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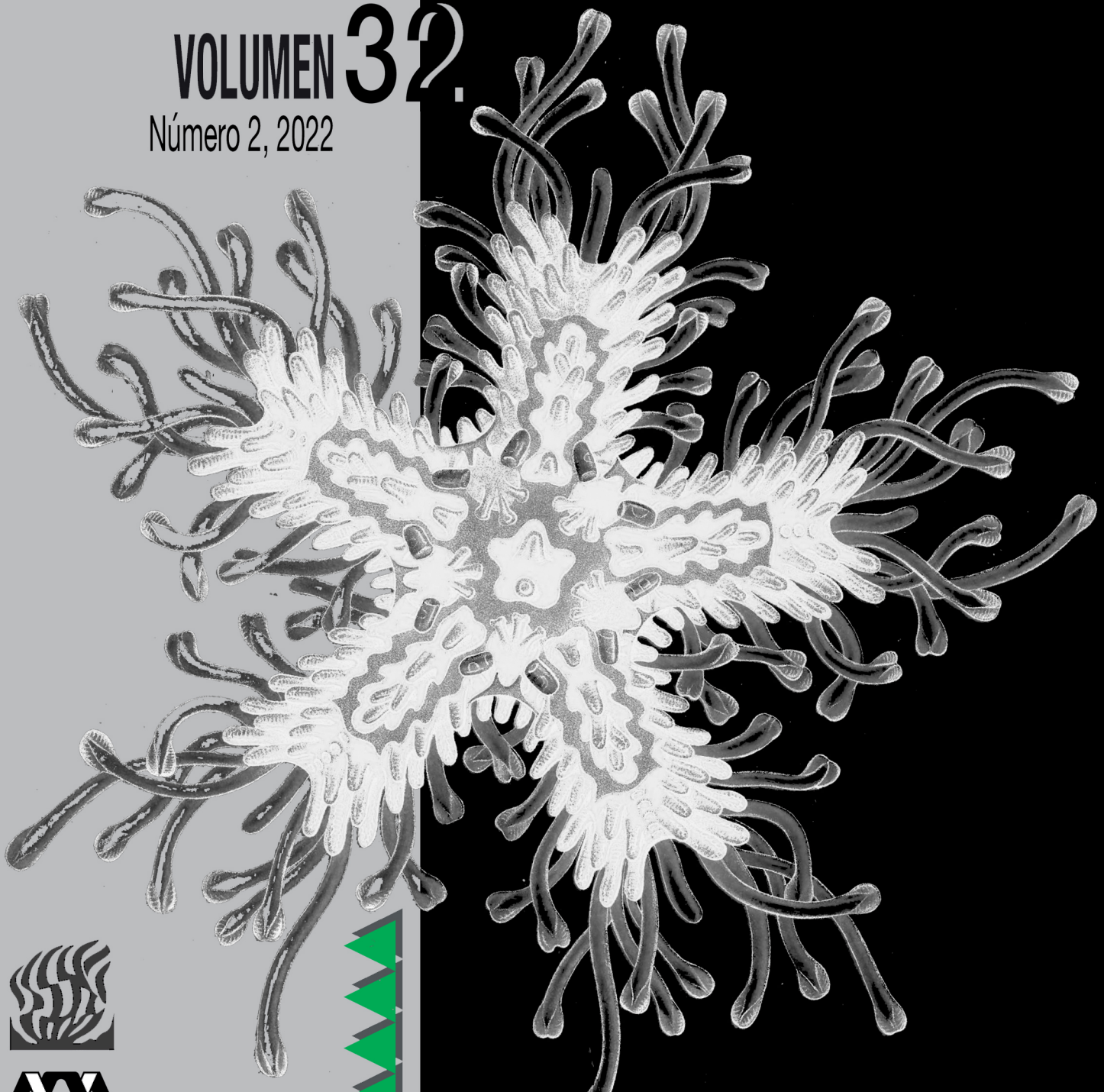

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Estimación de la densidad y abundancia de la nutria neotropical (*Lontra longicuadis annectens* Olfers, 1818) en el Sistema Lagunar de Alvarado, Veracruz

Density and abundance estimation of the neotropical otter (*Lontra longicuadis annectens* Olfers, 1818) in the Alvarado Lagoon System, Veracruz

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RESUMEN

Antecedentes. En México la nutria neotrópical (*Lontra longicaudis annectens*, Olfers, 1818) se encuentra ampliamente distribuida y sus poblaciones están amenazadas. **Objetivos.** Estimar la densidad y abundancia de la nutria de río neotropical en el Sistema Lagunar de Alvarado (SLA), Veracruz. **Métodos.** El SLA tiene una extensión de 51,960.52 hectáreas y está compuesto por lagunas costeras, lagunas interiores y ríos. Por medio de la técnica de muestreo a distancia con transectos sistemáticos, se estimó la distribución, densidad y abundancia de la nutria neotropical en el SLA. **Resultados.** Se obtuvieron un total de 25 avistamientos independientes de nutrias, siendo los ríos Limón, Blanco y Culebrilla donde se registraron con mayor frecuencia. Se estimó una abundancia de 934 (% C.V.= 20.45) nutrias para todo el SLA, con una densidad de 0.179 organismos / km^2 (% C.V.= 20.45). **Conclusiones.** Este estudio contribuye a determinar una primera estimación directa de la densidad, para esta especie en una región de México. La importancia de este estudio es la precisión con la que se estima la población de nutrias, lo que permite tener una información más robusta para tomar decisiones y acciones para el manejo y conservación de la especie.

Palabras clave: conservación, distribución, ecosistemas, fauna, Golfo de México.

ABSTRACT

Background. In Mexico, the neotropical otter (*Lontra longicaudis annectens*, Olfers, 1818) is widely distributed, and its populations are threatened. **Objectives**. To estimate the density and abundance of neotropical river otters in the Alvarado Lagoon System (SLA), Veracruz. **Methods.** The SLA has an extension of 51,960.52 hectares and is made up of coastal lagoons, interior lagoons, and rivers. We employed the distance sampling technique with systematic transects to estimate the distribution, density, and abundance of the Neotropical otter in the SLA. **Results.** A total of 25 independent otter sightings were obtained; the Limón, Blanco, and Culebrilla rivers are the areas with the most significant sights. An abundance of 934 (% C.V.= 20.45) otters was estimated for the entire SLA, with a density of 0.179 organisms/km2 (% C.V.= 20.45). **Conclusions.** This study contributes to determining the first direct estimation of density for this species in a region of Mexico. The importance of this study is the precision with which the otter population was estimated, which allows for more robust information to make decisions and to take actions for the management and conservation of the species.

Keywords: conservation, distribution, ecosystems, fauna, Gulf of Mexico.

INTRODUCCIÓN

La nutria de río (*Lontra longicaudis annectens*, Olfers, 1818) tiene una amplia distribución en el Continente Americano, desde el noroeste de México hasta el norte de Argentina (Larivière, 1999, Gallo-Reynoso, 2013, Rheingantz *et al*., 2017). En México se reporta una distribución amplia y continua (Gallo-Reynoso, 1997; Aranda, 2000; Gallo-Reynoso, 2013). Se encuentra en ríos grandes y medianos, en planicies costeras y arroyos de montaña (Gallo-Reynoso, 1997; Villa & Cervantes, 2003; Gallo-Reynoso, 2013), aunque soporta cambios en el ambiente e incluso puede ocupar zonas contiguas donde se realizan actividades humanas (Larivière, 1999; Mayagoitia-González *et al.,* 2013, Santiago-Plata *et al*., 2013). Su distribución varía en función del tipo de hábitat, disponibilidad de alimento, comportamiento y necesidades de la especie (Morrison *et al.,* 1998; Larivière, 1999).

Para el estado de Veracruz, se reportan nutrias neotropicales en los ríos Pescados y Actopan (Macías-Sánchez, 2003; Arellano-Nicolas *et al.,* 2012); también se han observado en el lago de Catemaco (González-Christen *et al*., 2013) y en la zona costera de Tuxpan (Grajales-García *et al*., 2019). Es muy difícil estimar el tamaño de sus poblaciones debido a que su comportamiento hace poco probable la observación de nutrias en su hábitat, lo que ha significado un obstáculo para estimaciones directas de la cantidad de especímenes. Por ello, se ha recurrido a realizar muestreos indirectos con base en excretas, huellas o madrigueras a partir de lo cual se han hecho estimaciones de su abundancia (Gallo-Reynoso, 1996; Gallo-Reynoso, 1997; Macías-Sánchez & Aranda, 1999; Sielfed & Castilla, 1999, Botello *et al*., 2006, Casariego-Modorell *et al*., 2006).

La abundancia de nutrias en México frecuentemente se ha estimado utilizando las metodologías propuestas por Gallo-Reynoso (1996) y por Macías-Sánchez (2003). Estas metodologías hacen una estimación de la abundancia relativa de las nutrias utilizando las excretas observadas para hacer el cálculo. De esta manera, se han hecho estimaciones de abundancia para el Río Yaqui, Sonora (0.34 nutrias/km; Gallo-Reynoso, 1996), La Vega Escondida, Tamaulipas (0.69 nutrias/km; Mayagoitia-González *et al*., 2013), zona costera de Tuxpan, Veracruz (0.94 nutrias/km - 0.14 nutrias/km; Grajales-García *et al*., 2019), Lago de Catemaco, Veracruz (0.97 nutrias/km - 0.49 nutrias/km; González-Christen *et al*., 2013), ríos Actopan y Los Pescados, Veracruz (3.10 nutrias/km - 1.2 nutrias/km; Macías-Sánchez, 2003), Reserva de la Biósfera Tehuacán-Cuicatlán, Oaxaca (0.689 nutrias/km; Duque-Dávila *et al*., 2013), Río Zimatán, Oaxaca (0.95 nutrias/km; Briones-Salas *et al.,* 2008), y Laguna de Términos, Campeche (0.86 ± 0.472 rastros/km; Santiago-Plata *et al*., 2013). La desventaja de estos cálculos es que no se puede saber el coeficiente de variación de las estimaciones y, por lo tanto, no se tiene certeza sobre el número de nutrias en los sitios estudiados.

Las poblaciones de nutrias han decrecido por la cacería, pérdida del hábitat y contaminación del agua (Gallo-Reynoso, 1986; Alho *et al*., 1988; Sierra & Vargas, 2002). Por ello, están catalogadas como "casi amenazada" por la Unión Internacional para la Conservación de la Naturaleza (UICN, 2021) y como amenazada por parte del gobierno de México de acuerdo con la NOM-059-2010 (SEMARNAT, 2010).

Los estudios de la nutria neotropical en el Estado de Veracruz son escasos y, cuando se han llevado a cabo, las estimaciones de abundancia han sido realizadas con métodos indirectos (Ruiz-Betancourt, 1992; Macías-Sánchez & Aranda, 1999; González-Christen, 2013; Grajales-García *et al*., 2019). Por lo anterior, el presente trabajo utiliza muestreos directos en un sistema fluvial navegable a partir de lo cual se pretende determinar la abundancia, densidad y distribución espacial de la nutria de río neotropical en el Sistema Lagunar de Alvarado, Veracruz.

MATERIALES Y MÉTODOS

Área de estudio

El Sistema Lagunar de Alvarado (SLA) es una planicie de inundación que abarca desde las coordenadas 18º 53'00" N; 95º34'00" O, hasta 18º25'00" N; 96º08'00" O, con una extensión aproximada de 51,960.52 hectáreas (INEGI, 2010). Se ubica en el centro del estado de Veracruz y comprende lagunas costeras salobres tales como la laguna de Alvarado, Buen País y Camaronera; lagunas interiores como la de Tlalixcoyan, Popuyeca y Las Pintas, y varios ríos como el Papaloapan, Acula, Blanco y Limón (Portilla-Ochoa, 2003). El SLA, fue declarado sitio Ramsar en 2004 (Ramsar, 2022).

Dentro del área existen comunidades vegetales representativas de la planicie costera como dunas costeras, espartales (*Spartina spartinae*), popales, diferentes tipos de palmas, encinos (*Quercus oleoides*, Schltdl & Cham, 1830), selva mediana subperennifolia con vegetación secundaria, selva baja caducifolia, acahuales, pastizales (naturales, inducidos y cultivados), vegetación acuática y subacuática, además de la comunidad de manglar (Portilla-Ochoa, 2003).

El Sistema Lagunar de Alvarado es una comunidad muy diversa, se han descrito 38 especies de moluscos, 26 familias de crustáceos, 44 especies de peces, por lo menos cinco especies de anfibios, 24 especies de reptiles, 346 especies de aves y al menos 15 especies de mamíferos (Portilla-Ochoa, 2003).

Navegaciones

Los muestreos se realizaron en una lancha de fibra de vidrio de 7.6 m de eslora y una manga de 1 1/2 m, impulsada por un motor fuera de borda de 75 hp. El diseño de muestreo se hizo con base en la metodología para diseño de transectos de sistemas complejos propuesto por Thomas *et al.* (2007) y Buckland *et al.* (2008). Los muestreos se realizaron por medio de transectos lineales sistemáticos, cubriendo casi la totalidad del área de estudio. Los cálculos de la densidad y la abundancia se elaboraron con el programa Distance v.5 (Thomas *et al*., 2005).

Los transectos se realizaron a una velocidad aproximada de 10 km / h; se mantuvo la observación continua hacia ambos lados de la embarcación para localizar a los organismos, utilizando un telémetro de caza Bushnell con brújula (precisión ± 0.9 m). Durante la navegación, se anotaron los siguientes datos: fecha, hora de inicio y final del transecto, hora de avistamiento, posición geográfica inicial, posición geográfica final (obtenida de un geoposicionador satelital o GPS (Garmin modelo GPS map 76CSx, precisión ± 3 m), condiciones climatológicas, luminosidad, visibilidad en km y observaciones generales.

Se registró el número de individuos por avistamiento, los movimientos de los animales con respecto a la embarcación, la zona donde se observaron, la distancia a la embarcación y el ángulo (con respecto a la proa de la embarcación) en el cual fueron avistados.

Análisis de los datos

Distribución

Se integraron en un sistema de información geográfica los avistamientos de las nutrias, lo que facilita su localización, la identificación de las áreas donde realizan actividades y distancia recorrida por cada una de las unidades de muestreo (White & Garrott, 1990). Una vez obtenidos los datos, se procesaron las coordenadas y se registraron sobre un mapa digitalizado del área.

Densidad

La determinación de la densidad se calculó a través de la siguiente fórmula:

$D = N / A$

Donde:

N = número de organismos

A = tamaño del área

Este cálculo se realizó con el programa Distance v.5 (Thomas *et al*., 2005).

Abundancia

La abundancia se estimó utilizando la técnica de muestreo a distancia (Buckland *et al*., 2008). Mediante esta metodología se logró una estimación de la abundancia basada en avistamientos durante el transecto, a partir de lo cual se obtuvo un coeficiente de variación (el error estándar dividido por la media) de las estimaciones. Los parámetros básicos de la estimación fueron:

$N = (D) (A)$

Donde:

N = tamaño total de la población

D = densidad (número de animales por unidad de área)

A = tamaño del área de estudio

Con base en lo anterior se obtuvieron distancias perpendiculares a partir de distancias radiales, con sus respectivos ángulos. El modelo que se utilizó para la estimación de la densidad y de la abundancia considera que, a mayor distancia son detectados un menor número de animales. Así, el aumento en la precisión de le estimación del tamaño población pasa por incluir el número de animales que no se observaron durante el transecto mediante una función de detección (Begon, 1989; Buckland *et al*., 2008). El modelo que se seleccionó para el análisis fue el "Half-Normal/Polynomial" debido a que fue el que mejor se ajustó a nuestros datos. Con esta metodología se pudo calcular un coeficiente de variación para la población estudiada (Buckland *et al*., 2008). Para la estimación de densidad y abundancia el modelo requiere que, a distancias muy cercanas, la función de detección sea igual a uno (Buckland *et al*., 2008).

RESULTADOS

Se realizaron un total de 45 navegaciones en el SLA, lo que representa un esfuerzo total de navegación de 332.36 horas con un promedio de 7.38 h. por navegación. Lo anterior representa un total de 959 transectos lineales lo que representó 1,027.63 km recorridos, equivalentes al 92% del área de estudio.

En total se lograron observar 25 nutrias, siendo en el río Limón donde se observó el mayor número de organismos con un total de siete (28%), seguidos por los ríos Blanco y el río Culebrillas, cada uno con tres (12%); siguen en orden de importancia los ríos Martinela, Acula, Papaloapan y las lagunas de Alvarado y Pupuyeca donde se avistaron dos animales (8%) en cada uno; finalmente en las lagunas de Tlalixcoyan y Naranjos se observó un sólo un animal (4%) (Fig. 1).

La abundancia total de organismos, basada en el muestreo a distancia, fue estimada en 934 nutrias (% C.V.=20.45), lo que representa una densidad de 0.179 individuos / km^2 (%C.V.=20.45) (Tabla 1).

DISCUSIÓN

En Veracruz, las nutrias han sido observadas en el centro del estado en los ríos Pescados y Actopan (Macías-Sánchez, 2003), así como en el lago de Catemaco (Ruiz-Betancourt, 1992) y en el SLA (Silva-López, 2009). En este estudio se observó que estos animales tienen una distribución desigual en el SLA, percibiéndose con mayor frecuencia en los ríos Limón, Blanco y Culebrillas. Hall (1981) documentó un espécimen de nutria en el río Blanco, mientras que Gallo-Reynoso (1986) reportó pieles de éstas provenientes del río Limón. En este estudio, también se observaron en lagunas interiores como las de Popuyeca y Tlalixcoyan, coincidiendo con lo reportado por Silva-López (2009), quien mencionó que la nutria neotropical ocupa una amplia extensión en el SLA. Las observaciones de nutrias tuvieron lugar en sitios que se caracterizaron por estar alejados de los poblados y en un mejor estado de conservación con respecto a los espacios cercanos a las comunidades rurales. Los sitios donde se observaron con mayor frecuencia, podrían ser lugares con una mayor disponibilidad de alimento (Mason & Mcdonald, 1990) además de que podrían ser áreas con una amplia disponibilidad de refugios para la especie.

Para la zona centro del estado de Veracruz, Macías-Sánchez (2003) estimó una alta densidad de nutrias en los ríos Actopan (3.1 nutrias/km) y Pescados (1.2 nutrias/km). No obstante, estos datos no son comparables con el presente estudio debido a que Macías-Sánchez (2003) utilizó muestreos indirectos, recorriendo a pie 20 km a lo largo de la ribera de cada río, por lo que no se tiene certeza respecto a si existe una sobreestimación o subestimación de las poblaciones de nutria para esos ríos. Lo mismo sucede con las estimaciones elaboradas por Grajales-García *et al.* (2019) (0.94 nutrias/km - 0.14 nutrias/km) para la zona costera de Tuxpan y por González-Christen *et al.* (2013) para el Lago de Catemaco (0.97 nutrias/km - 0.49 nutrias/km) quienes también utilizaron métodos indirectos para estimar la abundancia de nutrias, por lo que la incertidumbre de las estimaciones no fue cuantificada y, por tanto, la precisión de la estimación es desconocida.

En la vertiente del Pacífico, Casariego-Madorrell *et al.* (2006), estimaron una abundancia de 495 nutrias, utilizando métodos indirectos, en un sector de tres ríos en el estado de Oaxaca, que corresponden aproximadamente a 60,000 hectáreas, similar a nuestra área de estudio SLA. De igual forma, las diferencias metodológicas (estimaciones a partir de observación directa vs. observaciones indirectas) impiden hacer una comparación válida entre las áreas.

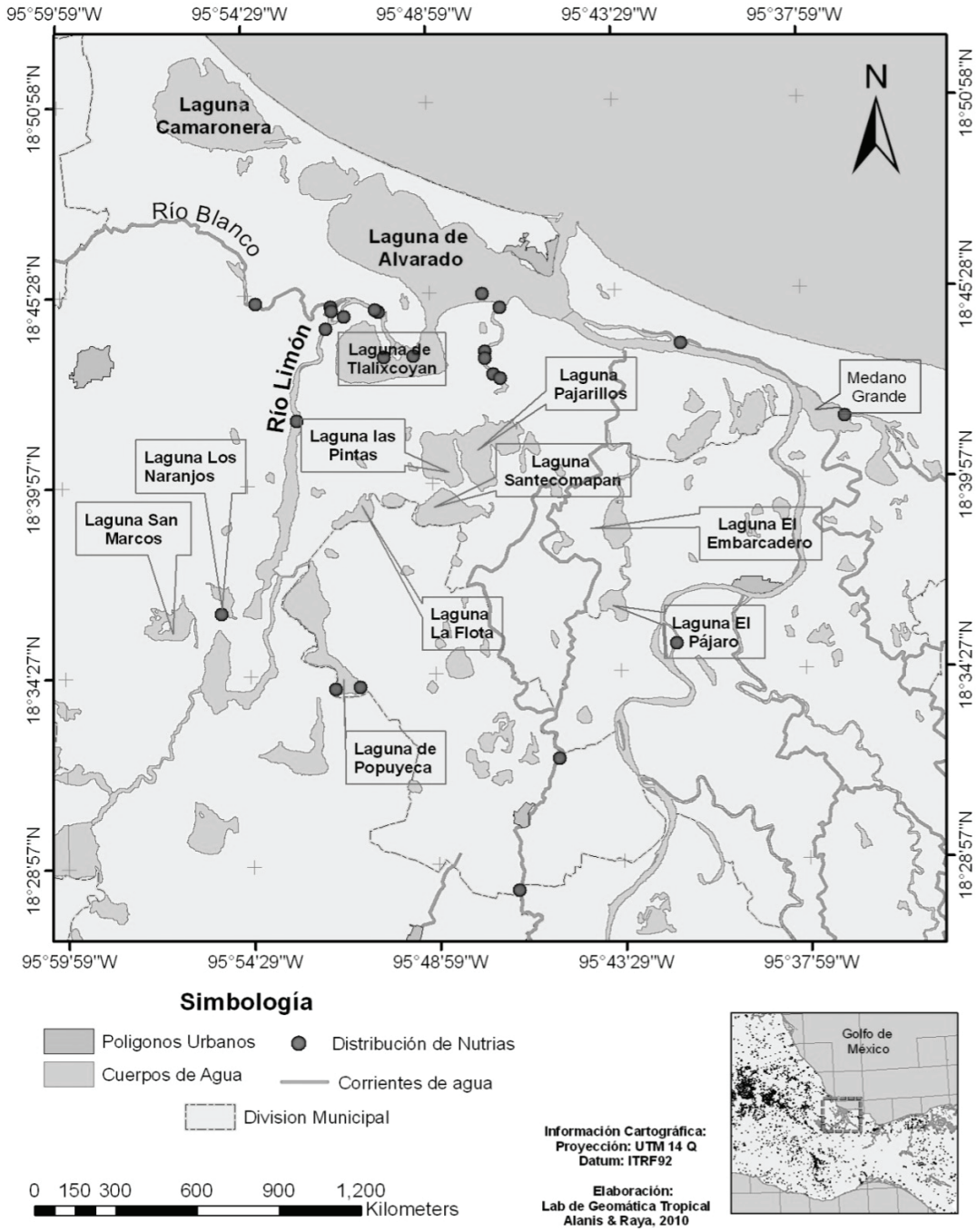

Figura 1. Ubicación geográfica de las 25 nutrias avistadas en el SLA.

