investigated with 15 studies in 25 different water bodies, followed by STX's, CYN's and NOD (Table 1). This result agrees with global research about cyanotoxin accumulation and distribution, where MCYST's are the most studied (Flores *et al.,* 2018), and highlights the need for more comprehensive studies including other cyanotoxins in Latin America.

Taxonomic groups and species accumulating cyanotoxins. Of the groups and species studied accumulating cyanotoxins in freshwater environments in Latin America, fish comprised most of the entries (90% of entries). Other entries corresponded to invertebrates: 2 entries for both snails and zooplankton (4% each group) and one entry for shrimps (2%). Mexico and Argentina are the countries where the invertebrate species accumulating cyanotoxins have been studied. In Mexico, Berry & Lind (2010) reported concentrations of STX's and CYN's accumulated in the Lake Catemaco endemic snail species, *Pomacea patula catemacensis* (Baker, 1922), locally known as "tegogolos", a relevant species because it is both the target of local and commercial fishing. That study was the first report showing evidence of accumulation of STX's and CYN's in any organism in México, and for CYN's the first report in Latin America. Also in México, two studies analyzed concentrations of cyanotoxins in zooplankton, an ecologically important group which can act as a vector of cyanotoxins, since some zooplankton directly consume cyanobacteria, and also are consumed by other zooplankton or fish (Sotton *et al.,* 2014). Berry *et al.,* (2012) found accumulations of STX's and CYN's in copepods (mainly *Mesocyclops* and *Arctodiaptomus*) from Lake Catemaco, while Zamora-Barrios *et al.,* (2019) reported MCYST's accumulation in copepods (*Acanthocyclops*) and cladoceran (*Daphnia* and *Bosmina*) from Lake Zumpango. In both studies, the concentrations of the cyanotoxins in zooplankton were notably higher than the concentrations in fish tissues. Zooplankton may ingest toxins directly from cyanobacteria and/or absorb dissolved cyanotoxins from water (Karjalainen *et al.,* 2005), which could explain their higher cyanotoxin concentration than fish, but this hypothesis should be tested. Finally, for invertebrates, Galanti *et al.,* (2013) carried out field exposures of the shrimp *Palaemonetes argentinus* (Nobili 1901) in San Roque Reservoir, Argentina, after a cyanobacteria bloom containing NOD. After three weeks of exposure in the reservoir, NOD was detected in *P. argentinus* tissues. Galanti *et al.,* (2013) noted that their study was the first report of NOD in South America freshwaters, and according to the present review, it is the first and unique report to date of accumulation of NOD in any freshwater organism. Also, it is the only report of the accumulation of cyanotoxins in shrimp in Latin America.

Table 2. Fish species included in studies of accumulation of cyanotoxins by commercial importance in Latin America. Countries where the studies were conducted and type of tissues analyzed for each fish species also included. References in table footnote. *indicates introduced species.

Species	Tissues analyzed	Countries	References
Commercial importance			
Astyanax caballeroi (Contreras-Balderas & Rivera-Teillery 1985)	muscle	Mexico	1
Chirostoma jordani Woolman 1894	whole fish	Mexico	2
Chirostoma sp.	Whole fish	Mexico	3
Coptodon rendalli (Boulenger 1897)*	liver, muscle, viscera	Brazil	4,5
Cyprinus carpio Linnaeus 1758*	liver, muscle	Mexico	3
Geophagus brasiliensis (Quoy & Gaimard 1824)	muscle	Brazil	6,7,8,19
Goodea sp.	viscera, muscle	Mexico	3
Hoplias sp.	muscle	Uruguay	9
Hypophthalmichthys molitrix (Valenciennes 1844)*	liver, muscle	Brazil	10
Mayaheros urophthalmus (Günther 1862)	muscle	Mexico	1
Odontesthes bonariensis (Valenciennes 1835)	liver, gills, brain, intestine, muscle	Argentina	11, 12
Oreochromis aureus (Steindachner 1864)*	muscle	Mexico	1
Oreochromis niloticus (Linnaeus 1758)*	liver, muscle, gills, intestine, gonads, bile	Brazil, Mexico	5, 13, 14, 15, 16, 17, 18
Plagioscion squamosissimus (Heckel 1840)*	liver	Brazil	10
Rhamdia sp.	muscle	Mexico	1
Vieja fenestrata (Günther 1860)	muscle	Mexico	1
Vieja sp.	muscle	Mexico	1
No commercial importance			
Dorosoma petenense (Günther 1867)	muscle	Mexico	1
Pseudoxiphophorus jonesii (Günther 1874)	muscle	Mexico	1
Thorichthys helleri (Steindachner 1864)	muscle	Mexico	1

[1]Berry *et al.,* 2012, [2]Zamora-Barrios *et al.,* 2019, [3]Berry *et al.,* 2011, [4] Magalhães *et al.,* 2001, [5]Deblois *et al.,* 2008, [6]Clemente *et al.,* 2010, [7]Calado *et al.,* 2017, [8]Calado *et al.,* 2019, [9]Morandi 2015, [10]Oliveira *et al.,* 2013, [11]Cazanave *et al.,* 2005, [12]Amé *et al.,* 2010, [13]Chellappa *et al.,* 2008, [14]Galvao *et al.,* 2009, [15]Vasconcelos *et al.,* 2013, [16]Hauser-Davis *et al.,* 2015, [17]Mendes *et al.,* 2016, [18]Lopes *et al.,* 2020, [19]Calado *et al.,* 2018.

Twenty fish species have been studied related to accumulation of cyanotoxins in Latin America, most of which are native species (70%) (Table 2). México is the country with more species (14), followed by Brazil (5). Argentina and Uruguay included only one species. Only four studies included more than one fish species and Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758), is the most studied species, included in 8 studies. Most of the species showing accumulation of cyanotoxins have commercial importance (80%) by aquaculture, commercial and/or subsistence fishing, highlighting the potential exposure of cyanotoxins to humans (Table 2). Muscle is the most analyzed fish tissue for determination of concentration of cyanotoxins in 16 species and 47 entries in database, followed by liver (6 species, 23 entries), other tissues analyzed in some species included gills, intestine, bile, brain, gonads or whole fish (Table 2, Table S1). The analysis of several types of tissues is important because they usually show distinct patterns of cyanotoxins accumulations, and eventually could produce different harmful effects in fish and on human health. For example, among the tissues, the concentrations of MCYST's are often reported to be higher in liver (and viscera) relative to concentrations in the muscle (Romo *et al.,* 2012, Flores *et al.,* 2018). For 20 of 21 entries of our database that reported both muscle and liver concentrations of cyanotoxins (corresponding to 6 species used in aquaculture or commercial fishing), the concentration in the liver was reported to be higher than in the muscle. This is in agreement with the global pattern of Flores *et al.,* (2008). This tendency is important because the liver, and the viscera in general, are normally not eaten and are discarded in aquaculture fish (e.g., Nile tilapia, which comprised 16 of the 20 entries), and it is a manner to reduce the risk of human exposure when eating fish that has accumulated cyanotoxins. There are two studies including whole fish consumption in México. The fish genus *Chirostoma*, locally known as "charales", is widely captured in lakes and reservoirs in Central Mexico; they are small fish which are sold whole, fresh or dried, and are part of the culinary traditions of that region. Berry *et al.,* (2011) from Lake Pátzcuaro and Zamora-Barrios *et al.,* (2019) from Lake Zumpango, two eutrophic lakes with known presence of cyanobacterial blooms, reported high concentrations of MCYST's in whole *Chirostoma*, and suggested a particularly high potential for human exposure to food-derived cyanotoxins. Another case of the relevance of studying different tissues for cyanotoxins accumulations is presented by Hauser-Davis *et al.,* (2015). They studied the accumulation of MCYST's in Nile tilapia in the chronically contaminated and eutrophic Jacarepaguá lagoon, Brazil, finding a greater concentration of MCYST's in gonads than in liver. They suggested that this is of concern since this could signal potential reproductive problems in tilapia, which, as noted above, is an important product of aquaculture (Hauser-Davis *et al.,* 2015). All the above information highlights the relevance of studying several types of tissues depending on research goals. For example, from an ecological point of view of trophic transfer in natural ecosystems, it could be important to study cyanotoxins contains in whole organisms, probably separating different tissues. From a human health risk perspective, it could be relevant to study only muscle, if people are only eating fish fillets, for example.

Fish trophic habits may potentially influence the accumulation of cyanotoxins (Zhang *et al.,* 2009, Flores *et al.,* 2018). For example, fish that feed on cyanobacteria that produce toxins have a direct path of cyanotoxin exposure (i.e., planktivorous fish), which is not present in piscivores or omnivorous fish. Two studies in the database deal with this topic in Latin America. Berry *et al.,* (2011) analyzed the concentration of MCYST's in three fish species of different trophic habits in Lake Patzcuaro: the mainly phytoplanktivorous *Goodea* sp., the zooplanktivorous *Chirostoma* sp. and the omnivorous *Cyprinus carpio* Linnaeus 1758. All three species accumulated MCYST's, and its content correlated with fish trophic level, with concentrations of cyanotoxin measured as phytoplanktivorous > omnivorous > zooplanktivorous. The authors suggest that, although phytoplanktivorous zooplankton could be a source of MCYST's for the fish, and could increase the exposure for zooplanktivorous fish, the microfauna of the lake is characterized by species that are not efficient grazers on cyanobacteria. Also, Berry *et al.,* (2011) indicated that the accumulation of the cyanotoxin may be mainly associated with direct consumption of the cyanobacteria rather than to biomagnification to higher trophic levels. On the other side, Zamora-Barrios *et al.,* (2019) found a higher concentration of MCYST's in the zooplanktivorous *Chirostoma jordani* Woolman 1894 than in the omnivorous Nile tilapia (even liver) in Lake Zumpango. Indeed, future studies should specifically test the hypothesis of a relation between the trophic habit of fish and the accumulation of several cyanotoxins.

Fluctuations in bioaccumulation and depuration of cyanotoxins. Patterns of temporal changes of cyanotoxins bioaccumulation in the field have been analyzed in seven studies in Latin America focusing on MCYST (Magalhaes *et al.,* 2001, Cazenave *et al.,* 2005, Ame *et al.,* 2010, Oliveira *et al.,* 2013, Zamora-Barrios *et al.,* 2019) and STX's (Clemente *et al.,* 2010, Calado *et al.,* 2017). Among them, some studies compared the accumulation of cyanotoxins in fish tissues between dry and wet season, however, although there were differences, there was not an obvious pattern of accumulation associated with a particular season. For example, Ame *et al.,* (2010) found accumulation of MCYST's in muscle of *Odontesthes bonariensis* (Valenciennes 1835) during both wet and dry season. The authors found temporal changes in the concentrations of MCYST variants (MCYST–LR, –RR, –LA and –YR), and suggested the need for an intensive monitoring program in that lake to ensure the health of people living in its surrounding. Also in *O. bonariensis*, Cazenave *et al.,* (2005) found a higher concentration of MCYST's in wet season in muscle, liver and gills, while Zamora-Barrios *et al.,* (2019) sampled Nile tilapia in three sampling dates and *C. jordani* in six, finding a higher concentration of MCYST's in tissues after the rains in both species when the decomposition of the *Microcystis* bloom occurred. Another study (Morandi 2015) suggested temporal fluctuations in MCYST's accumulation, since toxin presence and cyanobacterial blooms were previously reported at the Reservoir Rincón del Bonete in Uruguay. However intense sampling in the muscle of *Hoplias* sp. found no toxins, highlighting the importance of sampling in different seasons. Contrastingly, Calado *et al.,* (2017) did not find differences in the concentrations of STX's between dry and wet season, and, Clemente *et al.,* (2010) reported no significant difference in the STX's concentrations in muscle among the three samplings seasons (summer, spring, autumn), both studies carried out in Alagados Reservoir, Brazil with *Geophagus brasiliensis* (Quoy & Gaimard 1824).