Tabla 1. Resumen estadístico de la densidad y abundancia de nutrias en el SLA.

Descripción	Unidades	% C.V.	G.L.	95%	Intervalo de Confianza
Densidad	0.1797	20.45	514.68	0.1207	0.2674
Abundancia	934.00	20.45	514.68	627.00	1,390.0

Nota: C.V. = Coeficiente de variación medido en porcentaje; G.L. = Grados de libertad.

La técnica de muestreo a distancia (Buckland *et al*., 2008) resultó de gran utilidad para obtener un estimado de la densidad de las nutrias, así como su abundancia en el SLA. Particularmente, porque permitió hacer estimaciones más precisas y porque posibilitó saber la precisión de los datos. Esta afirmación se apoya en los valores del coeficiente de variación, de la densidad y de la abundancia, que al ser menores al 30% representa un relativamente alto margen de confiabilidad de la información.

Las nutrias son consideradas especies bioindicadoras (Cruz-García *et al*., 2017), así que los estudios poblacionales de éstas son de suma importancia para conocer el estado de conservación del ecosistema en el que habitan. El SLA es vulnerable pues recibe descargas de desechos industriales, comerciales y de los centros urbanos a lo largo del sistema, lo cual, probablemente, afecta a las poblaciones de la nutria neotropical (Gallo-Reynoso, 1986; Alho *et al*., 1988; Sierra & Vargas, 2002).

La importancia de este estudio es la precisión con la que se pudo estimar la población de nutrias, lo que permite tener una base sólida para tomar decisiones para el manejo y conservación de la especie en el Sistema Lagunar de Alvarado. Es recomendable seguir estudiando las poblaciones de las nutrias con métodos directos para hacer una prospección del estado de sus poblaciones y determinar un adecuado plan para su conservación y la de los hábitats donde se encuentran.

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Effect of diet and temperature on the culture of *Alona guttata* (Sars, 1862) (Cladocera: Chydoridae) under laboratory conditions

Efecto de la dieta y temperatura en el cultivo de *Alona guttata* (Sars, 1862) (Cladocera: Chydoridae) en condiciones de laboratorio

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ABSTRACT

Background. Chydoridae is the most diverse family of cladocerans but information about their biology or life cycles is still limited, perhaps because of the hard task that their taxonomy involves or that culture in optimal conditions are not described for several species. **Goals.** This study examined the effects of culture conditions (algal concentration, algal species, and temperature) on the demography of *Alona guttata* (Sars, 1862) to obtained their maximal growth rates. **Methods.** Life table analysis with the chydorid *A. guttata* were performed as follows: five females per cohort (six replicas) were fed on either *Chlorella vulgaris* (Beijerinck, 1890) or *Nannochloropsis oculata* (Hibberd, 1981) at 0.5×10^6 or 2×10^6 cells/mL, reared at 20ºC or 25ºC and photoperiod 16:8 h (light: dark). Media was supplemented with an artificial substrate. Then, daily fertility and survival were assessed to estimate the demographic parameters: average lifespan (ALS), life expectancy at birth (LEB), generation time (GT), gross reproductive rate (GRR), longevity, net reproductive rate (NRR), and the intrinsic rate of population increase (r). **Results.** Significant effects were observed due to the three factors tested and their interactions. Increasing algal concentrations of either *C. vulgaris* or *N. oculata* promoted higher fertility and longer survival. Lower temperature extended the ALS and LEB when organisms were fed on the highest algal concentration. The highest r values were observed when *Alona* was fed on *N. oculata* at 2×10^6 cells/mL. **Conclusions.** The best culture conditions, in terms of the population growth rates of *A. guttata*, were provided by *N. oculata* at 2×10^6 cells/mL at 25°C with the supplementation of artificial substrate.

Keywords. Anomopoda, demographic parameters, fertility, life table analysis, survival

RESUMEN

Antecedentes. La familia Chydoridae es la más diversa de los cladóceros pero la información acerca de su biología o sus ciclos de vida es aún limitada, posiblemente por la dificultad que representa su taxonomía o porque las condiciones óptimas de cultivo no han sido descritas para varias especies. **Objetivos**. Este estudio examina los efectos de las condiciones de cultivo (concentración de algas, especie de alga, y temperatura) en la demografía de *Alona guttata* (Sars, 1862) para obtener sus mayores tasas de crecimiento. **Métodos**. Se realizaron análisis de tablas de vida con el quidórido *A. guttata* de la manera siguiente: cinco hembras por cohorte (seis réplicas) fueron alimentadas con *Chlorella vulgaris* (Beijerinck, 1890) o *Nannochloropsis oculata* (Hibberd, 1981), a una concentración de 0.5×10^6 o 2×10^6 *células/*mL, con una temperatura de 20°C o 25°C, y un fotoperiodo de 16:8 h (luz:oscuridad). Al medio de cultivo se adicionó un sustrato artificial. Posteriormente, la fertilidad y supervivencia diarias fueron evaluadas para estimar los parámetros poblacionales: promedio de vida (ALS), expectativa de vida al nacimiento (LEB), tiempo generacional (GT), tasa reproductiva bruta (GRR), longevidad, tasa reproductiva neta (NRR), y la tasa intrínseca de crecimiento poblacional (r). **Resultados.** Se observaron efectos significativos debidos a los tres factores evaluados y sus interacciones. El aumento de la concentración de las algas *C. vulgaris* o *N. oculata* incrementó la fertilidad y supervivencia. La menor temperatura extendió la ALS y la LEB cuando los organismos fueron alimentados con la concentración más alta de algas. Las r más altas se observaron cuando *Alona* fue alimentada con *N. oculata* a 2×10^6 células/mL. **Conclusiones**. Las mejores condiciones de cultivo, en términos de la tasa de crecimiento poblacional de *A. guttata*, fueron provistas por *N. oculata* a 2×10^6 cells/mL, a 25°C, con la adición del sustrato artificial.

Palabras clave: análisis de tabla de vida, Anomopoda, fertilidad, parámetros demográficos, supervivencia

INTRODUCTION

Chydoridae is the most diverse family within cladocerans, but information regarding their culture and biology is still scarce in comparison to other families like Daphniidae and Moinidae. In natural environments, fish larvae can feed on little size species like chydorids, which are preferably consumed over other large cladocerans, likely because of their ease to capture (Nunn *et al*., 2012). Chydorids, which inhabit littoral zones of water bodies, are generally associated to aquatic plants, periphyton, or sediment (Dole-Olivier *et al*., 2000; Masclaux *et al*., 2012). Thus, different media have been developed to culture chydorids in laboratory conditions, feeding these organisms on algae, bacteria, detritus, yeast, or combinations of these sources of organic matter (Martínez-Jerónimo & Gomez-Díaz, 2011).

Castilho *et al*. (2015) stated that the study of the life cycle of cladocerans offers a better understanding of their biology, and at higher hierarchy level provide information about population dynamics, their interactions with the surroundings, and their role within food webs and secondary production.

Alona guttata (Sars, 1862) is a cosmopolitan species, with records in several places around the world (Sinev & Siva-Briano, 2012; Sousa *et al*., 2014). Cortez-Silva *et al*. (2022) described the life history of *A. guttata* fed on *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen & O.M.Skulberg 1987, and the strategies that this species might follow to rapidly colonize temporal and transitory ponds. Alvarado-Suárez (2017) implemented this chydorid to nourish the goodeid fish *Poeciliopsis infans* (Woolman, 1894) and concluded that *A. guttata* is of high nutritional value but their usage is limited due to poor biomass production. Therefore, this study was aimed to assess the effect of the food source, algal concentration, and temperature on the demography of *A. guttata* through life table analysis and find the best culture conditions in terms of the population growth rates.

MATERIAL AND METHODS

Maintenance of chydorids. The strain of *A. guttata* has been maintained in the Laboratory of Aquatic Toxicology of the Universidad Autónoma de Aguascalientes (UAA) and was cultured with the following conditions: moderately hard reconstituted water (MHRW) (USEPA, 2002), photoperiod 16h light and 8h dark, fed once a day *ad libitum* with either the green algae *Chlorella vulgaris* (Beijerinck, 1890) or *Nannochloropsis oculata* (Hibberd, 1981), and temperature at either 20°C or 25°C ± 2ºC. Freshwater media was supplemented with 660 mg/L of artificial substrate, which consisted of 70% silica sand, 25% kaolin, and 5% dried and ground cattle manure. The complete mixture of the substrate was sterilized by autoclave at 15 psi for 20 min (Martínez-Jerónimo & Gómez-Díaz, 2011).

Algal biomass as food source for chydorids. *Chlorella vulgaris* and *N. oculata* were grown in Bold's Basal medium (Nichols, 1973) under aseptic conditions at 25ºC. Continuous illumination was provided by day-light fluorescent lamps (approximately 5000 lux). Algal biomass was collected during the exponential phase of population growth and separated from the culture medium by centrifugation at 5000 rpm for 5 min. Then, culture media for chydorids were supplemented with either *C. vulgaris* or *N. oculata* at low (0.5×10^6 cells/mL) and high densities (2×10^6 cells/mL).

Effect of diet and temperature on *Alona guttata*. Cohorts of five neonates (less than 24-h old) of *A. guttata* were placed separately in a 24-well polystyrene microplate; then, every well was supplemented with the corresponding algal suspension in MHRW and adjusted to a final volume of 2 mL. Temperature was controlled in bioclimatic chambers at either 20ºC or 25ºC ± 2ºC with a photoperiod of 16 h light and 8 h dark. To avoid desiccation, 200 µL of distilled water were added every other day to each well. Complete renewal of the culture media was performed once a week. Fertility and survival data were daily recorded until all individuals in the cohort had died. The neonates and dead organisms were removed and counted daily. All treatments consisted of six replicas (n = 6).

Life table analysis. Data of survival (l_x) and fertility (m_x) were used to determine: average lifespan (ALS) (days), maximum longevity (days), life expectancy at birth (LEB) (days), generation time (GT) (days), gross reproductive rate (GRR) (neonates/female), net reproductive rate (NRR) (neonates/female), and intrinsic rate of population increase (r) (1/days) (Pianka, 1978; Krebs, 1985):

Average lifespan $\mathrm{ALS} = \sum_{0}^{\infty} l_x$

Life expectancy at birth $\mathrm{LEB} = \frac{T_x}{l_x}$

Gross reproductive rate $\mathrm{GRR} = \sum_{0}^{\infty} m_x$

Net reproduction rate $\mathrm{NRR} = = \sum_{0}^{\infty} l_x m_x$

Generation time $\mathrm{GT} = \frac{\sum l_x m_x x}{NRR}$

The intrinsic rate of population increase (IRPI) was computed through iteration with the Euler-Lotka equation: $\sum_{x=0}^{n} e^{-rx} l_x m_x = 1$

Statistical Analysis. Results from the life table experiments were analyzed through three-way analysis of variance (ANOVA). Significant differences were established through Bonferroni´s multiple comparison test. Statistical analysis were performed in R-Studio v.1.0.143 and the packages a*gricolae* v.1.2-4 (De Mendiburu, 2016) and *ggplot2* v.3.2.1 (Wickham, 2016).

RESULTS

Figure 1 shows the survival curves for *A. guttata* raised at 20ºC or 25ºC and fed on 0.5×10^6 or 2×10^6 cells/mL of either *C. vulgaris* or *N. oculata*. The longer survival rates were recorded when organisms were reared at 20°C in medium supplemented with *N. oculata*. Chydorids fed on *N. oculata* (2×10^6 cells/mL) and grown at 20°C delayed until the day 40 of the test to exhibit the first females to die, while at 25°C dead organisms appeared since the day 30. Temperature alone seemed to have no significant effect on the survival of *A. guttata*, but it was influenced by the interaction of temperature×algal concentration and temperature×algal species.

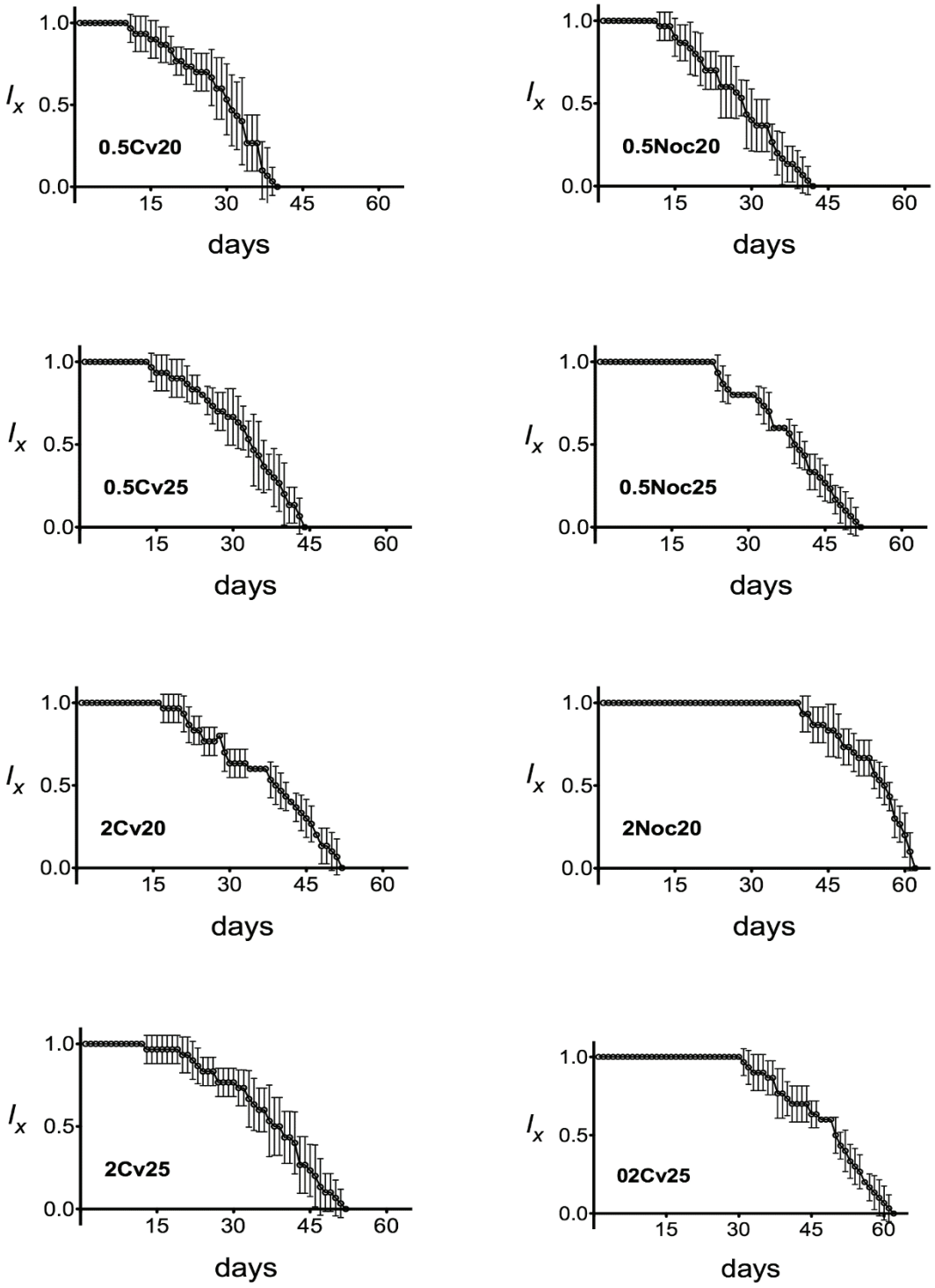

Figure 1. Survival (lx) of *Alona guttata* (Sars, 1862) fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981).

Error bars represent the confidence interval (P<0.05). All experiments were carried out with six replicates (n = 6). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius.

Figure 2 shows the daily fertility of the eight treatments. *Alona guttata* produced offspring during almost their entire life cycle, although the number of neonates per reproductive episode was in general low. Maximum values of longevity were recorded for every treatment, with the highest values (60.33 ± 0.8164 d) when organisms were fed on *N. oculata* at 2×10^6 cells/mL (Fig. 2). The highest longevity was recorded for the group fed on *C. vulgaris* (49.17 ± 1.8348 d). In general, the concentration of 0.5×10^6 cells/mL probed to be insufficient to promote longer longevity in *Alona*. These results were influenced mainly by the algal species and the algal concentration but were not significantly affected by temperature (Table 1).

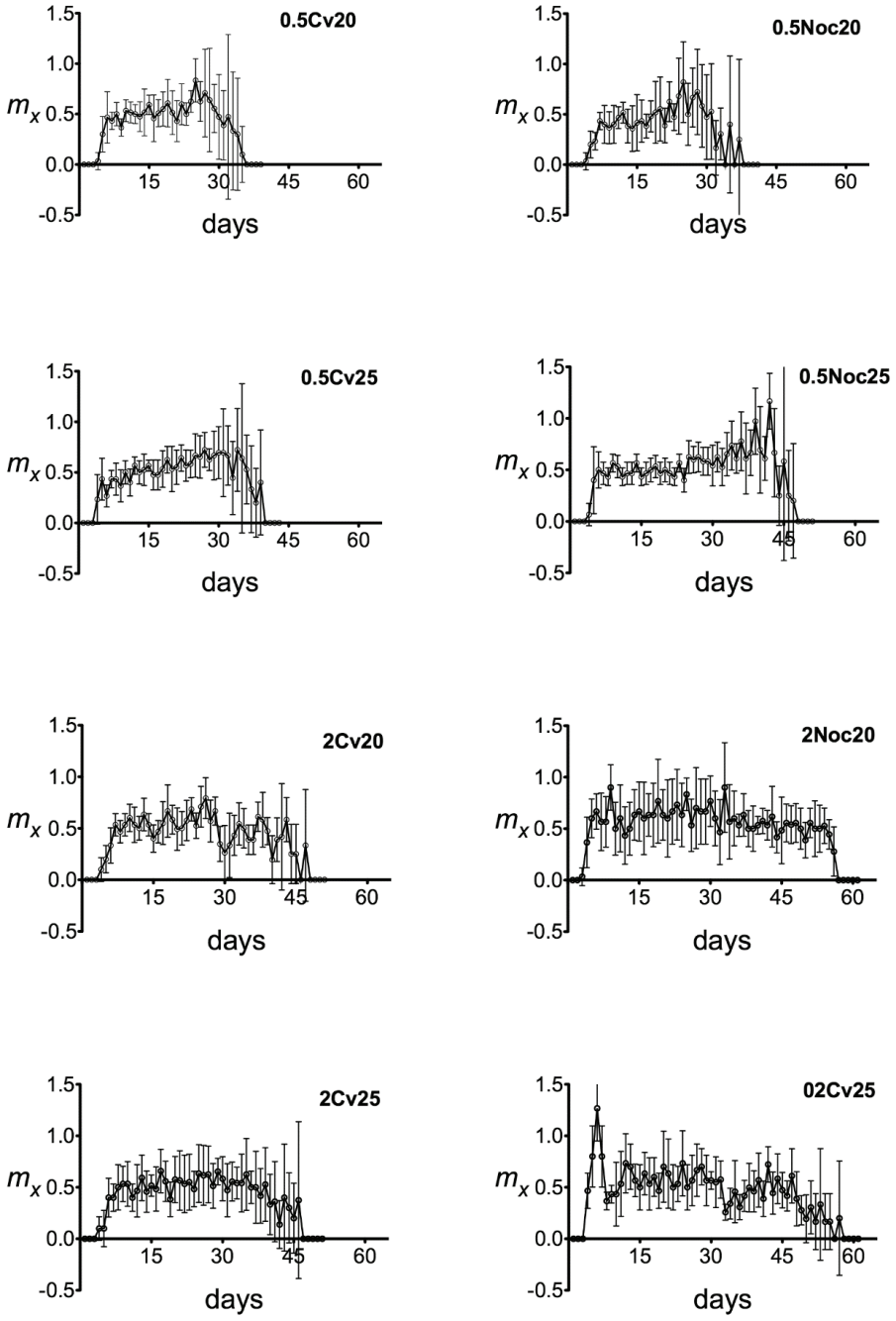

Figure 2. Fertility (mx) of *Alona guttata* (Sars, 1862) fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981).

Error bars represent the confidence interval ($P<0.05$). All experiments were carried out with six replicates (n = 6). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius.

Table 1. Analysis of variance performed on the demographic responses of *Alona guttata* fed on *Chlorella vulgaris* or *Nannochloropsis oculata*. ALS, average lifespan (days); longevity, maximum longevity recorded by replicate (days); LEB, life expectancy at birth (days); GT, generation time (days); GRR, gross reproductive rate (neonates/female); NRR, net reproductive rate (neonates/female); IRPI, intrinsic rate of population increase, 1/days.