Two studies included a long sampling period: Magalhaes *et al.,* (2001) sampled the accumulation of MCYST's in tissues of *Coptodon rendalli* (Boulenger 1897) for 40 months and Oliveira *et al.,* (2013) determined the accumulation of MCYST's in the phytoplanktivorous fish *Hypophthalmichthys molitrix* (Valenciennes 1844) during one year. Sampling in both studies was every two weeks. Six distinct phases based on MCYST's accumulations in different tissues were identified in Magalhães *et al.,* (2001). Although MCYST's were not detected in

Table 3. CYN's and STX's concentration (µg/kg) in muscle of different fish species by water body in Latin America including the corresponding daily intake by an adult and child (µg/kg body weight). Adult intake calculated based on consuming 300g by an 70kg body weight (bw) person and child intake based on consuming 200g by an 30kg bw person. Intake in bold exceed the ARfD of 0.5 µg/kg bw for STX's equivalents (EFSA, 2009). *denotes the use of reliable analytical methods

Cyanotoxin/Fish species	Water body	Toxin conc. (µg/kg)	Daily intake adult (µg/kg bw)	Daily intake child (µg/kg bw)	Reference
CYN's					
Astyanax caballeroi (Contreras-Balderas & Rivera-Teillery 1985)	Catemaco Lake, México	0.81	0.003	0.005	Berry *et al.,* 2012
Thorichthys helleri (Steindachner 1864)		0.15	0.001	0.001	
Mayaheros urophthalmus (Günther 1862)		0.26	0.001	0.002	
Dorosoma petenense (Günther 1867)		0.8	0.003	0.005	
Pseudoxiphophorus jonesii (Günther 1874)		1.26	0.005	0.008	
Oreochromis aureus (Steindachner 1864)		0.09	0.0004	0.001	
Rhamdia sp.		0.24	0.001	0.002	
Vieja fenestrata (Günther 1860)		0.81	0.003	0.005	
Vieja sp.		0.42	0.002	0.003	
STX's					
Geophagus brasiliensis (Quoy & Gaimard 1824)	Alagados Reservoir, Brazil	48	0.206	0.320	Calado *et al.,* 2017
		187.3	**0.803**	**1.249**	Calado *et al.,* 2019*
		12.2	0.052	0.081	Clemente *et al.,* 2010
Astyanax caballeroi (Contreras-Balderas & Rivera-Teillery 1985)	Catemaco Lake, México	0.71	0.003	0.005	Berry *et al.,* 2012
Thorichthys helleri (Steindachner 1864)		0.06	0.0003	0.0003	
Mayaheros urophthalmus (Günther 1862)		0.32	0.001	0.002	
Dorosoma petenense (Günther 1867)		0.33	0.001	0.002	
Pseudoxiphophorus jonesii (Günther 1874)		0.36	0.002	0.002	
Oreochromis aureus (Steindachner 1864)		0.03	0.0001	0.0001	
Rhamdia sp.		0.1	0.0004	0.001	
Vieja fenestrata (Günther 1860)		0.3	0.001	0.002	
Vieja sp.		0.22	0.001	0.001	

viscera and liver in some phases, they were always detected in muscle, with different concentrations of MCYST's depending on the phase (Magalhaes *et al.,* 2001). For *H. molitrix,* Oliveira *et al.,* (2013) found that during the drought months (April–September), the concentrations of toxins in muscle and liver were higher than in other months of the study period.

In general, the studies summarized here concerning fluctuations of cyanotoxins accumulation could reflect variations associated with type of cyanotoxin, accumulating species and/or geographic zone, which could be addressed in future analyses. Also, they highlight the need of site-specific studies of fluctuations of cyanotoxins accumulation in fish for a better understanding of potential human exposure by consumption of fish during annual changes in weather patterns.

Four studies analyzed the depuration of cyanotoxins (i.e., reduction or elimination of cyanotoxins after stop exposure during a specific time), finding contrasting results. First, Galvao *et al.,* (2009) analyzed in Nile tilapia from an artificial lake in Brazil the presence of MCYST's and STX's, NOD, ATX's and CYN's, and its depuration. Only STX variants were detected in fish from the lake and, after a depuration time of five days without food in clean running water, the fish completely eliminated the STX's in muscle and liver (Galvao *et al.,* 2009). The authors suggested that depuration is a simple process that can be readily adopted by Nile tilapia producers as a way to eliminate STX's. In contrast, Calado *et al.,* (2017, 2019) analyzed the depuration of STX's in *G. brasiliensis* from Alagados Reservoir, Brazil, during 40 and 90 days respectively, finding that although there was a reduction of STX's in depurated fish, toxins were still present in fish muscle. Calado *et al.,* (2017) found a reduction in the percentage of specimens with the STX variant in the depurated fish, while the concentrations of the gonyautoxin 2 (GTX2) variant increased. They suggested that STX variant could be transformed to GTX2 and such transformation may decrease the toxicity of cyanotoxins to fish, since GTX2 is less toxic than STX. In addition, Calado *et al.,* (2019) determined the concentrations of STX's in the water and fish feces during the depuration time, suggesting that the fish biotransformed and eliminated STX's during the detoxification process and that the elimination of these toxins is possible but it takes a long time. Finally, Calado *et al.,* (2018) studied the depuration of MCYST's in *G. brasiliensis* during

90 days from Iraí Reservoir, Brazil. They found that MCYST's concentrations increased in fish muscles during the depuration time at around 30 days, then MCYST's decreased but were still present in fish muscle at 90 days when the experiment finished. Calado *et al.,* (2018) argued that when MCYST's enter into cells they can be bound to phosphatase proteins and glutathione (GSH), and, during the depuration process, the toxins were metabolized and released. Thus, they initially increased in fish muscles, and finally MCYST's were excreted via feces and urine.

Future studies should focus on depuration patterns of other species and cyanotoxins, in order to determine the utility of the depuration process in reducing the exposure to humans when consuming fish exposed to cyanotoxins. Also, the suggested pattern of depuration of cyanotoxins by aquatic organisms in the field is promissory. It provides an option for species in natural environments to recover after a potential change in conditions of the waterbodies (e.g., after a management program to stop or reduce the eutrophication caused by human activities).

Potential human intake of cyanotoxins in Latin America. Several routes of human exposure have been recognized from water bodies containing cyanotoxins: use of drinking water, skin or nasal mucous membrane contact during recreational activities (e.g., swimming, canoeing or bathing), consumption of irrigated vegetables or fruits, consumption of aquatic organisms including fish, oral intake of cyanobacterial dietary supplements and dialysis (Drobac *et al.,* 2013, Ibelings *et al.,* 2021). In the case of the present review related to accumulation of cyanotoxins in freshwater organisms with some of them used as food, a tolerable daily intake (TDI) for lifetime has been recommended as a provisional guideline value for human consumption of contaminated organisms based on body weight (bw). For MCYST's the recommended TDI is 0.04 µg/kg bw by World Health Organization (WHO) (Falconer *et al.* 1999), and for CYN's of 0.03 µg/kg bw (Humpage & Falconer, 2003, Ibelings & Chorus, 2007). Also an acute reference dose (ARfD) of 0.5 µg STX's equivalents/kg bw by the European Food Safety Authority (EFSA, 2009) associated to the no-observed- adverse-effect level (NOAEL). In addition for MCYST's, Ibelings & Chorus (2007) calculated 2.5 µg/kg bw for the maximum tolerable intake to avoid an acute exposure (ATI) by a single consumption and 0.4 µg/kg bw for short term exposure tolerable intakes (STI), for example during a cyanobacterial bloom.

As noted in Section 3.1.1 above, almost all the Latin America studies of bioaccumulation of cyanotoxins showed limitations and flaws in the analytical methods employed, precluding a reliable risk assessment due to the consumption of contaminated fish based on these studies. However, in a screening sense and to alert to the risk of cyanotoxins through food in the region, we calculated potential human intakes for studies in the database which reported potential daily intakes and compared them with some guideline values.

We recalculated the potential human intake of cyanotoxins by assuming an adult to weigh 70 kg, consuming 300 g of fish (muscle or whole fish as usually eaten) and for a child weighing 30 kg and consuming 200 g of fish. We compared the potential intake values calculated from field studies with TDI, STI and ATI thresholds. When studies reported concentrations in tissues by sample points in a waterbody, several seasons, or different fish stages, we calculated the intake considering the higher concentrations reported. When reported several water bodies in a study for the same species, we calculated the daily intake for each water body.

Based on the only study reporting accumulation of CYN's in fish species in Latin America (Berry *et al.,* 2012), no CYN's concentration in muscle of the nine species reported from Catemaco Lake, México exceeded the TDI suggested (Humpage & Falconer, 2003; Ibelings & Chorus, 2007) (Table 3). For STX's, four studies (including a total of 10 species) reported intake calculations (Table 3), however, only Calado *et al.,* (2017) included concentrations in tissues exceeding the ARfD for *G. brasiliensis* from Alagados Reservoir, Brazil for both adult and child intake (exceeding 1.6 and 2.5-fold respectively).

Studies reporting intakes of MCYST's included 10 species in several waterbodies from Brazil, Mexico and Argentina (Table 4). From all these countries, some studies reported fish intakes exceeding TDI, some even exceeding STI and ATI. In Argentina, Cazanave *et al.,* (2005) reported potential intakes of MCYST's due to consumption of *O. bonariensis* from San Roque Reservoir to be higher than TDI, while for the same species from Los Padres Lake, Ame *et al.,* (2010) found intakes lower than TDI (Table 4). Most of the studies, including potential human intakes of MCYST's, come from Brazil for four species and 18 water bodies (Table 4). Among them, Oliveira *et al.,* (2013) reported the highest concentrations in muscle and, consequently, the highest daily intakes for a fish (*H. molitrix*) in Latin America, which exceed by 168 and 261-fold the TDI respectively for adult and child. In this case, the intake also exceeded the ATI, and represented a potentially high risk of intoxication by consumption of this planktivorous species. Contradictingly, *H. molitrix* was introduced in Paranoá Lake as an attempt to reduce the amount of cyanobacteria, and became an important issue of health risks for its consumption by local people (Oliveira *et al.,* 2013).

The MCYST's intakes by consumption of Nile tilapia were reported in 19 cases (18 from Brazil), and in 10 of them exceeded the TDI (Table 4). Moreover, it is notably the study of Lopes *et al.,* (2020) where the MCYST's intakes of Nile tilapia from eight water bodies exceeded even the ATI. From Mexico, the MCYST's intake by consumption of *Goodea* sp. and *Chirostoma* sp. from Pátzcuaro lake (Berry *et al.,* 2011) and by *C. jordani* from Zumpango lake (Zamora-Barrios *et al.,* 2019) exceeded the TDI (also the STI for *Goodea* sp.). Related to differences between adult and child intakes, two studies including Nile tilapia from Brazil (DeBlois *et al.,* 2008) and *C. jordani* (Zamora-Barrios *et al.,* 2019) from Mexico showed intakes exceeding TDI for child but not exceeding for adults, highlighting the higher risk to children of exposure to harmful levels.