	Factor or interaction	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
ALS	Algal species	1	585.20	585.20	86.207	< 0.001	***
	Algae concentration	1	2,192.40	2,192.40	322.966	< 0.001	***
	temperature	1	7.70	7.70	1.131	0.294	
	Algal species × algae concentration	1	588.00	588.00	86.619	< 0.001	***
	Algal species × temperature	1	9.40	9.40	1.379	0.247	
	Algae concentration × temperature	1	162.80	162.80	23.983	< 0.001	***
	Algal species × algae concentration × temperature	1	40.30	40.30	5.942	0.019	*
	Residuals	40	271.50	6.80			
Longevity	Algal species	1	574.10	574.10	123.903	< 0.001	***
	Algae concentration	1	2,352.00	2,352.00	507.626	< 0.001	***
	temperature	1	36.80	36.80	7.932	0.007	**
	Algal species × algae concentration	1	225.30	225.30	48.633	< 0.001	***
	Algal species × temperature	1	0.70	0.70	0.162	0.689	
	Algae concentration × temperature	1	161.30	161.30	34.82	< 0.001	***
	Algal species × algae concentration × temperature	1	0.30	0.30	0.072	0.79	
	Residuals	40	185.30	4.60			
LEB	Algal species	1	585.20	585.20	86.207	< 0.001	***
	Algae concentration	1	2,192.40	2,192.40	322.966	< 0.001	***
	temperature	1	7.70	7.70	1.131	0.294	
	Algal species × algae concentration	1	588.00	588.00	86.619	< 0.001	***
	Algal species × temperature	1	9.40	9.40	1.379	0.247	
	Algae concentration × temperature	1	162.80	162.80	23.983	< 0.001	***
	Algal species × algae concentration × temperature	1	40.30	40.30	5.942	0.019	*
	Residuals	40	271.50	6.80			
GT	Algal species	1	38.00	38.00	23.624	< 0.001	***
	Algae concentration	1	287.00	287.00	178.412	< 0.001	***
	temperature	1	2.13	2.13	1.327	0.256	
	Algal species × algae concentration	1	22.12	22.12	13.754	< 0.001	***
	Algal species × temperature	1	8.80	8.80	5.469	0.024	*
	Algae concentration × temperature	1	44.93	44.93	27.929	< 0.001	***
	Algal species × algae concentration × temperature	1	15.82	15.82	9.832	0.003	**
	Residuals	40	64.34	1.61			
GRR	Algal species	1	0.50	0.50	0.12	0.731	
	Algae concentration	1	148.50	148.50	35.662	< 0.001	***
	temperature	1	318.10	318.10	76.38	< 0.001	***
	Algal species × algae concentration	1	1.90	1.90	0.467	0.498	
	Algal species × temperature	1	161.10	161.10	38.68	< 0.001	***
	Algae concentration × temperature	1	11.30	11.30	2.724	0.107	
	Algal species × algae concentration × temperature	1	104.30	104.30	25.052	< 0.001	***
	Residuals	40	166.60	4.20			

	Factor or interaction	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
NRR	Algal species	1	254.80	254.80	180.248	< 0.001	***
	Algae concentration	1	875.50	875.50	619.253	< 0.001	***
	temperature	1	1.30	1.30	0.896	0.349	
	Algal species × algae concentration	1	404.80	404.80	286.343	< 0.001	***
	Algal species × temperature	1	9.90	9.90	7.003	0.012	*
	Algae concentration × temperature	1	106.20	106.20	75.12	< 0.001	***
	Algal species × algae concentration × temperature	1	33.70	33.70	23.813	< 0.001	***
	Residuals	40	56.60	1.40			
IRPI	Algal species	1	0.00696	0.00696	48.194	< 0.001	***
	Algae concentration	1	0.01844	0.01844	127.632	< 0.001	***
	temperature	1	0.00109	0.00109	7.541	0.009	**
	Algal species × algae concentration	1	0.02120	0.02120	146.711	< 0.001	***
	Algal species × temperature	1	0.00108	0.00108	7.452	0.009	**
	Algae concentration × temperature	1	0.00009	0.00009	0.654	0.424	
	Algal species × algae concentration × temperature	1	0.00039	0.00039	2.71	0.108	
	Residuals	40	0.00578	0.00014			

From the life table analysis, *Alona guttata* showed the longest ALS values when feeding on algal densities of 2×10^6 cells/mL (Fig. 3). Significant interactions were recorded for algal species×algal concentration ($P<0.01$), algal concentration×temperature ($P<0.01$), algae species×algae concentration×temperature ($P<0.05$) (Table 1). The ALS presented values from 26.97 (SD = 3.2752) to 53 d (SD = 1.8199), with *N. oculata* as the best food source for this chydorid when it was supplemented at 2×10^6 cells/mL.

The life expectancy at birth (LEB) was mainly affected by the algal species and algae concentration; thus, obtaining the highest LEB value (52.50 d) with *N. oculata* (2×10^6 cells/mL) at 20ºC (Fig. 3). Significant interactions were registered for algae concentration×algal species, algae concentration×temperature, and for the three factors tested (Table 1).

The highest GT (27.28 ± 0.8018 days) was recorded when chydorids fed on *N. oculata* (2×10^6 cells/mL) and reared at 20ºC (Fig. 3). The lowest GTs were observed with the organisms fed on either *C. vulgaris* or *N. oculata* at 0.5×10^6 cells/mL. The two temperatures tested caused no significant differences in the GT of *A. guttata*, although their interactions significantly affected the GT (Table 1).

GRR was influenced by the algal concentration of both algal species (Table 1); then, organisms fed on 0.5×10^6 cells/mL produced fewer neonates than the organisms fed on 2×10^6 cells/mL. Thereafter, *N. oculata* at the highest algal density promoted *A. guttata* to have more neonates during their whole life cycle (Fig. 3).

The NRR registered the highest value with the females fed on *N. oculata* at 2×10^6 cells/mL (28.86 ± 0.8547 neonates/female), while those organisms fed on *C. vulgaris* produced fewer neonates, even when *C. vulgaris* was supplemented at 2×10^6 cells/mL (Fig. 3).

Finally, the treatment that promoted the higher rates of population increase was *N. oculata* at 2×10^6 cells/mL and temperature at 25°C ($r = 0.2656 \pm 0.0163$). The fed on *C. vulgaris* did not reach the same population growth rates in comparison to the groups fed on *N. oculata*. *Alona guttata* fed on *C. vulgaris* exhibited similar growth rates than those females fed on *N. oculata* at 0.5×10^6 cells/mL (Fig. 3).

DISCUSSION

Although there are several reports on chydorid species, information on their life cycle or reproductive biology is still scarce. Therefore, the main contributions of this study are: a) the insights on *A. guttata* asexual reproduction since neither males nor ephipia were detected while carrying out the different experiments during this research; and b) the culture conditions that promoted the highest rates of population increase to obtain higher number of individuals for further usage, as food source for early life stages of fish (Alvarado-Suárez, 2017) or as test organisms in environmental toxicology (Garza-León *et al*., 2017; Osorio-Treviño *et al*., 2019).

The maximum survival of *A. guttata* was comprised within values reported for other chydorids (Table 2). The longest survival within the family Chydoridae were reported for organisms grown at 5°C, reaching values of about 100 d. In the subfamily Aloninae survival has registered values of up to 90 d. For instance, Cortez-Silva *et al*. (2022) reported that longevity of *A. guttata* reached 37 d as maximum (30.9 d average) when chydorids were fed on *R. subcapitata*, but we found that this species can survive longer when feeding on either *C. vulgaris* or *N. oculata*. It is worth pointing out that food source and temperature influenced longevity of organisms and several publications reported no more than one food source or temperature. Although several results showed long survival of chydorids, some others might be explored to improve the survival of organisms.

The NRR in chydorids can take values from 6 to 135 neonates per female, with the highest reproduction rates in the genus *Eurycercus* (Table 2). In *A. guttata* the NRR was influenced by the algae concentration and the algal species, reaching the highest values at 2×10^6 cells/mL with *N. oculata* as food source. On this regard, Muro-Cruz *et al*.

(2002) found no significant differences in the NRR of *Coronatella rectangula* (Sars, 1861) (formerly *Alona rectangula*) when feeding on *C. vulgaris* at 0.5 and 2×10^6 cells/mL (NRR = 14.72 ± 0.62 and 13.22 ± 0.77 neonates/female, respectively); however, *A. guttata* showed significant differences when fed on increasing algal concentrations, reaching higher reproductive rates, GRR and NRR, when organisms were fed on *C. vulgaris* or *N. oculata* at 2×10^6 cells/mL, which improved the reproductive performance of *A. guttata*.

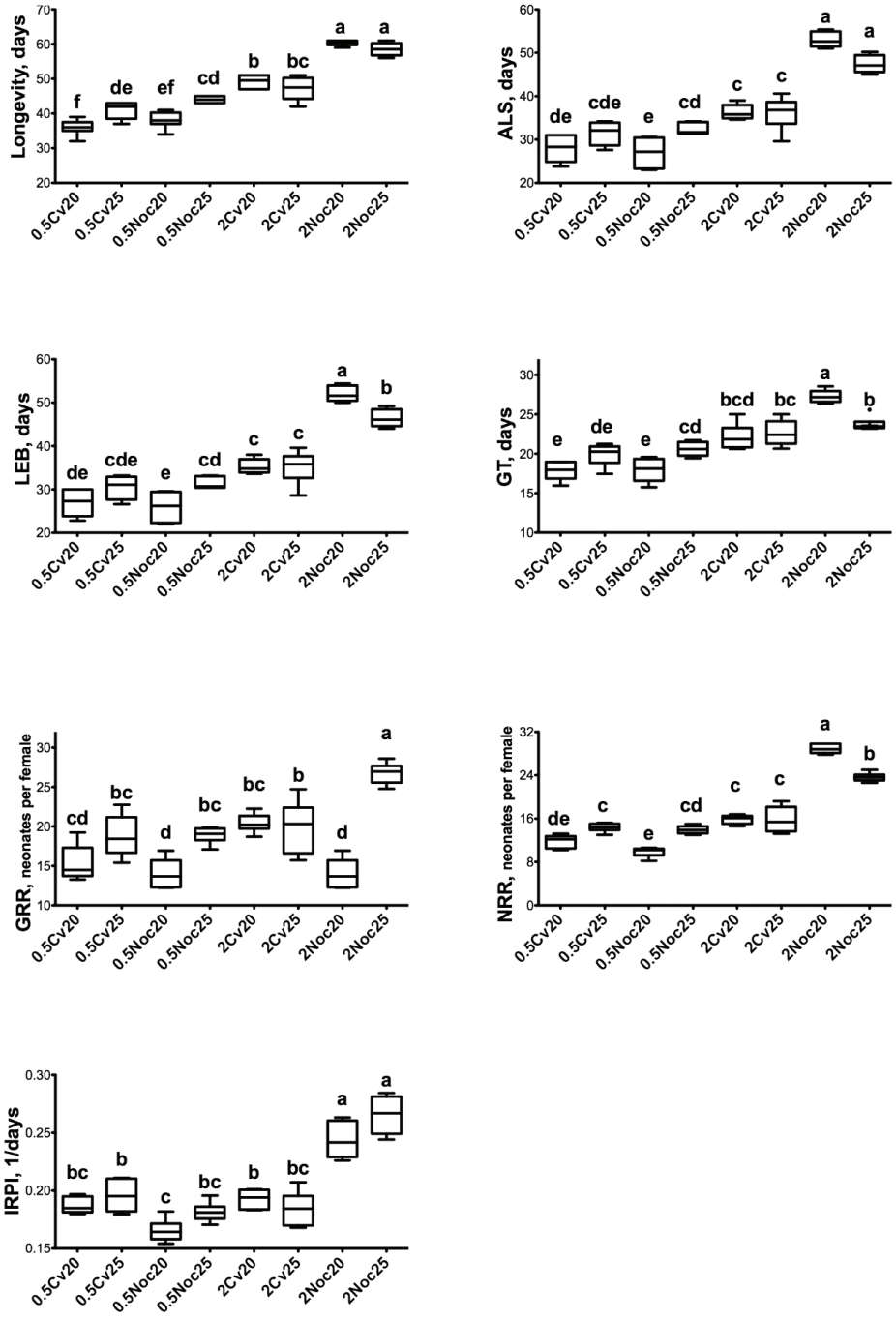

Figure 3. Demographic responses of *Alona guttata* fed on *Chlorella vulgaris* (Beijerinck, 1890) and *Nannochloropsis oculata* (Hibberd, 1981). ALS = average life span, GT = generation time, LEB = life expectancy at birth, GRR = gross reproductive rate, NRR = net reproductive rate, and IRPI = rate of population increase. All experiments were carried out with six replicates (n = 6). 0.5 = 0.5×10^6 cells/mL; 2 = 2×10^6 cells/mL; Cv = *C. vulgaris*; Noc = *N. oculata*; 20 and 25 = temperature in Celsius. Significant differences were established by factorial ANOVA and multiple comparison test of Bonferroni ($P<0.05$)

Table 2. Comparison of the life cycle and demographic responses of chydorids from this study and reported in the literature. GT, generation time (days); GRR, gross reproductive rate (neonates/female); NRR, net reproductive rate (neonates/female); IRPI, intrinsic rate of population increase; T, temperature

Species	T (°C)	Survival (d)	GT	GRR	NRR	IRPI	Reference
Acroperus harpae (Baird, 1834)	5 – 25	9 – 119	5.3 – 70.4				Bottrell, 1975
Alona affinis (Leydig, 1860)	5 – 20	37 – 144	16.9 – 72.9				Bottrell, 1975
A. guttata (Sars, 1862)	22	37					Cortez-Silva *et al.*, 2002
	20 – 25						This study
A. iheringula (Sinev & Kotov, 2004)	25	54	5.0				Silva *et al.*, 2014
Alonella excisa (Fischer, 1854)		73					Sharma & Sharma, 1998
Chydorus pubescens (Sars, 1901)	23.6	31	4.3				Santos-Wisniewski *et al.*, 2006
C. sphaericus (Müller, 1776)	5 – 20	23 - 96	8.9 – 38.5				Bottrell, 1975
	15 – 25		10.3 – 24.9		19.04 – 34.96	0.143 – 0.268	Keen, 1967 (cited by Hann 1985)
Coronatella rectangula (Sars, 1862)	25	26.92 – 30.82	10.2 – 10.6	18.0 – 18.8	19 – 27.12	0.25 – 0.26	Muro-Cruz *et al.*, 2002
	23.6	28.4	4.2				Viti *et al.*, 2013
Disparalona rostrata (Koch, 1841)	10 – 19	30 – 80	18.8 – 35.5				Robertson, 1988
Euryalona orientalis (Daday, 1898)	28-30	24					Venkataraman, 1990
Eurycercus lamellatus (Müller, 1776)	5 – 20	42 – 162	19.1 – 100.5				Bottrell, 1975
E. longirostris (Hann, 1982)	16 – 24	16 – 19	16.9 – 28.3		10.45 – 13.25	0.083 – 0.153	Hann, 1985
E. vernalis (Hann, 1982)	16 – 24	16 – 17	16.5 – 32.5		8.53 – 10.12	0.071 – 0.130	Hann, 1985
	10 – 25	35.50 – 79.7	15.2 – 53.2		69 – 135	0.08 – 0.310	Lemke & Benke, 2004
Graptoleberis testudinaria (Fischer, 1851)	5 – 20	23 – 95	9.5 – 44.6				Bottrell, 1975
Karualona muelleri (Richard, 1897)	23 – 26	17				0.180	Panarelli *et al.*, 2019
Leydigia acanthocercoides (Fischer, 1854)	29	23	4.7				Murugan & Job, 1982
L. ciliata (Gauthier, 1939)	28-30	46					Venkataraman, 1990
L. leydigi (Schödler, 1863)	5 – 19	21 – 120	10.1 – 63.9				Robertson, 1988
L. louisi mexicana (Kotov, Elías-Gutiérrez & Nieto, 2003)	20 – 25	58 – 97	18.2 – 36.8		17.34 – 24.86	0.120 – 0.190	Martínez-Jerónimo & Gómez-Díaz, 2011
Oxyurella longicaudis (Birge, 1910)	23	47	7.50				Castilho *et al.*, 2015
Pleuroxus aduncus (Jurine, 1820)	25	16 – 44	7.4 – 14.4	1.04 – 13.8	6.47 – 0.53	-0.091 – 0.149	Nandini & Sarma, 2000
P. denticulatus (Birge, 1879)	15 – 25		8.4 – 25		4.92 – 10.96	0.096 – 0.174	Keen, 1967 (cited by Hann 1985)
P. uncinatus (Baird, 1850)	5 – 20	31 – 132	13.6 – 78.3				Bottrell, 1975

The GT in chydorids varies significantly as a function of food source, food density, and temperature, taking values from some days (about 10 d) up to 100 d (Table 2). The longest GT were reported for organisms within the subfamily Chydorinae, like *Acroperus harpae* (Baird, 1834) and *Eurycercus lamellatus* (Müller, 1776) when they were grown at 5 °C, but increasing temperature to 25°C promoted significantly lower values of 5.30 d and 19.08 d, respectively. In the subfamily Aloninae, the longest GT was reported for *Alona affinis* (Leydig, 1860) (72.88 d at 5°C) while *C. rectangula* presented the shorter GT (4.16 d at 23.6°C) (Table 2). Thus, the GT of *A. guttata* (17.82 ± 1.15 to 27.28 ± 0.80 d) is similar to the values reported for other chydorid species. Bottrell (1975) stated that the interaction of abundant food supply and increasing temperature promote shorter times for every developmental stage in chydorids, thus, the GT can be shorten as it was observed within the group of *A. guttata* fed on *N. oculata* (2×10^6 cells/mL). On the other side, Muro-Cruz *et al.* (2002) found no significant differences for the GT of *C. rectangula* fed on *C. vulgaris* at either 0.5×10^6 or 2×10^6 cells/mL.

For chydorids, the intrinsic rate of population increase (*r*) is generally accepted to take low values, which are in most cases below or near 0.2/d (Martínez-Jerónimo & Gómez-Díaz, 2011; Nandini *et al*., 2007), with the exception of some species of the genus *Eurycercus*, which can reach high values for chydorids of up to 0.310/d (Table 2). Furthermore, the intrinsic rates of population increase are affected by temperature, for instance, Hann (1985) found that increasing temperatures promoted higher reproductive rates in chydorids by decreasing egg maturation times; therefore, in four species the highest *"r"* values were recorded at 24 – 25ºC. Despite *A. guttata* presented high growth rates in comparison to *Pleuroxus aduncus* (Jurine, 1820) 0.15/d (Nandini & Sarma, 2000), or very similar to those of *C. rectangula* or *Karualona muelleri* (Richard, 1897) (Muro-Cruz *et al*., 2002; Panarelli *et al*., 2019), such values are below the intrinsic growth rates that other cladocerans like *Moina* sp. can reach (Sipaúba-Tavares & Bachion, 2002; Deng & Xie, 2003; Rodríguez-Estrada *et al*., 2003).

In relation to algal density, some cladoceran species are adapted to either low or high densities; for instance, *C. rectangula* and *P. aduncus* showed lower growth rates at increasing algal densities (Nandini & Sarma,2000; Muro-Cruz *et al*. 2002). Nevertheless, these authors tested a single algal species. In our study, *A. guttata* exhibited higher growth rates with increasing algal density, with *N. oculata* as a better food source over *C. vulgaris*.

The algal species has also significant effects on the reproduction and survival of *A. guttata.* In this study we observed that either *C. vulgaris* or *N. oculata* were good food source, which promoted a higher performance than the alga *R. subcapitata*.

In the literature, the genera *Chlorella* and *Nannochloropsis* have been described to synthesize high amounts of polyunsaturated fatty acids (PUFA) in comparison to other genera like *Raphidocelis* (Patil *et al*., 2007); therefore, feeding on these algae could serve as a better food source that provides the essential nutrients to enhance reproduction and survival (Schlotz *et al*., 2012).

Up to date, three publications have reported the survival and fertility of *A. guttata.* On the one side, Garza-León *et al.* (2017) and Osorio-Treviño *et al.* (2019) carried out partial life table analysis with similar algal densities (*N. oculata* at 2×10^6 cells/mL) and temperature (25°C) than the present study; an on the other side, Cortez-Silva *et al.* (2022) studied the life cycle but used *R. subcapitata* as food source and temperature within the interval here tested. In these studies, neither of them employed any substrate in the culture media, and despite *A. guttata* survived and produced offspring in those conditions, we found out that the inclusion of substrate improved the performance of these chydorids, increasing their rate of population growth and survival. It has been demonstrated that not all chydorids grow better on a diet composed solely of microalgae but rather on one of detritus or bacteria (Smirnov, 1962; Vijverberg & Boersma, 1997), although a mixed diet could be more important for chydorids nutrition (Lemke *et al*., 2007).

In conclusion, *A. guttata* can feed on either *C. vulgaris* or *N. oculata*, increasing their longevity and fertility when algal density is high (2×10^6 cells/mL). Temperature had significant effects on the life history of *A. guttata*, but is was the interaction with the algal density and algal species which produced more significant effects on the performance of *A. guttata*. The continuous production of offspring and long survival in *A. guttata* allowed intrinsic growth rates that are among the highest within the family Chydoridae, which might suggest this species to be cultured for further purposes like aquaculture (since some *Alona* species form part of fish diets) or ecotoxicological studies, which have already been reported in the literature.

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Ecological aspects of the Neotropical otter, *Lontra longicaudis annectens* (Major, 1897), in La Lagartera Lagoon, Campeche, Mexico

Aspectos ecológicos de la nutria neotropical, *Lontra longicaudis annectens* (Major, 1897) en la laguna La Lagartera, Campeche, México

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ABSTRACT

Background. Several works conducted in Mexico have addressed the Neotropical otter (*Lontra longicaudis annectens*), a protected subspecies (NOM-059-SEMARNAT-2010). **Objectives**. To know the abundance and distribution of the neotropical otter in the State of Campeche in La Lagartera Lagoon (Palizada River). **Methods**. During 2017 were searched the lagoon looking for indirect evidence on mangrove, collected and analyzed scats in order to identify preys. **Results**. Were traveled 1.49 km in each climatic season (nortes, dry, and rainy seasons), on board a small vessel, recording a total of 99 signs (85 scats, five feeding sites, five dens, and four vocalizations). We recorded the distribution of these signs across the sampling area and estimated a relative abundance of 0.30 otters/km. The analysis of 85 scats collected on logs or roots recorded six major prey groups: fish (40 %), crustaceans (26 %), reptiles (15 %), mollusks (11 %), mammals (4 %), birds (3 %) and other unidentified prey (1%). **Conclusions**. No evidence was found to suggest a variation in the consumption frequencies of the groups of prey by season. The presence and consumption of the "sailfin catfish" was also recorded in all seasons. The presence of four native species and five new records of prey fish was confirmed from otoliths contained in feces. This information highlights the ecological relevance of the species.

Keywords: distribution, feeding habits, Palizada river, relative abundance.