Similar to the summarized data reported by Ibelings and Chorus (2007) on their review of accumulation of cyanotoxins in freshwater seafood and its consequences for public health, there are variations in cyanotoxins intakes depending on fish species and water body, in many cases exceeding the TDI, and some doses exceeding ATI. For example, Deblois *et al.,* (2010) found intakes exceeding TDI for Nile tilapia, but lower (and not exceeding TDI) for *C. rendalli* in Furnas Reservoir, suggesting that certain fish known to feed on cyanobacteria might be safer for consumption than others, which could be considered in the formulation of public health guidelines. Indeed, more research is needed in order to evaluate the health problems for people consuming exposed fish on a local scale and for particular waterbodies, and its correlation to the provisional guideline values suggested. Also, specific factors of water bodies and consuming habits of people including the frequency and for which time spans people will be exposed by their diet, the du-

ration of cyanotoxins occurrence in the water bodies where people get the fish, and other exposure routes acting synergistically, together with other risk conditions of expose people (i.e., health condition, age), will influence the potential risk for humans (Ibelings and Chorus 2007). Finally, reliable analytical methods for cyanotoxins concentrations in tissues of expose organisms should be implemented in future assessment of risk to human health in Latin America, even more considering the summarized patterns of risk in some cases using screening methods.

Future directions. The bioaccumulation of cyanotoxins and potential impacts on environment and human health constitute a complex scenario, where biological processes, but also social, economic, cultural, management, conservation and regulatory factors are involved. Some of these factors are out of the scope of the present review, however, we identified some future directions in order to reduce specifically for Latin America the potential harmful effects of cyanotoxin on the environment and humans.

Table 4. MCYST's concentration (µg/kg) in muscle of different fish species by country and water body in Latin America, including the corresponding daily intake by an adult and child (µg/kg of body weight). Adult intake calculated based on consuming 300 g by a 70 kg body weight (bw) person and child intake based on consuming 200 g by a 30 kg bw person. Intakes in bold exceed the TDI of 0.04 µg/kg bw for a lifetime suggested by the World Health Organization (Falconer *et al.* 1999). (All concentration for muscle except for *Chirostoma jordani* and *Chirostoma* sp., which includes the whole fish). *Denotes the use of reliable analytical methods, [a]Exceed the "short-term" daily intake of 0.4 µg/kg bw, [b]Exceded maximum tolerable intake to avoid an acute exposure by a single consumption of 2.5 µg/kg bw.

Country/Species	Water body	Toxin conc. (µg/kg)	Daily intake adult (µg/kg bw)	Daily intake child (µg/kg bw)	Reference
Argentina					
Odontesthes bonariensis (Valenciennes 1835)	Los Padres Lake	4.9	0.021	0.033	Ame *et al.,* 2010*
	San Roque Reservoir	50	**0.214**	**0.333**	Cazanave *et al.,* 2005*
Brazil					
Coptodon rendalli (Boulenger 1897)	Furnas Reservoir	1.7	0.007	0.011	DeBlois *et al.,* 2008
	The Jacarepaguá lagoon	337.3	**1.44[a]**	**2.24[a]**	Magalhães *et al.,* 2001
Hypophthalmichthys molitrix (Valenciennes 1844)	Paranoá Lake	1570	**6.72[b]**	**10.46[b]**	Oliveira *et al.,* 2013
Geophagus brasiliensis (Quoy & Gaimard 1824)	Iraí Reservoir	4.6	0.01	0.03	Calado *et al.,* 2018
	Acauã Reservoir	0.006	<0.002	<0.002	Mendes *et al.,* 2006
	Acauã Reservoir	0.37	<0.002	0.002	Vasconcelos *et al.,* 2013
	Araçagi Reservoir	0.019	<0.002	<0.002	Mendes *et al.,* 2006
	Aracoiaba pond	570	**2.44[a]**	**3.8[b]**	Lopes *et al.,* 2020
	Boqueirão do Casi Reservoir	0.018	<0.002	<0.002	Mendes *et al.,* 2006
	Cacimba de Varzea Reservoir	0.03	<0.002	<0.002	Mendes *et al.,* 2006
	Camalau Reservoir	0.16	<0.002	<0.002	Vasconcelos *et al.,* 2013
	Castanhão pond	1040	**4.45[b]**	**6.93[b]**	Lopes *et al.,* 2020
	Cordeiro Reservoir	0.005	<0.002	<0.002	Mendes *et al.,* 2006
Oreochromis niloticus (Linnaeus 1758)	Cordeiro Reservoir	0.23	<0.002	<0.002	Vasconcelos *et al.,* 2013
	Funil Reservoir	6.1	0.026	**0.041**	DeBlois *et al.,* 2008
	Furnas Reservoir	11.7	**0.05**	**0.078**	DeBlois *et al.,* 2008
	Furnas Reservoir	710	**3.04[b]**	**4.73[b]**	Lopes *et al.,* 2020
	Ilha Solteira Reservoir	350	**1.5[a]**	**2.33[a]**	Lopes *et al.,* 2020
	Juara lake	625	**2.67[b]**	**4.16[b]**	Lopes *et al.,* 2020
	Linhares lake	740	**3.17[b]**	**4.93[b]**	Lopes *et al.,* 2020
	Orós pond	655	**2.81[b]**	**4.36[b]**	Lopes *et al.,* 2020
	The Jacarepaguá lagoon	2.75	0.011	0.018	Hauser-Davis *et al.,* 2015
	Tres Marias Reservoir	780	**3.34[b]**	**5.2[b]**	Lopes *et al.,* 2020
México					
Chirostoma jordani Woolman 1894	Zumpango Lake	7	0.03	**0.046**	Zamora-Barrios *et al.,* 2019
Chirostoma sp.	Pátzcuaro Lake	18.5	**0.079**	**0.123**	Berry *et al.,* 2011
Cyprinus carpio Linnaeus 1758	Pátzcuaro Lake	4.99	0.021	0.033	Berry *et al.,* 2011
Goodea sp.	Pátzcuaro Lake	157	**0.67[a]**	**1.047[a]**	Berry *et al.,* 2011
Oreochromis niloticus (Linnaeus 1758)	Zumpango Lake	2	0.008	0.013	Zamora-Barrios *et al.,* 2019

As highlighted in the present review, bioaccumulation of cyanotoxins have been accessed only in four countries in Latin America, a region comprising ~20 countries and investigations have focused mainly on MCYST's, for example, bioaccumulation of CYN's and NOD have only been analyzed twice and once from México and Argentina, respectively. Accordingly, Zurawell *et al.,* (2005) and Scarlet *et al.,* (2020) stated that some cyanobacterial toxins, including CYN's, are not routinely monitored around the world, and thus the range of toxins concentrations in natural systems is not known. There is a need for studies of bioaccumulation and monitoring, including less studied cyanotoxins and in more water bodies in more countries in Latin America, for a better understanding of potential risk of exposure to cyanotoxins. Aquaculture is a common activity in the region (Morales & Morales, 2006) and, as reported in the studies analyzed here, 80% of the species showing accumulation of cyanotoxins have commercial importance. To what extent the aquaculture constitutes an important route of transfer of cyanotoxins to humans is still unknown, and it should be determined in future studies. Also, as noted by Cantoral Uriza *et al.,* (2017), it is important to investigate unknown cyanobacteria species from tropical areas in Latin America to access their potential toxicity.

From an ecological point of view, there is evidence of behavioral alterations caused by cyanotoxins exposure in fish (Malbrouck & Kestemont 2006), including a decrease in locomotor activity and altered reproductive behavior (reduction in the spawning activity and success) (Baganz *et al.,* 1998). It is still unknown how changes in behavior associated with cyanotoxins exposure may affect fish populations in the wild (e.g., increasing or decreasing populations) and future studies could focus on the potential threat to conservation of native species by cyanotoxin exposure. Also, the trophic transfer of cyanotoxins has been widely documented (Ferrão-Filho & Kozlowsky-Suzuki *et al.,* 2011, Lance *et al.,* 2014), however, comprehensive analyses of food-chain effects of cyanotoxins (Zurawell *et al.,* 2005) and potential negative impacts in a wide range of organisms (i.e., insects, other vertebrates than fish) in freshwater natural systems have not been evaluated and constitute a pending area of research in Latin America. In this sense, the implementation of new approaches, including ecotoxicological signals using omics analyses, allowing the investigation of thousands of molecular responses of the cell to cyanotoxins at the same time (Marie 2020), will be particularly useful in future studies.

The present review summarized the current knowledge of cyanotoxins accumulation in freshwater organisms in Latin America, integrating information of major topics studied in the region, including groups and species accumulating cyanotoxins, fluctuations and depuration of cyanotoxins, and potential human intake of cyanotoxins from field studies. A further understanding and reduction of the harmful effects of cyanotoxins on the environment and on human health, specifically for Latin America, is a promising field for future research. Additionally, in the face of the population increase and watershed modifications in the region, combined with climate change and eutrophication that promotes cyanobacterial blooms, the study and monitoring of bioaccumulation of cyanotoxins should be mandatory. Also, assessing, monitoring and/or managing cyanobacterial/cyanotoxins risks in water-use systems is lacking for most of the countries in the region. This scenario indicates the need for more efforts to generate scientific research, but also, this research needs to be linked with national and local level management policies.

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NOTA CIENTÍFICA

Recruitment of *Pocillopora* coral on experimental tiles in the Mexican Pacific

Reclutamiento del coral *Pocillopora* en placas experimentales en el Pacífico mexicano

Alvarado-Rodríguez J. Fernando*[1,2], Calderon-Aguilera, Luis. E.[1], Cabral-Tena, Rafael. A.[1]

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[1] Laboratorio de Esclerocronología y Ecología de la Zona Costera, Departamento de Ecología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California. Carretera Ensenada – Tijuana No. 3918, Zona Playitas, Ensenada, B. C., 22860, México

[2] Laboratorio de Biodiversidad Marina, Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo. Avenida San Juanito Itzícuaro s/n, Col. Nueva Esperanza, Morelia, Michoacán, 58337. México.

***Corresponding author:**
J. Fernando Alvarado-Rodríguez: e-mail: jfalvarado15@gmail.com

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ABSTRACT

Background. The recruitment of branching corals in the eastern Pacific is poorly understood despite being of paramount importance to the dynamics of coral populations. Experimental studies provide a non-destructive means to evaluate recruitment and compare settlement materials. **Goals.** To study *Pocillopora* recruitment on PVC and terracotta tiles in Bahía Ixtapa-Zihuatanejo (Mexican Pacific). **Methods.** We deployed 40 square (10 x 10 x 0.5 cm) experimental (20 PVC and 20 unglazed terracotta) tiles arranged as Calcification/Accretion Units at the Islote Zacatoso reef. **Results.** We observed two coral recruits at the edges of terracotta tiles, presumably due to light availability, and no recruits on horizontal sides, which may have been due to siltation stress, predation, or biofouling. No recruits were found on PVC tiles. **Conclusions.** Our findings indicate that the coral recruitment is low in the study area and that the terracotta tiles may be a better experimental substrate than PVC tiles to assess pocilloporid coral recruitment in the Mexican Pacific; however, further studies are needed to clarify this assumption.