RESUMEN

Antecedentes. En México, existen diversos trabajos sobre la nutria neotropical (*Lontra longicaudis annectens*), subespecie protegida (NOM-059-SEMARNAT-2010); sin embargo, son pocos los trabajos realizados en el estado de Campeche. **Objetivos**. Conocer la abundancia y distribución de esta especie en laguna "La Lagartera" (río Palizada). **Métodos**. Durante 2017 realizaron recorridos en la zona de estudio buscando evidencias indirectas de las nutrias en troncos de mangle analizando heces para conocer las presas. **Resultados**. Se recorrieron 1.49 km en cada temporada climática (nortes, secas y lluvias), a bordo de una embarcación menor, obteniéndose un total de 99 rastros (85 heces, cinco comederos, cinco madrigueras y cuatro vocalizaciones). Se registró la distribución de estas evidencias dentro del área de muestreo y se estimó una abundancia relativa de 0.30 nutrias/km. El análisis de 85 heces colectadas sobre los troncos y/o raíces, registró seis grupos principales de presas: peces (40 %), crustáceos (26 %), reptiles (15 %), moluscos (11 %), mamíferos (4 %), aves (3 %) y otros no identificados (1 %). **Conclusiones**. No se encontró evidencia que determine una variación en las frecuencias de consumo de los grupos presas por temporada. También se registró la presencia y consumo del pez diablo en todas las temporadas. Se confirma por medio de otolitos obtenidos en las heces, la presencia de cuatro especies nativas y cinco nuevos registros de peces presa. Con esta información se resalta el valor ecológico de la especie.

Palabras clave: abundancia relativa, distribución, hábitos alimentarios, río Palizada.

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INTRODUCTION

Mexico is one of the countries harboring a huge biodiversity; it ranks first in the Americas and third worldwide in terms of the number of mammals (PROFEPA, 2020). However, mammals currently face conservation issues derived from the drastic disruption of ecosystems, pollution of tributaries, habitat destruction and fragmentation. These have affected multiple species by displacing them from their original habitats and even causing local extinctions when wildlife does not encounter suitable conditions for survival. Additional factors affecting wildlife include indiscriminate poaching, resulting in marked reductions in their populations. Therefore, natural ecosystems — and the species living in them — should be preserved, since each organism within populations and communities have a significant role in the functioning of ecosystems (Cruz-García *et al*., 2017).

The Neotropical otter, *Lontra longicaudis annectens* (Major 1897), also known as water dog *"perro de agua"* in Spanish (Gallo-Reynoso, 1989), is listed as a nearly threatened species on the Red List of the International Union for the Conservation of Nature (Rheingantz & Trinca, 2015) and as endangered of extinction in the Convention on International Trade in Endangered Species of Wild Fauna and Flora, Appendix I (CITES, 2018). In Mexico, it is listed as a "threatened" species in NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

The earliest records of this subspecies in the State of Campeche Gallo-Reynoso (1989, 1997), reporting its presence from indirect signs (tracks, skins examined, latrines, scats, resting sites, dens, and interviews with fishers in 1978 in the Usumacinta River Delta. The usumacinta delta is made up by the Champotón River and the high reaches of rivers such as Candelaria, Samaria, Chumpán, Del Este Lagoon, and Palizada, which flow into Laguna de Términos and by the San Pedro River, which forms the limit with the State of Tabasco. Later, Ramírez-Pulido *et al*. (2017) published a literature collection of mammals in Mexico during the period 2000-2010, stating that no scientific publications were available for *L. l. annectens* in the State of Campeche. Santiago-Plata *et al.* (2013) confirmed the presence of Neotropical otter at La Veleta road, between the federal highway between Villahermosa and Cd. del Carmen, and the San Pedro and San Pablo River, through indirect signs (footprints, grooming sites, latrines, and scats) and direct sightings of individual otters. In the same year, Guzmán-Soriano *et al*. (2013) recorded the presence of the subspecies from the finding of two skulls, one in the Calakmul Biosphere Reserve and the other in the Venustiano Carranza river southeast of Candelaria, in addition to one sighting in the Caribe River.

There are only four records of Neotropical otter presence in the Palizada River prior to this work. Corresponding to Gallo-Reynoso (1997), who found tracks and dens. Later on, an otter skin obtained in the Palizada area, was donated by a fisherman from Palizada town in 2016, to the Mammal Collection of the *Centro de Estudios en Desarrollo Sustentable y Aprovechamiento de la Vida Silvestre* (Center for Studies in Sustainable Development and Wildlife Use at Universidad Autónoma de Campeche (CEDESU-UAC). Vázquez-Maldonado *et al.* (2018a, 2018b), found scats, latrines, and obtained four observations of Neotropical otters in lagoons adjacent to the Palizada River (La Sangría, La Lagartera and Las Coloradas) and in Laguna de Términos.

In Mexico, several studies have been carried out to evaluate the habitat of the Neotropical otter and determine its biological and ecological features. *Lontra longicaudis annectens* is recognized as a carnivore, generalist, and top predator in riparian ecosystems (Gallo-Reynoso *et al*., 2008); its recorded diet is based primarily on fish and crustaceans (Gallo-Reynoso, 1997; Macías-Sánchez & Aranda, 1999; Monroy-Vilchis & Mundo, 2009). Sánchez & Gallo-Reynoso (2007) state that this subspecies is likely to be primarily a predator that prefers certain types of prey, displaying flexibility in feeding preferences according to prey abundance and availability. Whether this prey substitution can be sustained in the long term remains unknown; for example, foraging on plants, mollusks, insects, amphibians, reptiles, birds, and mammals has been recorded (Gallo-Reynoso, 1989, 1997; Macías-Sánchez & Aranda, 1999; Ramón, 2000; Santiago-Plata, 2009; Arellanes-Licea & Briones-Salas, 2003; Casariego-Madorell, 2004; Soler-Frost, 2004; Díaz-Gallardo *et al.,* 2007; Duque-Dávila, 2007; Monroy-Vilchis & Mundo, 2009; Rangel-Aguilar & Gallo-Reynoso, 2013; Grajales-García *et al*., 2019; García-Silva *et al*., 2021). Thus, feeding habits allow determining the diversity of prey in the area where Neotropical otter thrive (Casariego-Madorell *et al*., 2008; Rheingantz *et al*., 2017).

The above highlights the importance of conducting research on Neotropical otter in the State of Campeche, to contribute to the knowledge of this species in this riparian habitat that includes the deltaic system with the greatest flow in México (Cotler-Ávalos *et al*., 2010). The objective of this work is to contribute ecological knowledge on Neotropical otter in La Lagartera lagoon (located adjacent to the Palizada River), Campeche, within the Laguna de Términos Flora and Fauna Protection Area (APFFLT); particularly, this study estimated population density, distribution of indirect signs (scats, latrines, feeding sites, and dens, among others). An additional objective was to describe its feeding habits by analyzing scats and food residues collected in latrines and feeding sites. This information will support proposals on conservation strategies for the Neotropical otter within and outside the Laguna de Términos Flora and Fauna Protection Area.

MATERIALS AND METHODS

The study comprised three climatic seasons during the year 2017, herein named as nortes, dry and rainy, as they are the main seasons in La Lagartera Lagoon, adjacent to the Palizada River, Campeche. Palizada River is the largest tributary of the Usumacinta River Delta (Coll de Hurtado, 1975); it is a long and narrow stream with multiple short-radius bends and a mean annual discharge volume of 11.9×10^9 m^3 (Soberón-Chávez & Yáñez-Arancibia, 1985). La Lagartera Lagoon is located between parallels 18° 22' 38.92" and 18° 22' 11.24" North, and between meridians 91° 51' 43.32" and 91° 51' 52.96" West (Fig. 1), comprising an area of approximately 0.38 km^2 calculated by GIS tools (QSIG 2.0 software), of which the entire perimeter of 1.49 km was surveyed. The "La Lagartera" lagoon is part of the Laguna de Términos Flora and Fauna Protection Area (APFFLT), which was decreed as a Natural Protected Area on 6 June 1994 (https://simec.conanp.gob.mx/ficha.php?anp=118&reg=5).

The Palizada-Del Este fluvial lagoon system has a salinity between 0 and 8 ‰, surface water temperature between 22 °C and 31 °C (Ayala-Pérez, 1989), and water transparency of 1.0 ± 0.23 m (Muciño-Márquez *et al*., 2017). The vegetation in the system includes submerged hydrophytic angiosperms, supralittoral hydrophytes typical of wetlands, reed, tulles, and annual and perennial grasses (Vera-Herrera *et al*., 1988a, 1988b). In addition, the surrounding vegetation includes well-developed riparian mangrove (10 - 25 m), dominated by black mangrove (*Avicennia germinans*), followed in abundance by red man-

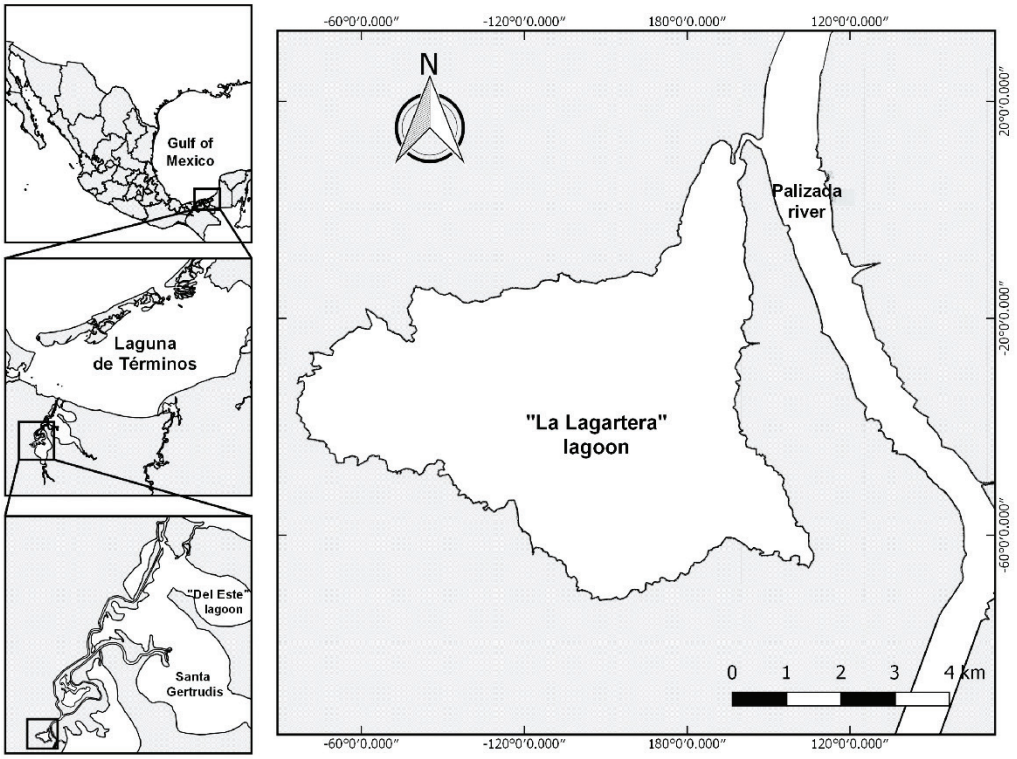

Figure 1. Location of the study area: La Lagartera Lagoon, adjacent to the Palizada River, Campeche, Mexico (APFFLT).

grove (*Rhizophora mangle*) and, in a smaller proportion, white mangrove (*Laguncularia racemosa*) (Jardel *et al*., 1987).

Field surveys in the study area comprised three visits, one per climatic season. Since the study area is regularly flooded and covered by dense vegetation, surveys were carried out onboard of a 7.6 m long fiberglass boat with a 50 HP outboard motor. A search for indirect signs of the presence of Neotropical otters was carried out on the shore of the lagoon and in areas that could be walked. Each evidence found was georeferenced (Garmin GPSMAP® 78s) and photographed (Ricoh WG-4 SR Adventure Proof digital camera with SanDisk 32GB memory card). Kayaks were used in shallow or closed vegetation areas where navigation with the boat was impossible. In sites where otter signs were found, surface water temperature, depth (Hawkeye manual echosounder), and salinity (ATAGO refractometer) were recorded.

Biological material was collected manually and placed in resealable Ziploc plastic bags labeled with the date, and sample number. All collected samples were placed in a cooler while transfer to "Laboratory No. 1" of the Faculty of Natural Sciences (Facultad de Ciencias Naturales) at Universidad Autónoma del Carmen (UNACAR), where they were kept refrigerated until their analysis. To collect potential prey, some fishes were caught by a fisherman in the study area at the end of sampling during the nortes season; potential prey collected were also placed in resealable bags and in a cooler for transfer to the laboratory.

Scats and food residues collected at feeding sites were washed with a soap solution (1:10) and left to stand for 24 hours to neutralize any bacterial activity and agglutinate organic matter residues. Afterward, these were washed with running water through a sieve (0.500 mm mesh) and the material retained was placed on aluminum paper trays and oven-dried (Felisa model FE-292AD) at 61 °C to dry; it was then placed in previously labeled manila paper envelopes. Identifiable components and structures in each sample were sorted out according to animal taxa under a stereo microscope (Iroscope model ES-24 PLIT), using dissection needles and forceps.

The food items found were sorted out as scales, bones, hairs, feathers, and other items, and were placed individually in properly labeled plastic vials. Structures larger than these vials (collected mainly from feeding sites) were stored in paper envelopes.

Once otoliths were separated from the rest of the scat components, they were cleaned with damp towels to remove any debris and determine whether their state of conservation allowed identification. Fish otoliths were identified, either individually or in pairs (left and/or right), based on characteristics such as shape, acoustic sulcus, and cauda. These were compared with four types of reference materials: 1) photographic catalogs contained in the *Anàlisi de formes d'Otòlits* (AFORO, for its acronym in Catalan) database (http://aforo.cmima.csic.es) and in the *"Catálogo de otolitos Sagitta de peces del Golfo de México"* (Catalog of *Sagitta* Otoliths of Fish from the Gulf of Mexico) by Martínez-Pérez *et al.* (2019); 2) list of fish species recorded for the Palizada-Del Este system and Laguna de Términos (Ramos-Miranda *et al*., 2006; Ayala-Pérez *et al*., 2015); 3) otoliths of the Collection of otoliths of the Faculty of Natural Sciences, UNACAR, obtained from the stomach content of stranded bottlenose dolphins (*Tursiops truncatus*); and 4) otoliths extracted from fish captured in La Lagartera Lagoon by us and identified to species. Once otoliths were identified to the extent possible, a unique key was

assigned for each otolith or pair of otoliths identified, which were integrated according to the guidelines of the Collection mentioned above.

Regarding the ecological aspects, we calculated the relative abundance of otters based in terms of number of fresh scats present in sampling sites per individual on sampling site (dark green to black scat, with a musk odor, soft consistency not disaggregated, sometimes moist pasty) (pers. obs.). The calculated figure was compared with the baseline relative abundance index (number of scats per kilometer [NE]), and the bounded abundance index (estimate of the number of otters per kilometer [AN]), respectively (Gallo-Reynoso, 1996; Macías-Sánchez, 2003) using a defecation rate of three defecation events (i.e., scats) per day (TD = 3) as established by Gallo-Reynoso (1989). The results on relative abundance were analyzed with the statistical program IBM SPSS Statistics 25 applying the Kruskal-Wallis and Chi square (X^2) non-parametric indices to determine the statistical significance of the difference between scat records and climatic seasons (nortes, dry and rainy), after testing the data for normality (Shapiro-Wilk) and homoscedasticity (Levene).

Ecological niche breadth was calculated using the Levins index (Levins, 1968; Jaksic & Marone, 2007), where *Pi* is the relative frequency of the species.

The feeding habits of the Neotropical otter were determined by estimating the Frequency of Occurrence (*FO*) of the different prey categories and the various structures within the same group, using the equation: $FO = fi/N \times 100$, where *fi* is the number of scats containing a given prey category and *N* is the total number of scats analyzed. Also, the Percentage of Occurrence (*PO* was calculated using the equation: $PO = fi/ft \times 100$, where *fi* is the number of scats containing a given prey category and *ft* is the total number of occurrences of all prey categories in all scats (Macías–Sánchez & Aranda, 1999). Last, a Kruskal-Wallis test was performed to determine the statistical significance of the differences in the consumption of prey groups between seasons (Díaz-Gallardo *et al*., 2007).

The location and spatial distribution of indirect signs (scats, latrines, feeding sites and dens) served to produce maps by climatic season using the software QGIS 2.0.

RESULTS

A total of 99 indirect signs of Neotropical otter were recorded in the study area, of which 85 were scats; most were found over tree roots, branches and logs, the majority on white mangrove trees that were either fallen or on the lagoon shore (86 %), five were found in dens (5 %), and five in feeding sites (5 %), also we registered four vocalizations (4 %). The number of indirect signs by climatic season were 37% in nortes, 35 % in dry season, and 28 % in rainy season; there were no significant differences in the percentage of indirect signs between climatic seasons ($X^2 = 3.856$, *d. f.* = 98, $p = 0.696$).

The analysis of indirect signs observed by climatic season showed that the Percentage of Occurrence (*PO*) was mostly represented by scats (Table 1).

A total of 11 latrines were identified and 85 otter scats were recorded on them; only four fresh scats were found during rainy a nortes seasons on 4.47 kilometers surveyed. These records were used to estimate the relative abundance indices of *L. l. annectens* in La Lagartera lagoon area (Table 2). The analysis by climatic season considering only fresh scats revealed that the highest relative abundance occurred in the nortes season, with 0.67 otters/km (Td = 3), and the lowest in the rainy season, with 0 otters/km (Table 2). However, the Kruskal-Wallis analysis yielded no significant difference in relative abundance between climatic seasons ($X^2 = 6.592$, *d. f.* = 2, $p = 0.159$). Therefore, the estimation of relative abundance of Neotropical otter was based on the number of indirect signs: number of scats (nE = 85) and number of latrines (nL = 11); this led to the estimation of 6.34 otters/km corresponding to 19.02 scats/km and 2.46 latrines/km. The Kruskal-Wallis test revealed no significant differences in the number of scats between climatic seasons ($X^2 = 2.000$, *d. f.* = 2, $p = 0.368$). Finally, the analysis based on fresh scats yielded an overall estimate of 0.30 otters/km (TD = 3)

In this work, five feeding sites were recorded, one for the nortes season, two for the dry season and two for the rainy season. In such feeding sites, two genera were identified based on bone remains: first the genus *Pterygoplichthys* (sailfin catfish, "*pez diablo"* in Spanish) and second, *Cathorops melanopus* (the dark sea catfish, "*bagre*" in Spanish).

Table 1. Percentage of occurrence (*PO* %) of the different signs of Neotropical otter per climatic season.

Indirect signs	Percentage of Occurrence (PO) Climatic season		
	Nortes	Dry	Rainy
Scats	86.49	85.72	85.19
Dens	8.11	5.71	7.41
Feeding sites	2.70	5.71	7.41
Vocalizations	2.70	2.86	-
Total	100	100	100

Table 2. Rates of relative abundance of Neotropical otter based on total and fresh scats, assuming a defecation rate of 3 scats per day (TD = 3).

Climatic season (Total scats – Fresh scats)	Total scats otters/km	Fresh scats otters/km
Nortes (32 – 3)	7.16	0.67
Dry (30 -1)	6.71	0.22
Rainy (23 – 0)	5.15	0.0
Total (85 – 4)	6.34	0.30

Six main prey groups were identified from the 85 scats analyzed: fish were the most frequently consumed group (40 %), followed by crustaceans (26 %), reptiles (15 %), mollusks (11 %), mammals (4 %), birds (3 %), and miscellaneous remains grouped under "other", including very small fragments of polychaetes and unidentified material (1 %).

Ten families of fish were identified: Batrachoididae, Gerreidae, Gobiidae, Paralichthyidae, Cynoglossidae, Cichlidae, Triglidae, Loricariidae, Poeciliidae, and Eleotridae. It should be mentioned that, from a total of 238 separate otoliths, only 145 (61 %) could be identified. In the case of the genus *Pterygoplichthys*, identification was achieved not only through otoliths, but also from other structures such as vertebrae, pectoral and dorsal fin spines, and dermal plates. As for crustaceans, two families were identified (Portunidae: *Callinectes*, and Palaemonidae: *Macrobrachium*); for reptiles, only the Squamata Order was identified, particularly the suborder Serpentes; for mollusks, two classes were identified: Bivalvia and Gasteropoda; finally, regarding birds and mammals, remains were determined only to class (Table 3).

The ecological niche breadth based on fish (the group where the largest number of species was identified) using the Levins index, indicate low dominance (0.14) and high diversity (0.86), that is, *L. l. annectens* is a generalist subspecies with a broad niche, consistent with the findings reported by other authors (Gallo-Reynoso, 1989, 1996, 1997; Macías-Sánchez & Aranda, 1999; Ramón, 2000; Arellanes-Licea & Briones-Salas, 2003; Casariego-Madorell, 2004; Díaz-Gallardo *et al.*, 2007; Duque-Dávila, 2007; Grajales-García *et al.*, 2019).

The consumption of prey groups was similar in the nortes and dry seasons, both with six well-defined groups: mollusks, crustaceans, fish, reptiles, birds, and mammals. However, in the rainy season, the prey groups consumed decreased from six to five, with mammals and the "other" group missing in this season. Fish, crustaceans, and reptiles were the groups with the highest percentage of occurrence in the three seasons. The statistical analysis of food preference according to climatic season showed no differences in prey consumption frequencies (X^2 = 9.775, *d.f.* = 6, p = 0.636); fish was the main prey group consumed during the three seasons (59 % nortes, 58 % dry, and 78 % rainy) (Fig. 2).

Table 3. Types of prey found and identified, to the lowest taxonomical level possible, in Neotropical otter scats at "La Lagartera" Lagoon, Campeche. From: WoRMS (2018) and FishBase (Froese & Pauly, 2018).

Phylum	Subphylum	Class	Family	Genus	Species
Mollusca		Bivalvia	Unidentifiable		
		Gastropoda	Unidentifiable		
Arthro-poda	Crustacea	Malacostraca	Portunidae	*Callinectes*	*Callinectes* sp.
			Palaemonidae	*Macrobrachium*	*Macrobrachium* sp.
Chordata	Vertebrata	Actinopterygii	Batrachoididae	*Opsanus*	*Opsanus beta* * (Goode & Bean, 1880)
			Gerreidae	*Eucinostomus*	*Eucinostomus melanopterus* * (Bleeker, 1863)
			Gobiidae	*Awaous*	Awaous sp. **
					Awaous banana ** (Valenciennes, 1837)
			Paralichthyidae	Unidentifiable	
			Cynoglossidae	*Symphurus*	*Symphurus* sp. **
			Cichlidae	*Cichlasoma*	*Cichlasoma* sp.**
					Cichlasoma urophthalmus * (Günther, 1862)
				Petenia	Petenia sp. **
					Petenia splendida * (Günther, 1862)
				Oreochromis	*Oreochromis niloticus* ** (Linnaeus, 1758)
			Triglidae	*Prionotus*	*Prionotus* sp. **
					Prionotus rubio ** (Jordan, 1886)
			Loricariidae	*Pterygoplichthys*	*Pterygoplichthys* sp. **
					Pterygoplichthys pardalis ** (Castelnau, 1855)
			Poeciliidae	*Poecilia*	*Poecilia* sp. **
			Eleotridae	*Gobiomorus*	*Gobiomorus dormitor* ** (Lacède, 1800)
		Reptilia	Order: Squamata, Suborder: Serpentes		
		Aves	Unidentifiable		
		Mammalia	Unidentifiable		

* Native species (Ramos-Miranda *et al.*, 2016; Ayala-Pérez *et al.*, 2015).