Keywords: Coral recruitment, experimental substrate, terracotta tiles, Mexican Pacific.

RESUMEN

Antecedentes. El reclutamiento de corales ramificados en el Pacífico oriental tropical está poco documentado, a pesar de la importancia que tiene para entender su dinámica poblacional. Los estudios experimentales proporcionan un medio no destructivo para evaluar el reclutamiento y comparar diferentes materiales. **Objetivo**. Estudiar el reclutamiento de *Pocillopora* en placas de PVC y de terracota en Bahía Ixtapa–Zihuatanejo, en el Pacífico mexicano. **Métodos**. Colocamos 40 placas cuadradas (10 x 10 x 0.5 cm), 20 de PVC y 20 de terracota armadas como Unidades de Calcificación/Acreción en el arrecife Islote Zacatoso. **Resultados**. Observamos dos reclutas de coral en la orilla de las placas de terracota pero ninguno en los lados horizontales, posiblemente debido a la disponibilidad de luz, el estrés por sedimentación, la depredación, o el sobrecrecimiento por biota incrustante. No encontramos reclutas en ninguna de las placas de PVC. **Conclusiones**. Nuestros hallazgos indican que el reclutamiento coralino es bajo en la zona de estudio y que las placas de terracota pueden ser un mejor sustrato experimental que las de PVC para evaluar el reclutamiento de corales pociloporidos en el Pacífico mexicano; sin embargo, se necesitan más estudios para corroborar esta suposición.

Palabras clave: Reclutamiento de corales, sustrato experimental, placas de terracota, Pacífico mexicano.

Larval dispersal and recruitment are crucial to the persistence of existing coral populations and the establishment of populations in new areas. In the eastern tropical Pacific, branching corals such as *Pocillopora* are the primary reef builders (Glynn *et al*., 2017), and fragmentation is the predominant mechanism of asexual reproduction and dispersal (Highsmith, 1982; Richmond, 1997). Although *Pocillopora damicornis* (Linnaeus, 1758) was thought to be sexually sterile in the eastern Pacific (Richmond, 1987), it can reproduce asexually and sexually (Chávez-Romo & Reyes-Bonilla, 2007; Carpizo-Ituarte *et al*., 2011).

Coral recruitment is indicative of sexual reproduction. In the face of anthropogenic and environmental disturbances, genetic diversity due to sexual reproduction is of paramount importance for the persistence of populations to adapt and persist over time (Glynn *et al.*, 1991; Richmond, 1997). Despite the importance of coral recruits, they are often difficult to detect *in situ* because of their small size, and live scans may underestimate overall recruitment compared to artificial substrate-based census methods (Harper *et al.*, 2021). Although artificial settlement tiles are a non-destructive means to study coral recruitment, both the material and orientation of the tiles may influence coral settlement and recruitment (Field *et al.*, 2007).

Herein, we provide evidence of the successful recruitment of *Pocillopora* branching corals at the Islote Zacatoso reef (Ixtapa-Zihuatanejo, Guerrero, Mexican Pacific; 17° 39' 14.5" N, 101° 37' 18.7" W). We deployed 40 square experimental (10 x 10 x 0.5 cm) tiles composed of two frequently used materials: polyvinyl chloride (PVC; 20 tiles) and unglazed terracotta (20 tiles). Tiles were arranged as Calcification/Accretion Units (CAUs) parallel to the sea floor to mimic horizontal exposed and natural cryptic substrates (Price *et al.*, 2012; Johnson *et al.*, 2022). All CAUs were deployed near healthy coral colonies within the reef in April 2019, with approximately 1 m between each one, at a 6 m depth (~ 30 cm above the seabed; Fig. 1A). After 15 months, the CAUs were recovered, and each tile was stored in a plastic bag and frozen until further processing. In the laboratory, each tile was rinsed to remove sediments and soaked in sodium hypochlorite for 24–48 h to degrade organic matter. The tiles were then oven-dried at 70 °C for 48 h, and coral recruits on the tiles were identified with a stereoscopic microscope.

We found two *Pocillopora* sp. recruits attached at the edges of terracotta tiles that were growing over encrusts such as bryozoans and barnacles (Fig. 1B). Corals did not settle on horizontally positioned tiles, which may have been due to siltation stress (Babcock & Davies, 1991; Te, 1992) or to avoid predation (Jokiel *et al.*, 2014; Doropoulos *et al.*, 2016). No recruits were observed on PVC tiles. These findings suggest that terracotta tiles facilitate coral recruitment (Harriott & Fisk, 1987) possibly because of their microstructure and similarity to reef substrata (López-Pérez *et al.*, 2007). Moreover, these results support the presence of active coral reproduction in the region (Carpizo-Ituarte *et al.*, 2011).

The recruitment of *Pocillopora* sp. is reportedly low in the Mexican Pacific. López-Pérez *et al.* (2007) observed only one *Pocillopora* recruit in 305 terracotta tiles, which were deployed at 45 degrees concerning the ocean floor over 12 months in Bahías de Huatulco, Oaxaca. In Bahía de La Paz, Baja California Sur, Cabral-Tena *et al.* (2018) recorded six *Pocillopora* recruits in 30 terracotta tiles deployed for ~ 3 months. Furthermore, other studies with terracotta tiles have not recorded pocilloporid recruits in the region (e.g., Medina-Rosas *et al.*, 2005 in Jalisco and Nayarit; Santiago-Valentín *et al.*, 2020 in the Islas Marias Biosphere Reserve).