** Potentially new records in the study area.

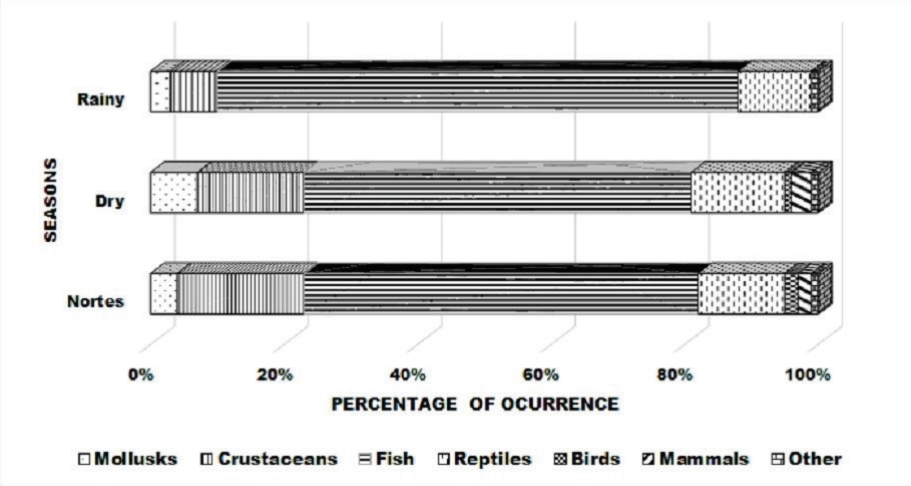

Figure 2. Variation in feeding habits of Neotropical otter according to the climatic seasons (nortes, dry, and rainy seasons).

DISCUSSION

Scats were the most representative indirect signs found over the three climatic seasons, located mainly in branches, trunks, logs, root systems and on the shore; some of these were on white mangrove (*Laguncularia racemosa*) trunks or roots, being the first record of the use of this tree species by Neotropical otter in the study area. The lowest number of scats recorded, were found during the rainy season, likely because rain washes away the scats deposited on the logs. In the nortes and dry seasons, it was possible to walk along the lagoon shoreline, where several dens were found. Although these observations were conducted under harsh conditions, such efforts should be continued, as this type of observational sampling yielded the highest number of records of dens. Of the two dens recorded in the dry season, one could be classified as a nursery (Fig. 4), since a feeding site and scats were found in the vicinity; also, this record coincides with the breeding period for the species (Gallo-Reynoso, 1989). During the rainy season, the water level rose and flooded the study area, making impossible to record evidence on the use of dens by otters. The type of vegetation and soil in the sampling area did not allow obtaining evidence from footprints or tracks.

The total number of indirect signs and their geographic location allowed the estimation of the abundance of the Neotropical otter, and to determine their distribution range in La Lagartera Lagoon, also to identify the main zones used by the otters in each season.

It is important to mention that anthropogenic activities like the burning of vegetation for clearing to allow the growth of grass during the dry season were observed on the lagoon's banks; however, vocalizations (indirect signs of otters) indicated that they were still present after burning in the area. The distribution of these signs along the perimeter of the lagoon was similar between climatic seasons (Fig. 3-5) indicating the even distribution, availability and abundance of food resources along the year.

La Lagartera Lagoon present freshwater characteristics during the three climatic seasons, with a variation of surface water temperature between 30.7 °C – 33.9 °C; this information matches the data recorded in the technical information of the subspecies base on PROY-NOM-059-ECOL-2000 (Mexican mammals in danger of extinction) (Gómez-Nisino, 2006).

If the estimated relative abundance (6.34 otters/km based on the total number of scats) is compared with other works for neotropical otters, the estimate obtained in the present study is slightly higher than estimates reported for other regions. This difference may derive from the fact that our analysis considered any scats identified, regardless of its degraded condition, which may have led to an overestimation of the number of individuals in the study area. The abundance figure derived from fresh scats only (0.30 otters/km), lies within the abundance range calculated for other regions with similar evidence (Gallo-Reynoso, 1996).

Considering the total area of the lagoon (0.38 km^2) and fresh-scat records, an estimate of 1.17 otters/km^2 was obtained, which is also within the range of previous estimates for Neotropical otter (Gallo-Reynoso, 1996) and more realistic one.

The Neotropical otter diet included six groups: fish, crustaceans, reptiles, mollusks, mammals, and birds, confirming that *L. l. annectens* is a generalist predator that probably consumes any potential preys available (Duque-Dávila, 2007), with fish as the main type of prey consumed in most cases (Table 3). The presence and consumption of *Pterygoplichtys* sp. and *Pterygoplichtys pardalis* were recorded in the three climatic seasons, indicates that Neotropical otter probably contributes to regulate the population size of the sailfin catfish, as mentioned by Vázquez-Maldonado *et al.* (2018a) but a bigger research effort will be necessary, including isotopic analyses as Juarez-Sanchez *et al.* (2019) have done with scats in the rivers of Guatemala. The identification of fish to species based on otoliths was difficult because no reference catalog of otoliths is currently available for the region. Some otoliths could not be identified to family because of their highly degraded status due to the digestion process, or were not *Sagitta* otoliths, which are the otoliths commonly used for the identification of fish species.

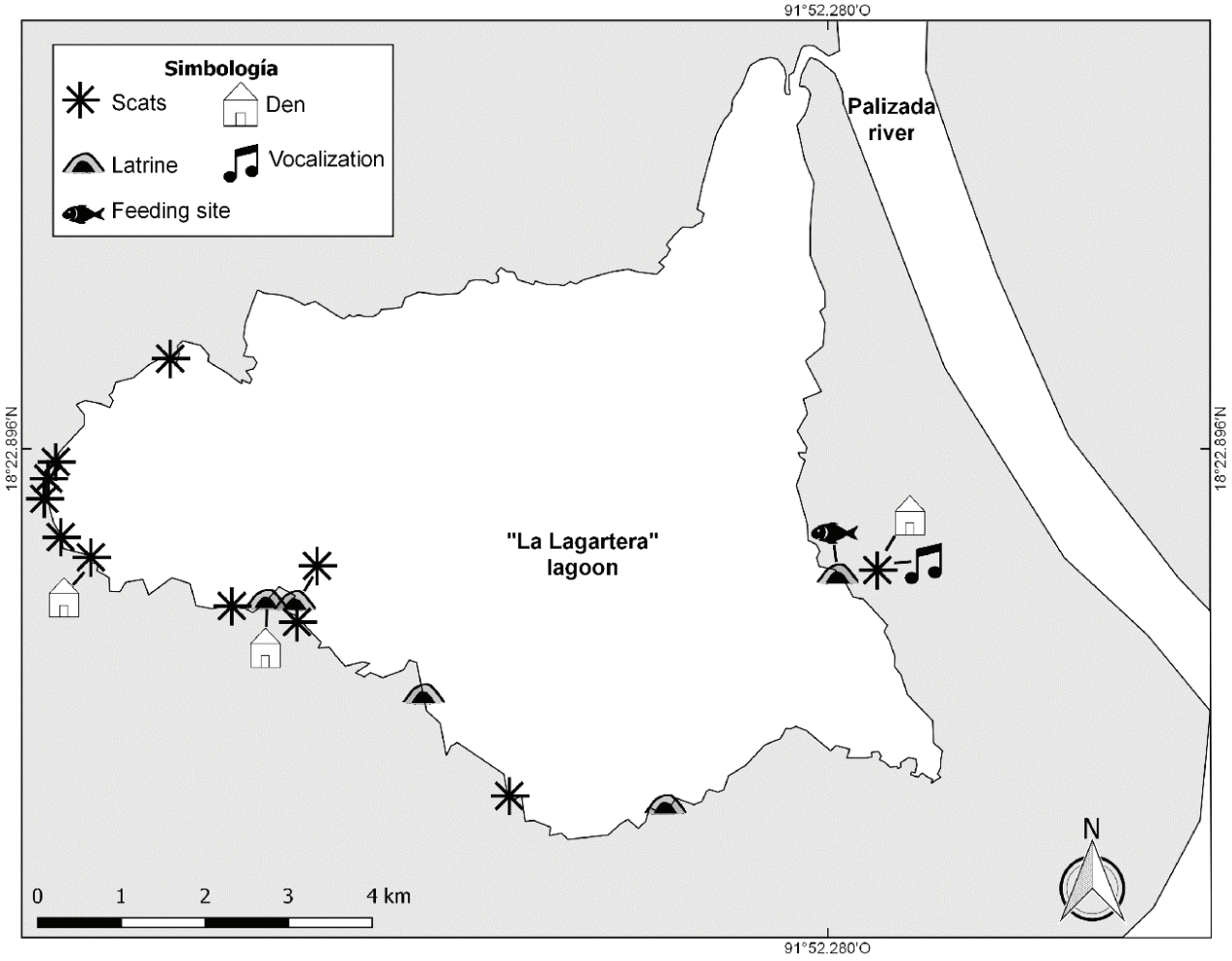

Figure 3. Distribution of Neotropical otter signs recorded for the study area in the nortes season.

Four of the prey fish species identified, (*Cichlasoma urophthalmus, Eucinostomus melanopterus, Opsanus beta*, and *Petenia splendada*) are native to the area according to the records of Ramos-Miranda *et al.* (2006) and Ayala-Pérez *et al*., 2015). Moreover, five new fish records were obtained for the study area: *Awaous banana, Gobiomorus dormitor, Oreochromis niloticus, Prionotus rubio*, and *Pterygoplichthys pardalis*; however, more analyses should be conducted for these species in the area, some of which are freshwater species not usually found in brackish environments.

Comparing the main groups of prey found in scats by other authors versus our findings in this study, the rank order in percentage of occurrence, in general and by climatic season, as recorded for La Lagartera Lagoon is the first report of its kind for the subspecies. In general, for the nortes and dry seasons, the rank order of frequency of consumption is: fish > crustaceans > reptiles; for the rainy season, the order is: fish > reptiles > crustaceans. These combinations of prey groups are different from those recorded in other regions of Mexico, such as the Sierra Madre del Sur (Gallo-Reynoso, 1989), Los Pescados River, Veracruz (Macías-Sánchez & Aranda, 1999), San Cipriano River, Tabasco (Ramón, 2000), Ayuquila River, Jalisco (Díaz-Gallardo *et al*., 2007), where the rank order is: fish > crustaceans > insects, or from the coastal zone of Tuxpan, Veracruz (Grajales-García *et al*., 2019), where the order is: crustaceans > fish > mollusks. However, this variation in the percentage of consumption of prey groups appears to be related to the type of river (narrow and rocky versus wide and muddy/sandy) or freshwater environment (river versus lagoon) (García-Silva *et al*., 2021), and even to anthropogenic pressures in the area, as well as to the time of the year when sampling was carried out and scats were collected. In La Lagartera Lagoon, no significant differences were found in the preference of prey by the Neotropical otter between climatic seasons.

Finally, otters showed certain tolerance to anthropogenic activities, as dens, scats, and vocalizations, were heard and recorded at the same sites where vegetation burning, and clearing was done in the previous season.

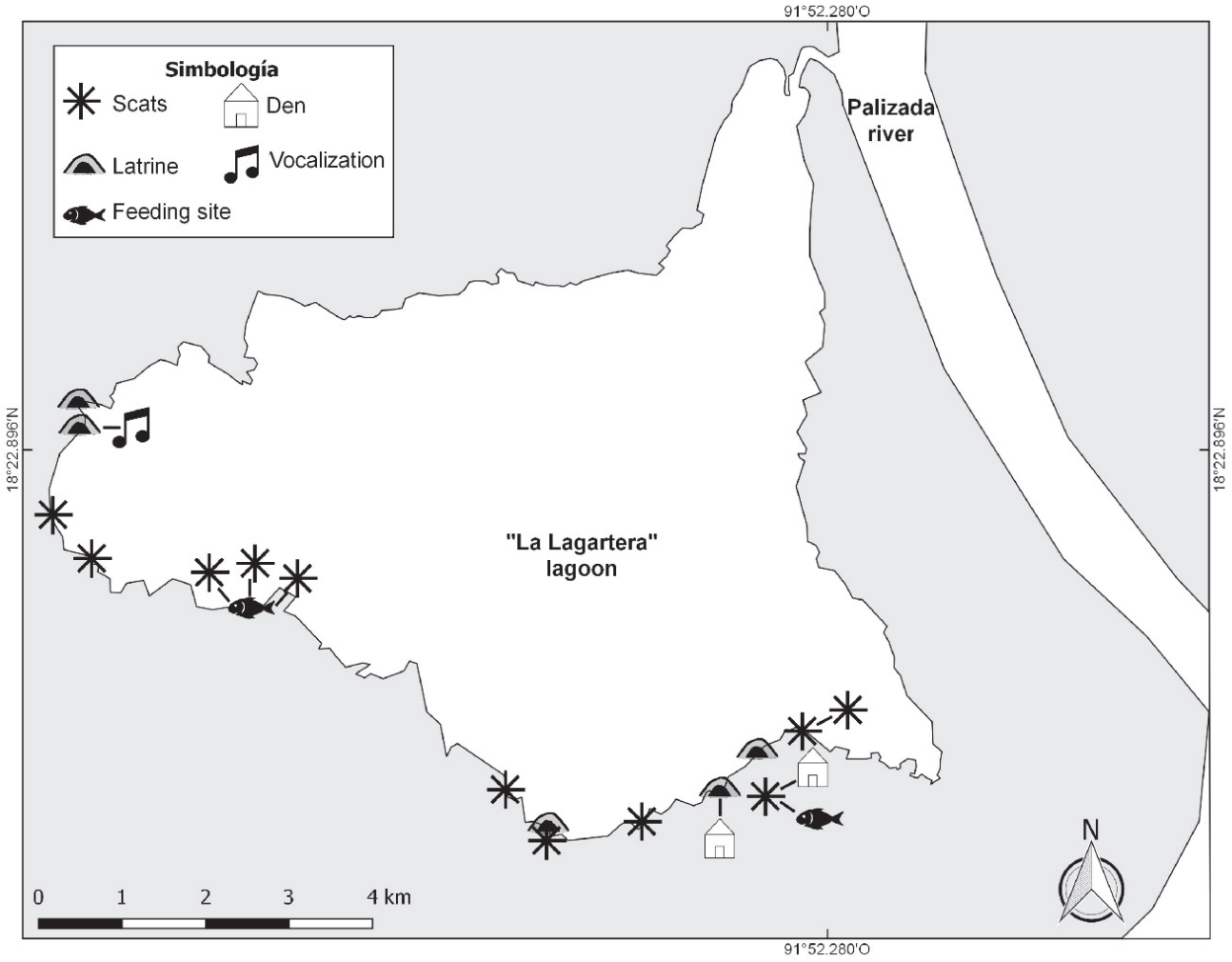

Figure 4. Distribution of Neotropical otter signs recorded for the study area in the dry season.

The species has been considered as an "umbrella species" (Gallo-Reynoso *et al.*, 2008) based on the conservation approach within the APFFLT Management Program. The efficiency of this strategy may be increased by including functional bonds between this species and other species in the same ecosystem (Bifolchi & Lodé, 2005), since this study shows that otters prefer sites with branches, root systems and logs. In addition, its position as a top predator highlights its important role in maintaining the functioning of ecosystems in aquatic environments (Grajales-García *et al.*, 2019), particularly, regarding the consumption of sailfin catfish, considered an invasive species. The results reported here indicate a central ecological role of the Neotropical otter in this transition zone between freshwater and brackish environments in the Laguna de Términos Flora and Fauna Protection Area.

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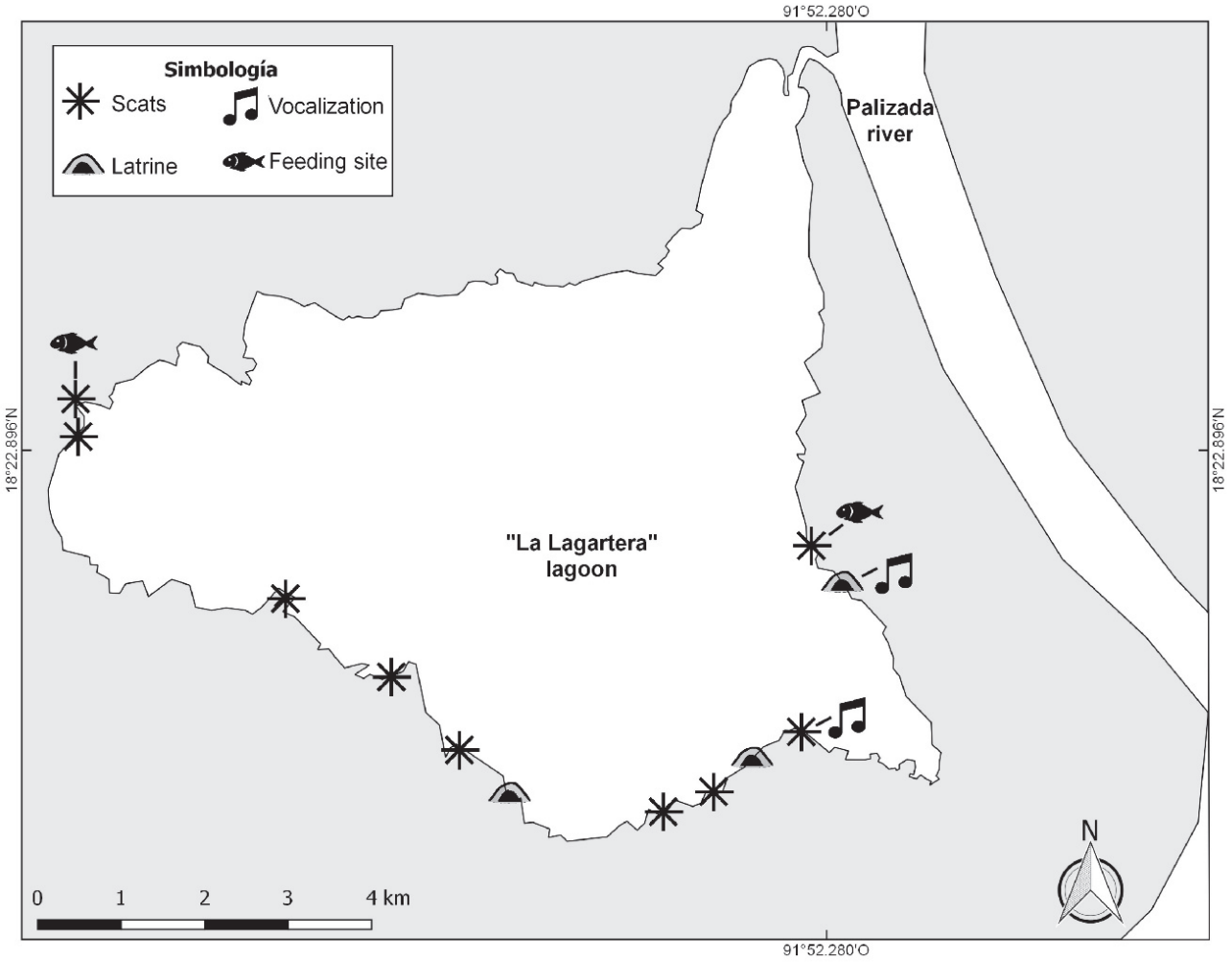

Figure 5. Distribution of Neotropical otter signs recorded for the study area in the rainy season.

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Actividad antibacteriana de bacterias aisladas de sistemas hidrotermales de Baja California Sur, México

Antibacterial activity of bacteria isolated from hydrothermal systems of Baja California Sur, Mexico

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RESUMEN

Antecedentes. Los sistemas hidrotermales son una fuente novedosa para el descubrimiento de nuevos compuestos antimicrobianos y/o metabolitos con potencial biotecnológico. **Objetivos.** En este sentido, en el presente trabajo se realizó una prospección de bioactividad de microorganismos aislados de sistemas hidrotermales someros con capacidad para inhibir el crecimiento de microorganismos de interés en salud humana, acuícola e industrial. **Métodos.** Se aislaron bacterias de dos sistemas hidrotermales ubicados en Bahía Concepción y uno en El Sargento, B.C.S. en época de lluvias y temporada de estiaje. Se evaluó la actividad antimicrobiana contra bacterias patógenas de humano, patógenas acuícolas y formadoras de biopelículas marinas, utilizándose la técnica de difusión en pozo. **Resultados.** El 73 % de las bacterias aisladas produjeron sustancias capaces de inhibir el crecimiento de al menos uno de los microrganismos evaluados. Solo se observó antagonismo para 15 de las 36 bacterias diana, donde *Photobacterium damselae* subsp. *damselae* fue la más sensible, observándose una inhibición de su crecimiento en presencia de más de la mitad de las bacterias marinas aisladas de sistemas hidrotermales (51 %). En cuanto a la actividad por sitio y temporada de obtención se observó un mayor número de aislados activos frente a los patógenos acuícolas, predominando los aislados de temporada de estiaje para Mapachitos y Santispac, mientras que en El Sargento el mayor número de aislados activos frente a este grupo de bacterias diana se obtuvo durante la temporada de lluvias. **Conclusión.** Las bacterias marinas aisladas de ambientes extremos sintetizan compuestos antimicrobianos que podrían ser utilizados en el campo de la salud y la industria.