In this study, Pocilloporid corals settled on the sides (i.e., vertical) of terracotta tiles but not in PVC tiles, even though the CCA, which is known to act as a preferential settlement substrate for reef-building coral larvae (Morse *et al.*, 1988; Heyward & Negry, 1999; Tebben *et al.*, 2015; Elmer *et al.*, 2018; Jorissen *et al.*, 2021; Tanvet *et al.*, 2022;), grew in both materials. Competent coral larvae can explore for a suitable substrate that maximizes their fitness (Morse & Morse, 1996). Moreover, perhaps coral spats settled on terracotta tiles because their texture resembles natural substrate conditions (López-Pérez *et al.*, 2007), and avoid predation and siltation stress on vertical sides (Babcock & Davies, 1991). PVC tiles might not be an attractive substrate for larvae of this coral branching, or if settlement occurs, we do not know which factors eliminate spats from tiles. Factors such as predation and competition for space with others encrusting organisms might be a possible response, but this question is beyond the objectives of the present work.

Although in other parts of the world PVC tiles appear to be a stable substrate for the recruitment of corals (e.g., Vargas-Ángel *et al.*, 2015; dos Reis *et al.*, 2016; Price *et al.*, 2019), to our knowledge, *Pocillopora* sp. recruits on PVC tiles has never been observed in the Mexican Pacific (Alvarado-Rodríguez *et al.*, 2019, 2021, 2022; Nava *et al.*, 2022; Orran-

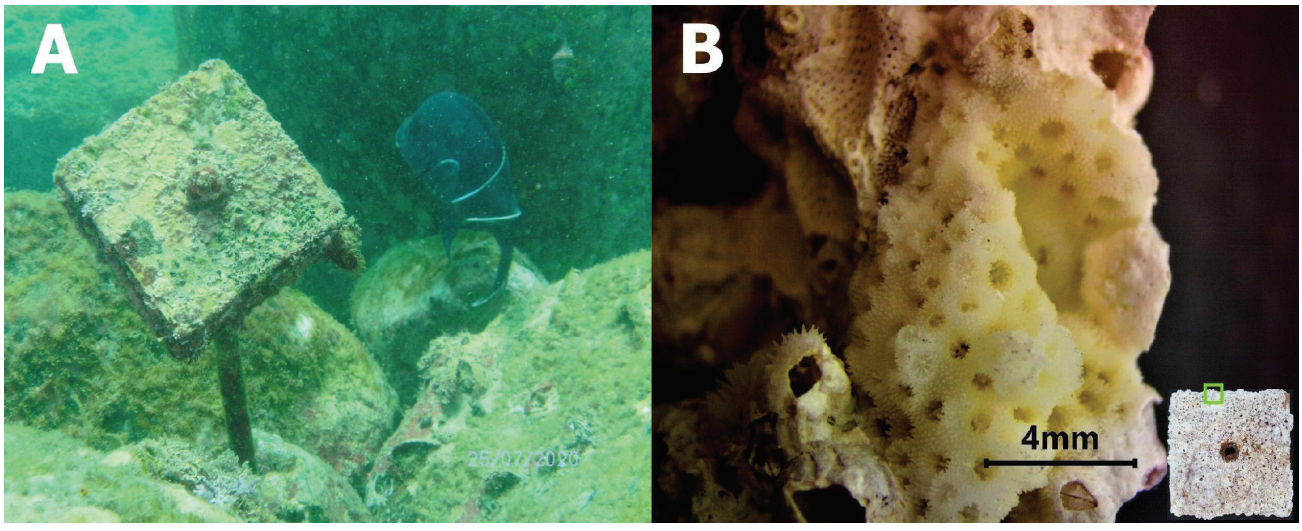

Figure 1. A) Photograph of a CAU (Calcification/Accretion Unit) after 15 months of deployment at Islote Zacatoso reef and B) *Pocillopora* sp. recruits overgrowing encrusting organisms; inset is a terracotta tile (lower right) showing the edge where the recruit attached (green square).

te *et al*., 2023; Medellín-Maldonado pers. comm. 2023 in Huatulco, Oaxaca; Pareja-Ortega pers. comm. 2023 in La Paz, Baja California Sur). We encourage terracotta tiles over PVC tiles in future studies of recruitment of branching corals in reefs of the Mexican Pacific because we have never observed coral recruitment in PVC tiles throughout several years of using this material while we found two recruits of *Pocillopora* coral for the first time using terracotta tiles.

It is also essential to evaluate multiple settlement tile orientations (e.g., 45 or 90 degrees concerning the ocean floor) because no scientific consensus exists that substrate orientation increases the chances of encountering coral recruits. For example, English *et al*. (1997) described the general procedure to study coral recruitment on terracotta tiles inclined to 45 degrees concerning seafloor, but no discussion about it is provided. In this regard, Glassom *et al*. (2004) argued that this orientation positively correlates with coral recruitment. However, horizontally deployed experimental substrates are common in coral recruitment studies (e.g., Doropoulos *et al*., 2016; Gallagher & Doropoulos, 2017; Elmer *et al*., 2018). More specifically, Harper *et al*. (2021) recommend including both sides (top and bottom) of horizontally deployed tiles and ensuring homogeneous rugosity due to coral recruits settling on both sides.

Like in this work, coral recruitment has occurred on lateral sides of the experimental substrates in other studies (e.g., Tomascik, 1991; Melo-Merino, 2009; Cameron & Harrison, 2020). These works conclude that predation and siltation effects are the main factors controlling larva settlement and argue that coral recruitment is proportionally greater on vertical or under surfaces than on upper surfaces (Babcock & Davies, 1991 and references therein). In particular, the recruitment of *Pocillopora* corals is so scarce that some works have adopted both material orientation strategies to increase chances for coral recruits encountering (e.g., Soong *et al*., 2003). In conclusion, more comparative studies in the Mexican Pacific are needed to clarify this issue.

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Hidrobiológica

Índice del volumen 33, 2023

Número 1

Número 2

Número 3

Hidrobiológica

Índice de autores

HIDROBIOLÓGICA

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VOLUMEN 33 Número 3 2023

Casa abierta al tiempo
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