Palabras clave: Antagonismo, Patógenos acuícolas, Patógenos de humanos, Biopelículas

ABSTRACT

Background. Hydrothermal systems are a novel source for the discovery of new antimicrobial compounds and/or metabolites with biotechnological potential. **Objectives.** In the present research, a bioactivity screening of microorganisms isolated from shallow hydrothermal systems with the capacity to inhibit the growth of microorganisms of interest in human, aquaculture, and industrial health was carried out. **Methods.** Bacteria were isolated from two hydrothermal systems located in Bahía Concepción and other in El Sargento, B.C.S. in rainy and dry season. The antimicrobial activity against human pathogens, aquaculture pathogens, and marine biofilm-forming bacteria was evaluated, using the well diffusion technique. **Results.** 73 % of the isolated bacteria produced substances capable of inhibiting the growth of at least one of the microorganisms evaluated. Antagonism was only observed for 15 of the 36 target bacteria, *Photobacterium damselae* subsp. *damselae* was the most sensitive, which was inhibited by 51 % of the marine bacteria. Regarding the activity by site and season of collection, a greater number of active isolates against aquaculture pathogens was observed, predominating the dry season isolates for Mapachitos and Santispac, while in El Sargento the highest number of active isolates was obtained during the rainy season. **Conclusion.** Marine bacteria from hydrothermal systems are able to produce antimicrobial metabolites that could be used in different areas e.g. health and industry.

Keywords: Antagonism, Aquaculture pathogens, Human pathogens, Biofilm

INTRODUCCIÓN

Los sistemas hidrotermales suelen estar relacionados con actividad volcánica costera (Tarasov *et al*., 2005), aunque también se pueden llegar a encontrar en márgenes continentales afectados por procesos activos de extensión tectónica (Prol-Ledesma & Canet, 2014; Rodríguez-Uribe *et al*., 2018), como es el caso de los que se encuentran en México. En Bahía Concepción, Baja California Sur, se localizan dos sistemas hidrotermales; uno de ellos en la zona del manglar de Santispac sobre la línea costera, y una ventila hidrotermal somera a 15 m de profundidad en la zona oceánica, conocida como Mapachitos.

Otro sistema hidrotermal en las costas de Baja California Sur está localizado en la línea de costa de Playa Agua Caliente en El Sargento, una zona de falla geológica con actividad hidrotermal, que es alimentada por canales subacuáticos costeros (Nava-Sánchez *et al*., 1995).

Los sistemas hidrotermales son calentados geotérmicamente y están enriquecidos con compuestos químicos inorgánicos; estas zonas presentan condiciones fisicoquímicas inusuales a otras áreas marinas, tales como: temperaturas elevadas, concentraciones altas de dióxido de carbono, ácido sulfhídrico, hidrocarburos y metales pesados; entre otras características que los hacen sistemas únicos (Lentini *et al*., 2014), por lo cual se han convertido en el objeto de estudio de diversos investigadores (Forrest *et al.,* 2005; Villanueva-Estrada *et al.,* 2005). Algunos de estos sistemas hidrotermales considerados como ambientes extremos presentan ventilas o fisuras en el sedimento marino de las que emanan fluidos, están ubicadas en diferentes sitios oceánicos y son divididas en ventilas profundas (mayores a 200 m) y someras (menores de 200 m) (Tarasov *et al*., 2005).

En los sistemas hidrotermales se han encontrado microorganismos extremófilos (afectos a ambientes inusuales como altas temperaturas y concentraciones de H_2S, hidrocarburos, metales pesados, entre otros), los cuales han sido estudiados con un enfoque ecológico para interpretar la función que ejercen en el medio (Van Dover, 2000). Estos sitios son influenciados por organismos fotosintéticos, de forma que se caracterizan como ambientes de alta energía que benefician a la microbiota (Giovannelli *et al.,* 2013).

La ecología microbiana se ha estudiado en varios sitios de sistemas hidrotermales, en donde evalúan las relaciones simbióticas, ecología fisiológica, adaptaciones al sulfuro, tolerancias térmicas y adaptaciones sensoriales, así como las formas en que se establecen las comunidades y por qué persisten en estos ambientes (Cerqueira *et al.* 2018; Meier *et al*., 2019; Mars-Brisbin *et al*., 2020; Dede *et al*., 2022); sin embargo, hay pocas investigaciones con un enfoque especial en la bioprospección. La adaptación a estos microhábitats, donde las condiciones son oscilantes, induce una serie de modificaciones en las vías metabólicas primarias y secundarias, por lo que se hipotetiza que los microbios afines a estos sitios son capaces de producir metabolitos estructuralmente únicos (Wilson & Brimble, 2009). Estas características han despertado el interés por el estudio de los sistemas hidrotermales, convirtiéndolos además en una fuente de microorganismos y/o metabolitos con potencial biotecnológico (Gugliandolo *et al.,* 2012) así como para el descubrimiento de nuevos compuestos antimicrobianos (Butler, 2008; Blunt *et al*., 2012; Rodríguez-Valdez, 2017). En este sentido, en el presente trabajo se realiza una bioprospección de microorganismos aislados de sistemas hidrotermales someros con capacidad para inhibir el crecimiento de microorganismos patógenos para el humano, patógenos acuícolas y de interés industrial.

MATERIAL Y MÉTODOS

Área de estudio. Los sistemas hidrotermales en Baja California Sur, México, son dados por fallas tectónicas (Leal-Acosta & Prol-Ledesma, 2016). En dirección NO-SE pasan por Mapachitos en Bahía Concepción (McFall *et al*., 1968), y por el estero del manglar Santispac, el cual cuenta con manantiales de fluidos hidrotermales que son mezclados con agua salada antes de su descarga (Prol-Ledesma *et al.,* 2004). Hacia el Sur del Estado, se encuentra la zona de la falla geológica activa El Sargento, en el abanico delta de Playa Agua Caliente, en donde se ha registrado actividad hidrotermal que atraviesa una larga extensión de playa alimentada por canales subterráneos con los fluidos termales (Nava-Sánchez *et al*., 1995).

Se recolectaron muestras de agua y sedimento en época de lluvias-verano y en estiaje-invierno en los dos sistemas hidrotermales dentro de Bahía Concepción: Mapachitos (26° 40’ 27.58” N; 111° 50’ 37.13” O) y Santispac (26° 45’46.21” N; 111° 53’ 36.91” O), además de muestras de sedimento en la línea de costa en Playa Agua Caliente, El Sargento (24° 6’ 51.01” N; 109° 59’ 54.80” O).

En Mapachitos las muestras se recolectaron mediante buceo SCUBA a una profundidad de 10 a 15 m, en donde la temperatura del agua osciló entre los 75 y 80 °C. Para llegar a esta zona de ventilas se utilizó una lancha con motor fuera de borda.

En Santispac las muestras de agua y sedimento se recolectaron en la zona de manglar a una profundidad aproximada de 2 m y en pozas de marea a 50 cm de profundidad. Las temperaturas en estas zonas oscilaron entre los 50 y 75 °C.

En Mapachitos y Santispac el sedimento se recolectó utilizando nucleadores de plástico de 15 cm de altura, previamente esterilizados, en las zonas de mayores temperaturas, registradas con un termómetro digital. Las muestras de agua se tomaron directamente de los fluidos hidrotermales en bolsas de polietileno estériles, registrando también la temperatura *in situ*. Las muestras se mantuvieron en una bolsa térmica a una temperatura promedio de 60 °C hasta su procesamiento.

En el Sargento, debido a que los fluidos hidrotermales son subterráneos, no se logró llegar hasta ellos, por lo que solo se tomaron muestras de sedimento en las zonas de playa influenciadas por el sistema hidrotermal excavando a una profundidad aproximada a los 2 m, que alcanzaban una temperatura de 70 °C, registrada mediante un termómetro de vástago. Se utilizó un nucleador de plástico de 15 cm de altura y las muestras se mantuvieron en una bolsa térmica a 60 °C para su transporte al laboratorio.

Aislamiento de bacterias. Se realizaron diluciones decimales en solución salina, en condiciones de esterilidad. Posteriormente se sembraron de forma masiva las diluciones 10^4 a 10^7 en cajas Petri con Agar Marino (AM: agar bacteriológico 17 g L^{-1}, peptona de carne 5 g L^{-1}, extracto de levadura 1 g L^{-1}, $FeSO_4$ 5 mg L^{-1} y agua marina filtrada y esterilizada), y fueron incubadas a 60 °C (temperatura máxima de la incubadora) de 24 a 96 horas. Al observarse crecimiento bacteriano, se aislaron individualmente las distintas colonias mediante la técnica de estría cruzada en AM, confirmando su pureza al observar en el microscopio estereoscópico una sola morfología, basados en la forma, borde, elevación y color, en caso de encontrar dos o más se repitió el proceso. Con el fin de conocer si las cepas eran termotolerantes o termófilas, éstas se sembraron por duplicado, colocándolas a temperatura de incubación de 35 y 60 °C. Cada uno de los aislados fue criopreservado con glicerol a -80 °C.

Actividad antibacteriana. Las bacterias aisladas se reactivaron en caldo marino a 60 °C durante 48 h. Posteriormente se centrifugó a 17530 g durante 30 min para obtener el sobrenadante.

Se analizó la actividad antibacteriana de las bacterias aisladas contra patógenos de humanos, patógenos acuícolas y bacterias marinas formadoras de biopelículas que forman parte de la colección del Laboratorio de Microbiología y Biología Molecular del CICIMAR-IPN (Tabla 1) mediante el método de difusión en pozo (NCCLS, 1993). Para ello, se preparó una suspensión celular de cada cepa diana en solución salina (2.5 %) ajustada a 1 x 10^8 cel mL^{-1}, se tomaron 100 µL y se realizó una siembra masiva en medio sólido AM, TSA o Müller-Hinton, dependiendo de la bacteria (Tabla 1). Posteriormente, se perforaron pozos de seis milímetros de diámetro en las placas previamente sembradas con cada una de las cepas diana. Se añadieron 50 µL del sobrenadante de los aislados de los sistemas hidrotermales a los pocillos y las placas se incubaron a 4 °C durante al menos una hora para permitir la difusión de las sustancias activas, seguido de incubación durante 24 h a 35 °C. Se realizaron tres réplicas para cada aislado, como control se utilizó estreptomicina (1 µg mL^{-1}). Se evaluó la presencia de zonas de inhibición alrededor de los pocillos.

Tabla 1. Cepas diana patógenas de humano, patógenas acuícolas y formadoras de biopelículas marinas.

CLAVE	IDENTIDAD	ORIGEN	INTERÉS	MEDIO DE CULTIVO
ATCC BAA-196	*Escherichia coli*	De referencia	Humano	MH
ATCC BAA-42	*Staphylococcus aureus*	De referencia	Humano	MH
ATCC 14990	*Staphylococcus epidermidis*	De referencia	Humano	MH
ATCC 14028	*Salmonella enterica*	De referencia	Humano	MH
ATCC 27853	*Pseudomona aeruginosa*	De referencia	Humano	MH
PA01	*Pseudomona aeruginosa*	De referencia	Humano	MH
ScH3	*Aeromonas caviae*	De referencia	Humano	MH
AH	*Aeromonas hydrophila*	*Puntis conchonius*	Acuícola	TSA
Cf	*Citrobacter freundii*	*Puntis conchonius*	Acuícola	TSA
Es	*Enterobacter sp*	*Puntis conchonius*	Acuícola	TSA
Sx	*Shewanella xiamenensis*	*Puntis conchonius*	Acuícola	TSA
1838	*Streptococcus sp*	*Litopenaeus vannamei*	Acuícola	TSA
1875	*Edwardsiella tarda*	*Oreochromis niloticus*	Acuícola	TSA
321	*Aliivibrio salmonicida*	*Salmo salar*	Acuícola	TSA
331	*Photobacterium damselae* subsp *damselae*	*Damselae damselae*	Acuícola	TSA
346	*Aeromonas salmonicida*	*Salmo salar*	Acuícola	AM
347	*Aeromonas hydrophila*	*Salmo gairdneri*	Acuícola	AM
375	*Shewanella haliotis*	*Litopenaeus sp.*	Acuícola	AM
527	*Streptococcus iniae*	*Inia geoffrensis*	Acuícola	TSA
696	*Listonella anguillarum*	*Gadus morhua*	Acuícola	TSA
G4	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
G6	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
G7	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
G9	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
G10	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
ATCC 17802	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
ATCC CVP2	*Vibrio parahaemolyticus*	*Litopenaeus vannamei*	Acuícola	AM
4M	*Alteromonas simiduii*	Biopelículas marinas *	Biopelículas	AM
6M	*Pseudoalteromonas ruthenica*	Biopelículas marinas *	Biopelículas	AM
11M	*Alteromonas mediterranea*	Biopelículas marinas *	Biopelículas	AM
12M	*Alteromonas macleodii*	Biopelículas marinas *	Biopelículas	AM
7A	*Alteromonas macleodii*	Biopelículas marinas *	Biopelículas	AM
8A	*Vibrio alginolyticus*	Biopelículas marinas *	Biopelículas	AM
9A	*Alteromonas macleodii*	Biopelículas marinas *	Biopelículas	AM
1D	*Alteromonas* sp.	Biopelículas marinas *	Biopelículas	AM
10D	*Aestuariibacter* sp.	Biopelículas marinas *	Biopelículas	AM

* Aisladas de probetas metálicas sumergidas. **MH:** Agar Müller Hinton, **AM:** Agar marino, **TSA:** Agar soya tripticaseina.

Identificación molecular. Solo las bacterias aisladas que presentaron mayor actividad y amplio espectro fueron identificadas molecularmente siguiendo la metodología de Sambrook & Russell (2006). Para ello, se inocularon las bacterias en agar marino por 24 h a 60 °C, posteriormente se tomaron las colonias y se colocaron en microtubos con agua libre de nucleasas, los cuales fueron centrifugados a 17530 g por 10 min y se descartó el sobrenadante. La pastilla obtenida se resuspendió en 575 µL de TE pH 8.0 (0.1 M Tris pH 7.6, 0.001 M EDTA pH 7.5), 30 µL de SDS al 10 % (Dodecil sulfato de sodio), 3 µL de proteinasa K (10 mg mL^{-1}), se mezcló y se incubaron por 2 horas a 37 °C. Después se les añadió 100 µL de NaCl 5 M, se mezclaron y se incubaron 10 min a 65 °C. Posteriormente se agregó 0.8 mL de fenol:cloroformo:alcohol isoamílico 25:24:1 (v/v/v) (Fenol:$CHCl_3$: $C_5H_{12}O$ Alcohol isoamílico) y se homogenizó, se centrifugó 10 min a 17530 g, se recuperó la fase acuosa y se colocó en un tubo nuevo. Fue añadido cloroformo:alcohol isoamílico, 24:1 (v/v), se mezcló y centrifugó 10 min a 17530 g, se recuperó el sobrenadante. Se adicionó 1 mL de isopropanol y se incubó a -20 °C por 24 h. Posteriormente se centrifugó a 17530 g a 4 °C por 20 min, se decantó y se agregaron 500 µL de etanol frío al 70 %, se centrifugó y se decantó el sobrenadante, se volvió a agregar 500 µL de etanol frío al 70 %, se centrifugó nuevamente y se decantó el sobrenadante. Se permitió la evaporación del etanol, posteriormente la pastilla de ADN se resuspendió en 100 µL de TE. Finalmente se agregaron 3 µL de ARNasa (10 mg mL^{-1}) y se incubó a 60 °C por 10 min. El ADN se almacenó a -20 °C hasta su uso. La concentración de ADN extraído se cuantificó en un espectrofotómetro UV-VIS de barrido de microgota (NanoDrop 2000, Thermo Sc).

La amplificación de las muestras de ADN se llevó a cabo mediante PCR, utilizando oligonucleótidos específicos para bacterias: 27F/1385R (GAGTTTGATCCTGGCTA/CGGTGTGTTCAAGGCCC) (Rheims *et al.*, 1996) o los oligos específicos para el género *Bacillus*: rpoB 1206F/rpoB 3202R (ATCGAAACGCCTGAAGGTCCAAACAT/ACACCCTTGTTACCGTGACGACC) (Ulyanova *et al.*, 2016). Se usó 1 µL de cada oligonucleótido (10 µM), 1 µL de dNTPs, 5 µL de amortiguador de PCR 10X, 2.5 µL de $MgCl_2$, 0.2 µL de Taq DNA polimerasa y agua libre RNAsas para ajustar a 50 µL el volumen de cada reacción. Finalmente se añadió la mezcla a cada tubo y se agregó el ADN (50 ng/µL). La reacción para el oligo 27F/1385R se llevó a cabo en un termociclador (MJ Mini, Biorad) bajo las siguientes condiciones: Desnaturalización inicial a 95 °C por 2 min, 29 ciclos de Desnaturalización a 95 °C por 1 min; alineamiento de oligos a 58 °C por 1 min; y extensión/síntesis a 72 °C por 1:30 min; finalmente, una última extensión a 72 °C por 10 min. Mientras que para los cebadores específicos para el género *Bacillus*, se utilizó el siguiente programa: Desnaturalización inicial a 95 °C por 3 min, 35 ciclos de Desnaturalización a 95 °C por 20 seg; alineamiento de oligos a 55.9 °C por 30 seg; extensión, síntesis a 72 °C por 1:30 min; finalmente, una última extensión a 72 °C por 5 min.

La calidad del producto de PCR resultante se evaluó en un gel de agarosa al 1 % con regulador de carga TBE 1X. La muestra (2 µL) se mezcló con buffer de carga (2 µL) a base de LB y GelRed en una proporción 1:8, respectivamente. Se utilizó un marcador de peso molecular de 1,000 pares de bases. La electroforesis se llevó a cabo a 75 V. Los productos de PCR se enviaron a secuenciar a la empresa MacroGen (Seúl, Corea del Sur). Las secuencias se editaron con el programa FinchTV, posteriormente se ensamblaron con el programa Codon Code Aligner y se compararon con otras secuencias genéticas en la base de datos del GenBank: http://www.ncbi.nlm.nih.gov/BLAST/.

RESULTADOS

De un total de 120 bacterias aisladas de los sistemas hidrotermales, se encontró que 87 de ellas (73 %) fueron capaces de inhibir el crecimiento de al menos uno de los microorganismos evaluados (Tablas 2 y 3), con variaciones en los tamaños de los halos desde 2 mm hasta 26 mm de diámetro (Figura 1), los controles con el antibiótico mostraron halos en un rango entre los 22 y 28 mm de diámetro. Solo se mostró antagonismo para 15 de las 36 cepas blanco, siendo las de interés acuícola las más sensibles a los compuestos producidos por las bacterias de los sistemas hidrotermales (Tabla 2). Se presentó una mayor actividad de los aislados contra *Photobacterium damselae* subsp *damselae* (331) siendo inhibida por un 51 % de las bacterias aisladas de los sistemas hidrotermales, considerándose como la cepa más sensible. Por otro lado, se observó una mínima sensibilidad de *Enterobacter* sp. (Es), ya que solo una bacteria aislada mostró actividad antagónica frente a ella (Tabla 2).

Se destaca la bacteria SL108 identificada como *Bacillus paralicheniformis* por presentar mayor actividad inhibitoria, con un halo de 26 mm de diámetro frente al patógeno *Listonella anguillarum* 696 (Tabla 2), frente a este mismo patógeno, la cepa *Bacillus firmus* (SRE61) mostró un halo de inhibición de 24 mm (Tabla 2).

Siete bacterias aisladas (6 %) fueron capaces de inhibir el crecimiento de al menos uno de los patógenos de humano evaluados, siendo más activas frente a *Staphylococcus epidermidis* ATCC14990 (Tabla 3).

Frente a bacterias formadoras de biopelículas marinas, ocho cepas aisladas de los sistemas hidrotermales fueron capaces de inhibir el crecimiento de los microorganismos evaluados, siendo la bacteria *Aestuariibacter* sp. (11M) la que mayor sensibilidad mostró y solamente con las bacterias aisladas durante temporada de lluvia.

Las bacterias aisladas de los sistemas hidrotermales que mostraron los mayores halos y que inhibieron el mayor número de cepas blanco fueron identificadas molecularmente como *Bacillus firmus* (SRE61), *B. sonorensis* (ME87), *B. paralicheniformis* (SL108), *Virgibacillus salarius* (SE88), y *Brevibacillus thermoruber* (ME99) (Tabla 4), siendo en su mayoría de temporada de estiaje.

El mayor número de cepas con actividad antimicrobiana fueron aisladas de El Sargento, de un total de 41 cepas aisladas, 38 (92 %) fueron activas frente a alguna de las cepas diana (Tabla 5).

De acuerdo con la temporada en que se obtuvo el aislado bacteriano hidrotermal, se observó que, de las 58 bacterias aisladas durante temporada de lluvias, 36 (62 %) mostraron capacidad de inhibir el crecimiento de al menos uno de los patógenos. En el caso de las 62 bacterias aisladas durante temporada de estiaje, 51 mostraron actividad (82 %) (Tabla 6).

Los aislados provenientes de Mapachitos predominaron durante la época de estiaje (24) con un 79 % de bacterias productoras de compuestos antimicrobianos, en El Sargento se aisló un menor número de bacterias durante esta temporada; sin embargo, se obtuvo un mayor porcentaje de bacterias con actividad antimicrobiana, siendo el más alto porcentaje de todas las localidades y temporadas de estudio. De Santispac se aisló el menor número de bacterias en la temporada de lluvias y solo el 35 % de ellas mostró actividad antimicrobiana (Tabla 6).

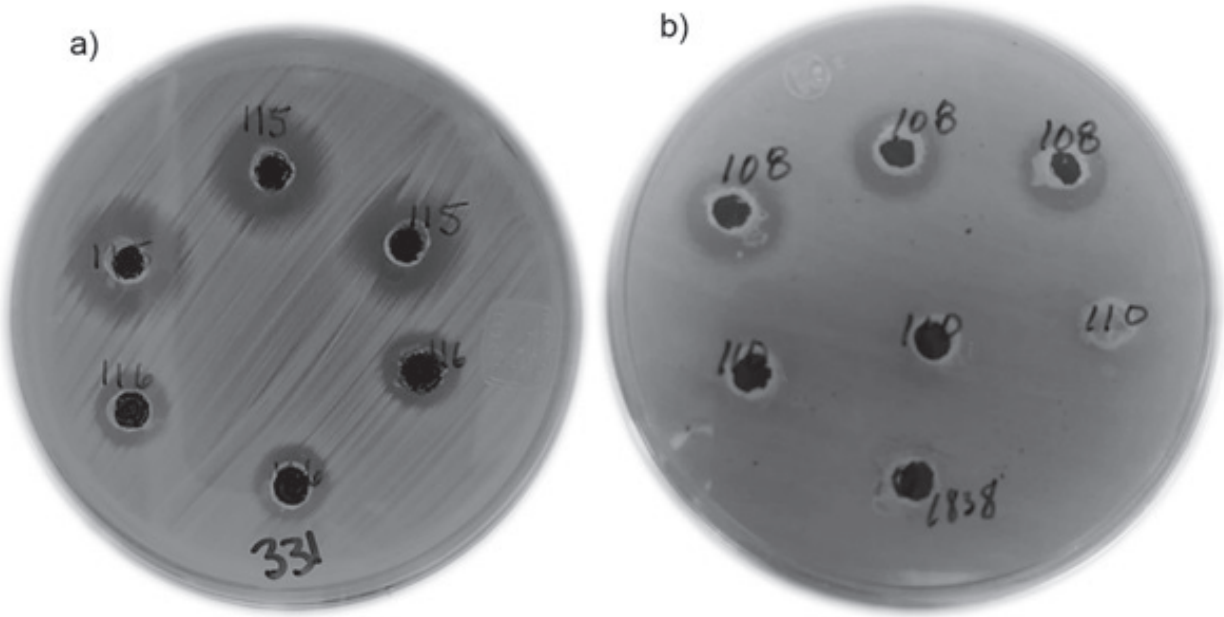

Figura 1. Halos de inhibición producidos por las bacterias aisladas de los sistemas hidrotermales frente a dos patógenos acuícolas; a) 331 (*Photobacterium damselae* subsp. *damselae*) y b) 1838 (*Streptococcus* sp).

De acuerdo con el número de aislados bacterianos con actividad antimicrobiana respecto al sitio, temporada de obtención y bacteria diana probada se observó un mayor número de aislados activos frente a los patógenos acuícolas, predominando los de la temporada de estiaje para Mapachitos y Santispac, mientras que en El Sargento el mayor número de aislados activos frente a este grupo de bacterias diana se obtuvo durante la temporada de lluvias. Por otro lado, se observó baja y en algunos casos nula actividad antagónica frente a bacterias formadoras de biopelículas y patógenos humanos (Figura 2).

DISCUSIÓN

Los microorganismos de ambientes extremos (extremófilos) son caracterizados por la capacidad de sobrevivir a ambientes hostiles con altas temperaturas, pH, presión, salinidad y otros parámetros físico-químicos o la combinación de los mismos (Antranikian *et al*., 2005; Ferrer *et al*., 2007; Jia *et al*., 2013), lo cual resulta en la producción de metabolitos con características únicas que pueden aplicarse en una amplia gama de procesos biotecnológicos (Maugeri *et al.,* 2009, 2010a, 2010b; Gugliandolo *et al*., 2012; Reed *et al*., 2013), como es el caso de bacterias aisladas de agua en ventilas hidrotermales someras de Portugal, que son capaces de degradar enzimas como las amilasas, asimismo, especies del género *Bacillus* aisladas de sedimentos de ventilas hidrotermales profundas en Okinawa son productoras de diversas proteasas (Mohandass *et al*., 2012; Sun *et al*., 2015). Estos sistemas son también considerados una fuente para el descubrimiento de nuevos fármacos, especialmente antibacterianos, por ello, las bacterias aisladas en los sistemas hidrotermales muestreados para este trabajo las vuelve candidatas para la exploración de compuestos con potencial biotecnológico.

Al igual que en el presente estudio, existen otros trabajos en donde se comprueba que los sistemas hidrotermales son sitios con gran diversidad de bacterias productoras de compuestos activos, como el realizado por Eyþórsdóttir (2007) quien encontró que 96 bacterias aisladas de organismos como esponjas, anemonas y algas de los sitios de ventila hidrotermal de Eyjafjörour, Islandia, mostraron actividad antimicrobiana frente a uno o más patógenos evaluados, 42 frente a *Escherichia coli*, 8 frente a *Enterococcus fecalis*, y 56 frente a *Candida albicans*. También, exopolisácaridos producidos por bacterias extremófilas de las ventilas de Isla Eolian, en el Mar Mediterráneo, han mostrado actividad antimicrobiana frente a bacterias Gram Positivas y Gram negativas como *S. aureus*, *E. coli* y *Pseudomonas aeruginosa*, así como contra el hongo *C. albicans* (Scala *et al*., 2019), dentro de estas bacterias activas, destacan las de los géneros *Bacillus* y *Geobacillus,* las cuales han sido registradas también por otros autores (Caccamo *et al*., 2000, Maugeri *et al*., 2002a, 2002b) demostrando ser potentes inhibidores microbianos (Ravindran *et al*., 2016).

Las especies del género *Bacillus* están adaptadas a ambientes cálidos y por lo general son capaces de colonizar nichos oligotróficos como es el caso de marismas, aguas termales y suelos desérticos (Khiyami *et al*., 2012). En el presente estudio, de 120 bacterias aisladas en las temporadas de lluvias y estiaje, 87 (73 %), fueron capaces de inhibir el crecimiento de al menos uno de los patógenos evaluados, las que mostraron mayor actividad en cuanto al tamaño del diámetro del halo de inhibición y el espectro frente a un mayor número de bacterias diana pertenecen al género *Bacillus* (*Bacillus paralicheniformis*, *Bacillus firmus*, y *Bacillus sonorensis*), coincidiendo con lo antes mencionado. De estas especies se conoce que, por ejemplo, *B. paralicheniformis* produce sustancias como antibióticos, vitaminas, aminoácidos y en-

zimas, adicionalmente han mostrado notable actividad antimicrobiana de amplio espectro, siendo eficaces contra bacterias, levaduras y hongos (Ahire *et al*., 2020). La cepa termofílica POD1 identificada como *B. paralicheliformis* aislada de una fuente termal de la India por Ojha *et al*. (2021) produce una lisozima con actividad frente a bacterias multirresistentes como *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* y *Lactobacillus reuteri.* También se ha reportado la producción de biosurfactantes con actividad antimicrobiana por cepas termotolerantes del género *Bacillus* aisladas de fuentes termales de Tailandia, entre ellas, *B. firmus, B. licheniformis, B. subtilis* y *B. pumilus* (Pakpitcharoen *et al*., 2008), y de péptidos antimicrobianos como bacteriocinas, producidos por *B. sonorensis* con actividad frente a *S. aureus* y *Listeria monocytogenes* (Chopra *et al*., 2014).

En el presente estudio, se identificó a las especies *Virgibacillus salarius* y *Brevibacillus thermoruber* como antagonistas de algunos de los patógenos diana. Otros autores han reportado que estas especies son productoras de compuestos que inhiben el crecimiento de patógenos multirresistentes (Radchenkova *et al*., 2011; Ayuningrum *et al*., 2020) y se menciona también que están emparentadas filogenéticamente con el género *Bacillus* (Hua *et al*., 2008; Yohandini *et al*., 2015), por lo que se puede inferir que estas cepas, al igual que *Bacillus*, producen diversos compuestos capaces de inhibir a diferentes microorganismos.

Gutiérrez-Almada *et al.* (2020) realizaron el aislamiento de las cepas bacterianas con las que se trabajó en el presente estudio, probando el potencial de extractos para inhibir la formación de biopelículas frente a dos cepas patógenas de humano (*Pseudomonas aeruginosa* PAO1 y *Aeromonas caviae*) y una cepa patógena de interés acuícola (*Vibrio parahaemolyticus),* por lo que, con los resultados de dicho trabajo, en conjunto con el presente estudio, se confirma su potencial para la producción de compuestos antibacterianos en diferentes campos de aplicación.

Tabla 2. Actividad antimicrobiana de las bacterias aisladas de los sistemas hidrotermales (SH) frente a patógenos acuícolas (Datos expresados en mm como la media ± desviación estándar de los ensayos por triplicado).

Aislados de SH	Cepas diana									
ID	**1838**	**1875**	**331**	**G7**	**346**	**347**	**696**	**375**	**Cf**	**Es**
ME40			2±0.0							
ME41							19±0.2			
ME42			4±0.0				20±0.1			
ME48			4±0.0							
ME50			4±0.0							
ME51			12±0.0							
ME52			10±0.0							
ME55			12±0.0				20±0.2			
ME73			10±0.0							
ME80							6±0.0			
ME81	2±0.0									
ME82	7±0.0		14±0.1				20±0.1			
ME87			11±0.1			2±0.0	20±0.2			10±0.0
ME89			9±0.0				10±0.1			
ME95	4±0.0						18±0.1			
ME96	2±0.0									
ME98	2±0.0	3	10±0.0						2±0.0	
ME99	9±0.0	2	23±0.3		6±0.0				3±0.0	
ME100			4±0.0							
ML102			2±0.0		4±0.0					
ML103			7±0.0							
ML104	6±0.0		8±0.0		7±0.0					
SE36			10±0.1							
SE38			8±0.0							
SE39			9±0.1							
SE43			8±0.0				20±0.5			
SE44			6±0.0							
SE46			2±0.0							
SE47			2±0.0							
SE53			8±0.0							

Aislados de SH	Cepas diana						
SE54			6±0.0				
SE88						18±0.1	
SE91			10±0.1		6±0.0	20±0.0	
SE92			12±0.2				
SE94	5±0.0	6±0.0	12±0.0				
SE97	2±0.0						
SL101			2±0.0				
SL105	4±0.0		12±0.1	7±0.0			
SL106			10±0.0	7±0.0	2±0.0		
SL108	7±0.0		10±0.0	6±0.0		26±0.3	
SRE56			2±0.0				
SRE57			4±0.0				8±0.0
SRE58			6±0.0				
SRE59			2±0.0				
SRE60			8±0.1			10±0.0	
SRE61			7±0.0			24±0.4	
SRE62			4±0.0			18±0.2	
SRE63			2±0.0				
SRE65			12±0.1				
SRE66			10±0.0				
SRE67			6±0.0				
SRE78					4±0.0	12±0.2	
SRE79						4±0.0	7±0.0
SRE83	2±0.0						
SRE84						10±0.3	
SRE85			10±0.0			20±0.3	
SRE86						7±0.0	
SRL107			12±0.0	5±0.0	7±0.0		
SRL109				2±0.0			
SRL110				2±0.0			
SRL111			8±0.0	6±0.0			
SRL112			8±0.0	8±0.0			
SRL113			8±0.0	4±0.0	2±0.0		
SRL114			10±0.0	4±0.0			
SRL115			12±0.3				
SRL116			6±0.0	6±0.0	4±0.0		
SRL117		6±0.0	10±0.0	8±0.0	5±0.0		
SRL118			4±0.0		2±0.0		
SRL119			10±0.0	9±0.3			
SRL68			8±0.0				
SRL69			10±0.0	2±0.0			
SRL70			6±0.0				
SRL71							
SRL72	4±0.0		2±0.0				
SRL74			8±0.0	4±0.0			
SRL75			14±0.2				
SRL76			7±0.0	4±0.0			
SRL77				2±0.0		14±0.5	

1838: *Streoptococcus* sp, 1875: *Edwardiella tarda*, 331 *Photobacterium damselae* subsp. *damselae*, G7: *Vibrio parahaemolyticus*, 346: *Aeromona salmonicida*, 347: *A. hydrophila*, 696: *Listonella anguillarum*, 375: *Shewanella haliotis*, Cf: *Citrobacter freundii*, Es: *Enterobacter* sp.

Tabla 3. Actividad antimicrobiana de las bacterias aisladas de los sistemas hidrotermales (SH) frente a patógenos de humanos y bacterias formadoras de biopelículas (Datos expresados en mm como la media ± desviación estándar de los ensayos por triplicado).

Aislados de SH	Cepas diana				
ID	**ScH3**	**ATCC14028**	**ATCC14990**	**10D**	**11M**
ME52			4±0.0		
ML10					4±0.0
ML11			4±0.1		6±0.2
ML12					2±0.0
ML14					2±0.0
ML19					2±0.0
ML20		2±0.0			5±0.0
ML9				20±0.5	
SE38			4±0.0		
SL23		4±0.0			6±0.3
SRE56					
SRE61			6±0.0		
SRL107	6±0.7				

ScH3: *Aeromonas caviae,* ATCC14028: *Salmonella enterica*, ATCC14990: *Staphylococcus epidermidis*, 10D: *Aestuariibacter* sp., 11M: *Alteromonas mediterranea.*

En cuanto a las cepas blanco, nueve de los patógenos acuícolas mostraron sensibilidad frente a las bacterias de origen termal, siendo *P. damselae* subsp *damselae* la más sensible con 61 casos de inhibición con halos desde 2 mm hasta 26 mm de diámetro. Este es uno de los patógenos bacterianos más devastadores en la acuicultura marina, al tratarse de una bacteria que prevalece a temperaturas de 37 °C utilizando los sedimentos marinos como reservorio y el agua de mar como vehículo de transmisión de infecciones. Como ejemplo de afecciones que ocasiona, se incluyen la pasteurelosis en organismos marinos y úlceras o septicemias en humano a causa de la exposición en heridas cutáneas superficiales en contacto al medio marino, en ambos casos puede llegar a provocar la muerte del individuo (Botella *et al.*, 2002; Rivas *et al.*, 2013). Otra cepa sensible fue *Listonella anguillarum* (*Vibrio*), una bacteria marina patógena de un gran número de organismos acuáticos, incluidas especies de importancia en la industria acuícola como camarón, bivalvos y peces, causando ulceraciones internas y externas, distención abdominal, letargo, pérdida de apetito, necrosis y eventualmente la muerte (Hickey & Lee, 2018). Por otro lado, bacterias formadoras de biopelículas como *Aestuariibacter* sp. se adhieren a sustratos sumergidos, como es el caso de embarcaciones, muelles, plataformas marinas y sistemas acuícolas; estas bacterias son pioneras en el proceso de sucesión de las comunidades que forman el conocido "*biofouling*" que incluye bacterias, microalgas, esporas, larvas y macroorganismos, lo cual causa deterioro y problemas económicos principalmente para la industria naviera, petrolera y acuícola (Moura *et al.*, 2018; Erni-Cassola *et al.*, 2020). Por lo que los resultados obtenidos en este estudio muestran un buen potencial para atender la problemática que causan estos microoganismos en las diferentes industrias.

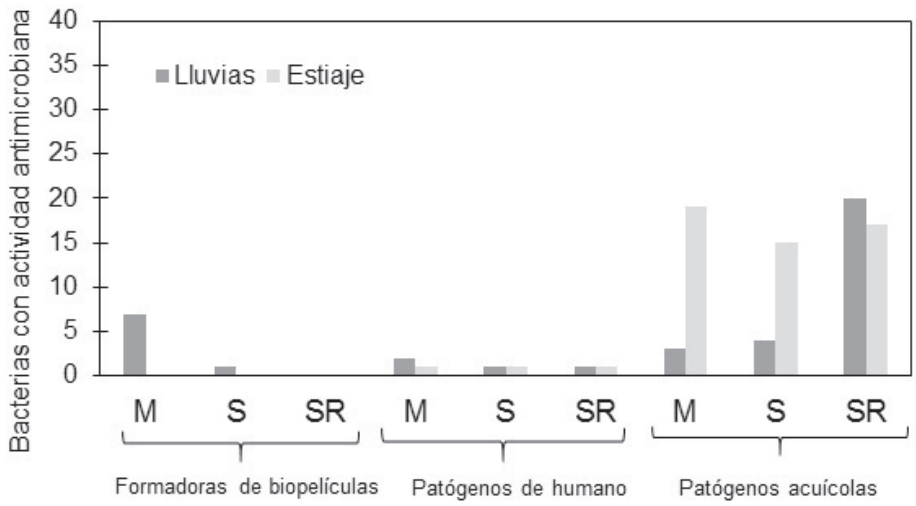

Figura 2. Número de bacterias con actividad antimicrobiana de acuerdo con el sitio de recolecta (M: Mapachitos, S: Santispac, SR: El Sargento), temporada de obtención y cepa diana.

Tabla 4. Identificación molecular de las bacterias aisladas de los sistemas hidrotermales que mostraron mayor actividad antibacteriana.

ID	Especie más cercana	% de identidad	Sitio de recolecta	Muestra	Temporada
SRE61	*Bacillus firmus*	100	El Sargento	Sedimento	Estiaje
ME87	*Bacillus sonorensis*	99	Mapachitos	Sedimento	Estiaje
SL108	*Bacillus paralicheniformis*	99	Santispac	Sedimento	Lluvias
SE88	*Virgibacillus salarius*	99	El Sargento	Sedimento	Estiaje
ME99	*Brevibacillus thermoruber*	98	Mapachitos	Sedimento	Estiaje

Tabla 5. Bacterias aisladas de sistemas hidrotermales que presentan actividad antimicrobiana frente a cepas blanco respecto al sitio de obtención.

Sitio	Bacterias aisladas	Número de bacterias con actividad antimicrobiana	Porcentaje de bacterias con actividad antibacteriana
Mapachitos	45	29	64
Santispac	34	20	58
El Sargento	41	38	92
Total	120	87	73

Tabla 6. Bacterias aisladas de sistemas hidrotermales que presentan actividad antimicrobiana frente a las cepas diana respecto a la temporada de obtención y sitio de recolecta.

	Bacterias aisladas		Número de bacterias con actividad antibacteriana		Porcentaje de bacterias con actividad antibacteriana	
Sitio	Lluvias	Estiaje	Lluvias	Estiaje	Lluvias	Estiaje
Mapachitos	21	24	10	19	48	79
Santispac	14	20	5	15	35	75
El Sargento	23	18	21	17	91	94
Total	58	62	36	51	62	82

Al parecer, las bacterias con actividad antagónica que se desarrollan en El Sargento no están afectadas por la época (lluvia o estiaje) pues en ambos casos se obtuvo un número considerable de este tipo de bacterias, sin embargo, para los otros sitios el estiaje parece influenciar la presencia de bacterias con bioactividad contra los patógenos probados. Esto pudiera estar relacionado a que El Sargento es una localidad con playas arenosas que cuentan con manifestaciones termales constantes a lo largo de su línea de costa (Nava-Sánchez *et al.*, 1995), con temperaturas registradas en el momento de las recolectas, que oscilan entre los 45 y 70 °C, sin presentar emanaciones con fluido termal que pudieran tener un efecto sobre las variaciones en estas comunidades microbianas.

Debido a que un mayor número de aislados fueron activos contra patógenos de peces, estos resultados nos brindan la oportunidad de dirigir futuros estudios para su uso potencial en sistemas acuícolas, tanto de agua dulce como salada, teniendo mayor interés en las cepas identificadas como *Bacillus firmus, Bacillus sonorensis, Bacillus paralicheniformis, Virgibacillus salarius* y *Brevibacillus thermoruber* que inhiben el crecimiento de bacterias patógenas como *Photobacterium damselae* subsp *damselae* y *Listonella anguillarum.*

Se concluye que las bacterias provenientes de sistemas hidrotermales poseen la capacidad de inhibir el crecimiento de patógenos acuícolas, humanos y formadoras de biopelículas marinas convirtiéndose en una alternativa para el uso de antibióticos.

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Research capability gaps hinder understanding of the impact of climate change on ecosystem services in the Latin American Pacific coast

Las brechas en la capacidad de investigación dificultan la comprensión del impacto del cambio climático en los servicios ecosistémicos en la costa del Pacífico latinoamericano

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ABSTRACT

Background. Coastal communities are highly dependent on ecosystem services, but the benefits and livelihoods people derive from natural ecosystems are directly and indirectly affected by climate change. The need for a mechanistic understanding of how components of climate change translate into measurable impacts on ecosystems and society is fundamental to the ability to manage, plan and mitigate for the most likely environmental futures, yet progress in this area in tropical and subtropical countries is frustrated by a lack of research capacity at the local and regional level. **Objectives.** Here, we investigate the research capacity of the countries along the Pacific coast, between Mexico and Chile, a region with an extensive coastline (23,191 km) that spans 11 countries of varying socio-economic development status and anticipated to be especially vulnerable to climate change. **Methods.** Specifically, our focus was to explore how the effects of climate change on ecosystem services (provision, regulation and cultural) may relate to research capacity and gross domestic product (GDP) in each country along the Pacific coast of the Americas. **Results.** We find that, since 1980, the number of peer-reviewed scientific studies relevant to this topic strongly correlates with GDP ($r = 0.90$, $p < 0.05$) and that research effort is an order of magnitude lower along the Latin American Pacific coast (13.8 studies 1000 km^{-1}) than in the neighbouring Californian coast (103 studies 1000 km^{-1}). **Conclusions.** Our results highlight the need to better develop the research in the Latin America Pacific, and for more work on the key links between climate change and ecosystem services.

Keywords: Food security, global warming, knowledge gap, natural hazards, poverty

RESUMEN

Antecedentes. Las comunidades costeras son altamente dependientes de los servicios ecosistémicos; sin embargo, los beneficios y el modo de vida de sus habitantes son afectados directa e indirectamente por el cambio climático. Por tanto, es necesario entender cómo el cambio climático se traduce en impactos medibles sobre la sociedad y los ecosistemas para implementar planes de manejo y de mitigación, pero no se cuenta con la capacidad de investigación local y regional para ello. **Objetivos.** Investigar la capacidad de investigación de los países latinoamericanos de la costa del Pacífico, desde México hasta Chile, una región de 23,191 km de largo, que comprende 11 países con diferente grado de desarrollo socio-económico y que serán especialmente vulnerables al cambio climático. **Métodos.** Específicamente, nos enfocamos en explorar como los efectos del cambio climático en los servicios ecosistémicos (provisión, regulación y cultural) se relacionan con la capacidad de investigación y el producto interno bruto de los países en la costa del Pacífico de Latinoamérica. **Resultados.** Encontramos que desde 1980 el número de estudios científicos publicados relacionados con el tema se correlaciona con el PIB ($r = 0.90$, $p< 0.05$) y el esfuerzo de investigación es un orden de magnitud menor en la costa de Latinoamérica (13.8 estudios por 1,000 km) que en la vecina costa de California (103 estudios por 1,000 km). **Conclusiones.** Nuestros resultados resaltan la necesidad de promover la investigación en la zona costera latinoamericana y de realizar más trabajos en aspectos clave de la relación entre cambio climático y servicios ecosistémicos.

Palabras clave: Brecha de conocimiento, calentamiento global, desastres naturales, pobreza, seguridad alimentaria

INTRODUCTION

The Millennium Ecosystem Assessment Report (MEA, 2005) highlighted the links between ecosystem and human well-being and the benefits as goods and services that humanity gets from nature, i.e., ecosystem services. What followed was a new impetus to consider ecosystem services (96% out of 35,284 publications since 2006, Web of Science retrieved May 4, 2020) but very few contributions (19%) that made explicit links between the provision of ecosystem services and climate change. Furthermore, although it is well known that species richness increases from the temperate regions to the tropics, and that biodiversity is fundamental to the natural capital on which many ecosystem services depend (Sandifer *et al*., 2015), even the most cursory examination of the emergent literature reveals that most of the studies were conducted in developed countries. Additionally, there is a negative correlation between species richness and wealth: the countries of Southeast Asia, Africa, and Latin America harbour high diversity but their gross domestic product (GDP) *per capita* is generally low. Moreover, these regions are considered disproportionately more vulnerable to the direct and indirect effects of climate change because they tend to "*include disadvantaged and vulnerable populations, some indigenous peoples, and local communities dependent on agricultural or coastal livelihoods (high confidence)"* (IPCC, 2018).

Marine environments are likely to be particularly vulnerable to climate change, as coastal and marginal seas host a disproportionately large fraction of productivity, and the marine environment is believed to harbor the highest biodiversity in the world (Mora *et al*., 2011). Rising atmospheric temperatures have increased global heat content in the upper 300 m of the oceans at a rate of about 0.04ºC decade^{-1} (IPCC, 2018), which has been linked to global sea-level rise (7-82 cm by 2100; (Siddall *et al*., 2009), and extensive areas of intertidal habitats are predicted to be lost due to a reduction of the intertidal zone (coastal squeeze) associated with thermal expansion (Gabler *et al*., 2017). Such physical changes will have significant implications for the future distribution and characteristics of coastal, intertidal and near-shelf ecosystems and their associated ecosystem properties (Gabler *et al*., 2017). Further, these systems are already compromised by multiple human activities, including overfishing, habitat destruction, and pollution (Cinner *et al*., 2020). Moreover, while coastal ecosystems are likely to experience a sizeable proportional change in their physical, chemical, and biological characteristics, fundamental differences exist between marine and terrestrial systems that lead to varying expectations in their response and adaptation time compared with terrestrial systems (Steele *et al*., 2019).

Here, we specifically investigate patterns of research capability on ecosystem services and climate change in the coastal ecosystems along the Latin American Pacific coast. This coast covers a total length of 23,191 km from the US-Mexico border to Tierra del Fuego, Chile, and extends across 11 countries (Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Peru, and Chile, figure 1). It encompasses numerous coastal ecosystems within a series of contrasting climate domains (from temperate to tropical) and encompasses four Large Marine Ecosystems (LME; California Current, Gulf of California, Pacific Central American Coast and Humboldt Current (Sherman, 1991, 2014). The coast is also particularly vulnerable to the consequences of climate change including sea level rise, rising sea surface temperatures (SST), changes to circulation patterns, ocean acidification and increasing frequency and strength of storms and hurricanes (Pérez-Maqueo *et al*., 2018; IPCC, 2018). Human activities that are likely to interact with or modify the ecological outcome of climate change are also prevalent, including extensive aquaculture systems replacing natural systems such as mangroves, tourism, unsustainable and unregulated fishing, untreated discharge of waste waters and agricultural run-off, and construction of harbour infrastructure (such as marinas and seawalls).

It is important to note that inadequacies and opportunities within the study of ecosystem services in Latin America has been highlighted before, but for different purposes. Balvanera *et al*. (2012, 2020) and recently Perevochtchikova *et al*. (2019) provide a comprehensive review and conclude that in Latin America ES supply and links to policy are most frequently assessed. However, the emphasis is placed on a limited number of services, namely carbon capture and water.

Dangles *et al*. (2016) showed that even when the studies were performed on sites located in LAC territories, only one third of the publications were led by Latin American authors, while Europeans authored 2.8 times more publications. Furthermore, those authors point out that only 11% of those studies dealt with marine ecosystems.

This gap is widening national expenditure in ocean science as a percentage of national research and development expenditure has fallen in Colombia (Balvanera *et al*., 2012) and except for Brazil, the most vigorous relative growth in scientific output has occurred in regions outside of South America (Valdés, 2017). Furthermore, food utilisation, access, and stability, which constitute significant food security challenges in the world, remain under-investigated in developing countries from Asia, Africa, and Latin America (Cruz-Garcia *et al*., 2016), and few assessments of the risk of ecosystem service provisioning under climate change have taken place across the region (Asmus *et al*., 2019).

One reason for this low number of studies, at least in part, is that the research capability in Latin America countries (LAC) is comparatively low; for example, in Mexico there are only 244 researchers million^{-1} population, whereas in the UK there are 4,400, and in USA there are 4,300 million^{-1} population (UIS-UNESCO, 2018). Here, motivated by the need to target research effort to areas most likely to be affected by climate change and facilitate directed efforts where most warranted, we provide an overview of 1) the extent of published research on ecosystem services and climate change in coastal ecosystems the Pacific coast of the LAC, and 2) the degree to which these publications correlate with socio-economic status, as quantified by GDP. We hope is that this contribution will be a first step in understanding how existing research capacities are being used to generate knowledge that support ocean management and policy, whilst identifying where further research emphasis is needed to best contribute towards attaining a sustainable future.

METHODS

We performed a systematic review to retrieve papers dealing with ecosystem services and climate change in one or more countries in Latin America. Then we explore the relationship among number of papers and selected geo-statistics of countries, including California, as a reference of what would be a developed country´s investment in research and development on the eastern Pacific coast.

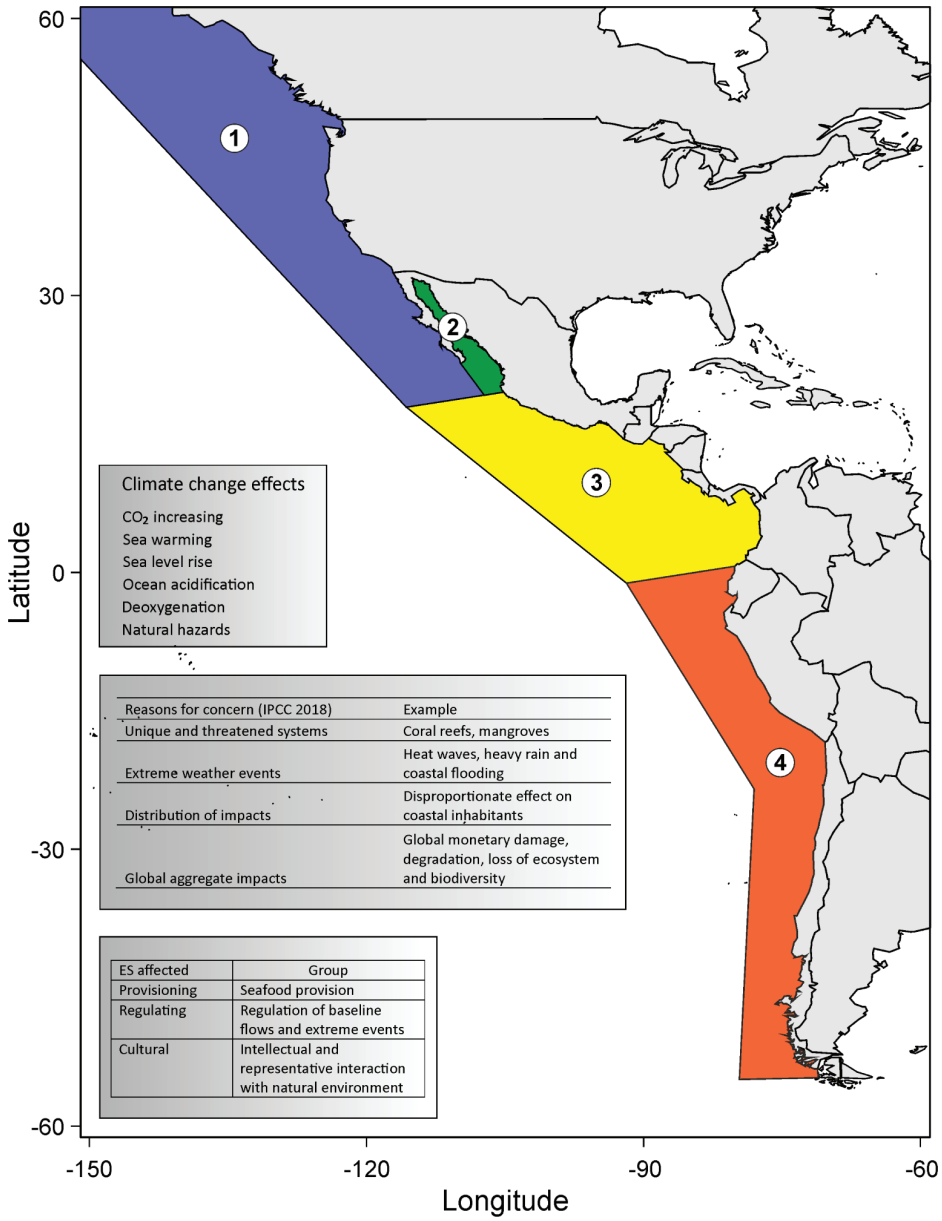

Figure 1. Climate change effects, reasons for concern, and ecosystem affected in coastal and marine ecosystems from Latin American countries. Large Marine Ecosystems are shown: 1. California Current; 2. Gulf of California; 3. Pacific Central American Coastal; 4. Humboldt Current.

Literature review. We searched the Thomson Reuters Web of Science collection (http://www.webofknowledge.com, accessed April 2020) using a 'Basic Search' across all databases with the search term ecosystem service* in the title, abstract, author keywords, and Keywords Plus of all document types, in all languages, for the publication years January 1980 – April 2020. This global search returned 35,359 contributions worldwide from marine and terrestrial systems (#1); we repeated the search but using 'climate change' as the search term and got 212,552 references (#2). We then combine #1 AND #2 and found 6,466 contributions (#3). Subsequently, we used the Advance Search option with the string ALL=("Chile" OR "Colombia" OR "Costa Rica" OR "Ecuador" OR "El Salvador" OR "Guatemala" OR "Honduras" OR "Mexico" OR "Nicaragua" OR "Panama" OR "Panamá" OR "Peru" OR "Perú" OR "Latin America" OR "América Latina" OR "South America" OR "Suramerica" OR "America del Sur" OR "Central America" OR "América Central" OR "Centroamérica" OR "Mesoamérica") NOT ALL=("Gulf of Mexico" OR FLORIDA) to g*et all* references relating to Latin American countries from the Pacific coast, obtaining 978,319 references (#4). Finally, we combined #3 AND #4 to get 616 contributions that meet our selection criteria of dealing with ecosystem services and climate change in one or more countries from Latin America. We manually screened the titles and abstract of the returned subset to retain only contributions focussing on the marine and coastal environment (n = 319, Table 1).

Table 1. Selected geo-statistics from Latin American countries (Full data and sources in appendix 2). Data for California for comparison.

Country	Studies on coastal ecosystem services	Researcher/ million people	Studies on ES/RD	Studies on ES/ Coastal inhab 10^5	HDI	Fisheries Landed Value in 10^3 USD	National Marine area %	Tourism as % of exports	Sea level Anomaly mm
California	139	4245	3.27E-02	0.67	0.92		16.00%	10	103.82
Mexico	130	252	5.16E-01	0.27	0.767	1,745,795	21.55%	5	121.144
Guatemala	1	14	7.11E-02	0.01	0.651	45,279	0.90%	11	177.45
El Salvador	2	66	3.03E-02	0.14	0.667	62,040	0.71%	16	155.61
Honduras	2	35	5.77E-02	0.22	0.623	63,700	4.58%	6	94.46
Nicaragua	1	70	1.42E-02	0.07	0.651	68,030	2.97%	9	111.72
Costa Rica	18	380	4.73E-02	6.95	0.794	37,171	2.61%	20	159.37
Panama	13	39	3.32E-01	1.67	0.795	153,258	1.68%	22	121.24
Colombia	22	89	2.49E-01	0.25	0.761	183,314	17.15%	13	101.99
Ecuador	27	401	6.74E-02	0.36	0.758	1,166,585	13.35%	8	143.88
Peru	19	140	1.35E-01	0.25	0.759	3,911,989	0.48%	9	117.37
Chile	84	502	1.67E-01	4.62	0.847	2,879,355	41.19%	5	78.82

To establish the relative importance of the search terms concerning other regions, we repeated our searches for California, a Pacific state in a highly developed country (the USA) with a population and economy comparable to a medium-sized developed country. A detailed analysis of references found in each query is presented in the online supporting information.

The rationale of proxy variables selection. We selected variables that take us to accomplish our aim to explore the extent each country could cope with climate change impacts based on their R&D capacity and economy. We also wanted to assess the vulnerability of people close to the coast. We selected classes of ecosystem services (ES) of the three sections (provisioning, regulation, maintenance, and cultural) *sensu* the Common International Classification of Ecosystem Services CICES (Haines-Young & Potschin, 2018) for comparison. We used fisheries landed value (FAO, 2018) as a proxy of the service class *Wild animals (terrestrial and aquatic) used for nutritional purposes;* percentage of marine protected areas in waters of natural jurisdiction of each country (UNEP, 2020) as a proxy of service class *Maintaining nursery populations and habitats (including gene pool protection)*, under the assumption that the larger the protected area, the larger the benefit of this ES. Finally, we used tourism percentage of exports (UNWTO, 2020) as a proxy of the ES class *Characteristics of living systems that enable activities promoting health, recuperation, or enjoyment through active or immersive interactions* to have an idea of how much of the country's economy depends on this ES and therefore how much would be affected by climate change. Tourism represents 8.8% of the gross domestic product of Latin America, about 299 billion dollars per year (https://wttc.org/en-gb/Research/Economic-Impact), and the sector creates one in four new jobs. For comparison, we normalised $\left(\frac{x_i}{x_{max}}\right)$ these three variables.

To overcome the lack of information for all countries in some other metrics, we used sea level rise (SLR; World Bank, 2020) as a proxy of the magnitude of climate change impact, under the assumption that the higher the rise, the stronger the impact. SLR and other oceanic climate changes will result in salinization, flooding, and erosion and affect human and ecological systems, including health, heritage, freshwater, biodiversity, agriculture, fisheries, and other services (Weissenberger & Chouinard, 2015; CIA, 2018; see details in supporting information (SI). Because higher SLR is detrimental, once normalized, we multiplied it by -1.

We weighted the number of published studies by the number of coastal inhabitants - Total population by country (CIA, 2018), the number of coastal inhabitants (CEDLAS, 2018), the number of researchers, and the length of coastline (SI). According to UIS (2018), researchers are professionals who conduct research and improve or develop concepts, theories, models, techniques, instrumentation, and operational methods. We acknowledge that the use of the number of researchers will overestimate research expertise, as not all national researchers investigate climate change and ecosystem services. In order to indicate whether research capacity is linked to the nation's wealth, metrics were correlated with the Gross domestic product (GDP) of the countries under study (World Bank, 2018).

To weigh the number of people in each country, we used the gross national income per capita (GNI, formerly GNP per capita), converted to U.S. dollars using the World Bank Atlas method, divided by the midyear population. GNI is the sum of value added by all resident producers plus any product taxes (fewer subsidies) not included in the valuation of output plus net receipts of primary income (compensation of employees and property income) from abroad (World Bank, 2018). As a proxy of well-being, we used the Human Capital Index (HCI), which is designed

to highlight how improvements in current health and education outcomes shape the productivity of the next generation of workers if children born today experience over the next 18 years the educational opportunities and health risks that children in this age range currently face; see details in SI. Despite both indices being correlated, we also used the Human Development Index (HDI) because it goes beyond the economy of each country and challenges national policy choices, asking how two countries with the same level of GNI per capita can end up with different human development outcomes (UNDP, 2020). The HDI is the geometric mean of normalized indices for three critical dimensions of human development: a long and healthy life, being knowledgeable, and having a decent standard of living; see details in SI.

RESULTS

We found that most studies (224/319, = 70%) investigating the impact of climate change on ecosystem services across the Latin America Pacific coast have been published since 2015.

As expected, the number of publications depends on the GDP of the country (adjusted $r^2 = 0.70$, $p < 0.01$), but decreases with the GNI per capita (adjusted $r^2 = 0.45$, $p < 0.01$), this is because Costa Rica and Panama have less GDP and publications than Mexico, but higher GNI per capita. The lack of knowledge from Central America is critical since there is only one paper from Guatemala and Nicaragua and just two from El Salvador and Honduras (Table 1). When weighted by km of coastline, research effort amounts to 13.8 studies 1000 km^{-1} of coastline in the Latin American Pacific, compared to 103 studies 1000 km^{-1} of coastline in California. There are 0.37 studies per 100,000 coastal inhabitants in Latin America, but with huge disparities: from 0.01 in Guatemala to 6.95 in Costa Rica (Table1).

The Pearson correlation matrix among variables is presented in Table 2. Number of studies is significantly correlated ($p < 0.05$) with HCI ($r = 0.66$) and % MPA (0.76) and so HDI with the research capacity (0.71) and studies per 100,000 coastal inhabitants (0.61).

Figure 2 depicts the relative importance of selected ES for the region; Seafood from wild animals and plants is of utmost importance to Peru, followed by Chile, Mexico, and Ecuador, but it is of almost negligible importance for the other countries. Chile has high coverage of MPAs, which help with *Maintaining Nursery Populations and Habitats and Gene pool protection*; levels are also high for Mexico, Colombia, and Ecuador. Peru has little in the way of MPAs despite depending so heavily on fisheries. Central America depends heavily on cultural services since tourism can account for as high as 22% of exports for Panama and 20% for Costa Rica. Ecuador has both high dependence on fisheries and high coverage of MPAs, an a strong dependence on tourism (Figure 2). Using SLR as the metric, vulnerability to climate change indicates that Guatemala, Costa Rica, El Salvador, and Ecuador are the worst affected (SI).

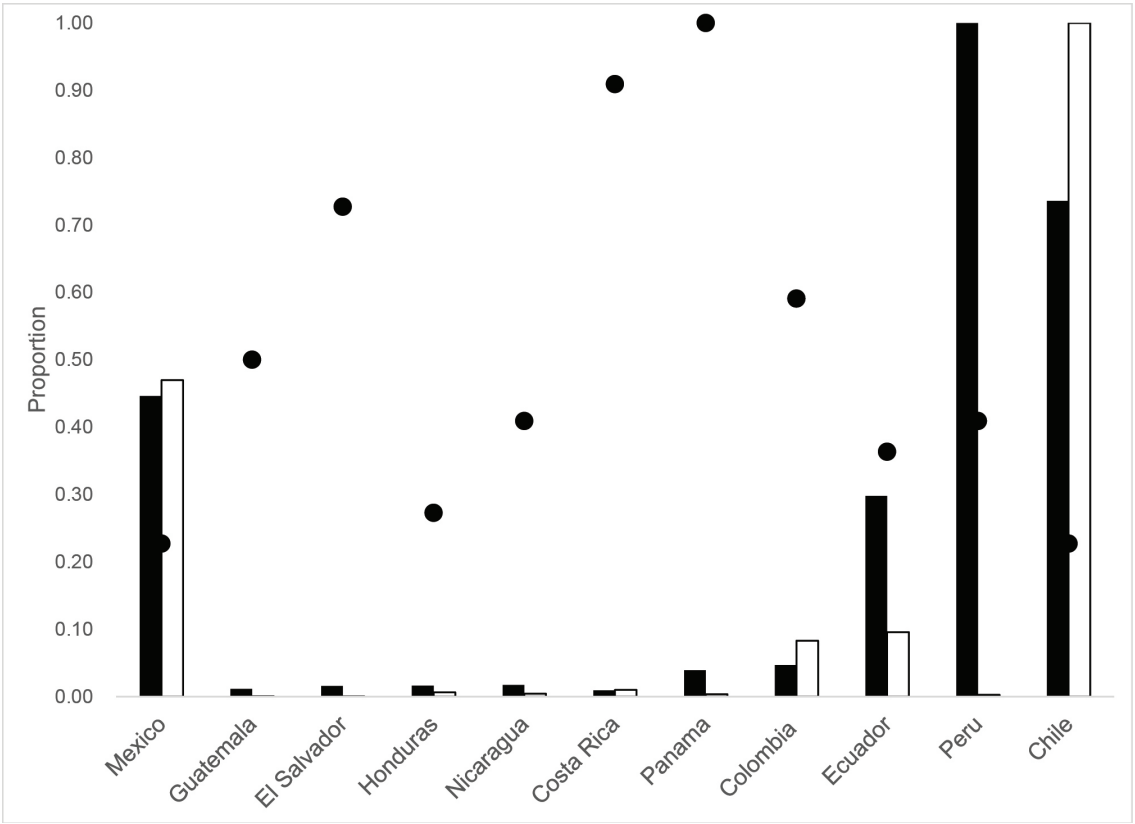

Figure 2. Max normalized value of fisheries landed (black bars), the proportion of marine protected area in waters of national jurisdiction of Latin American countries on the coast of the eastern Pacific (empty bars), and proportion of tourism to exports (black dots).

Table 2. Pearson's correlation matrix of variables. Numbers in **bold** are significant at $p<0.05$. Source of data in supplemental material.

	Studies on coastal ecosystem services	Total Population	Pop region coast	Researcher/ million people	Studies on ES/RD	Studies on ES/Coastal inhab 10^5	Length of coastline km	Studies on ES/1000 km coast	GDP millions USD	HDI	HCI	GNI per capita	Tourism as % of exports	Marine Area of National Jurisdiction km^2	National MPA km^2	National Marine area %	Fisheries Landed Value
Studies on coastal ecosystem services (ES)	1																
Total Population	**0.81**	1															
Pop region coast	**0.79**	**0.97**	1														
Researcher/ million people (RD)	0.57	0.12	0.11	1													
Studies on ES/RD	**0.74**	**0.81**	**0.77**	0.05	1												
Studies on ES/Coastal inhab 10^5	0.17	-0.2	-0.3	0.66	-0.1	1											
Length of coastline km	**0.97**	**0.72**	**0.68**	**0.62**	**0.68**	0.17	1										
Studies on ES/1000 km coast	0.44	0.33	0.25	0.53	0.35	0.6	0.34	1									
GDP millions USD	**0.9**	**0.98**	**0.96**	0.25	**0.84**	-0.1	**0.82**	0.38	1								
Human Development Index	0.56	0.22	0.14	**0.71**	0.49	**0.61**	**0.63**	0.53	0.34	1							
Human Capital Index	**0.66**	0.34	0.23	**0.86**	0.33	0.57	**0.71**	0.59	0.43	**0.86**	1						
GNI per capita	0.49	0.09	0.06	0.56	0.5	**0.69**	0.54	0.48	0.24	**0.9**	**0.6**	1					
Tourism as % of exports	-0.5	-0.4	-0.4	-0.3	-0.1	0.34	-0.5	0.09	-0.4	0.15	-0	0.31	1				
Marine Area of National Jurisdiction km^2	**0.95**	**0.62**	0.57	**0.7**	0.58	0.28	**0.98**	0.38	0.74	**0.63**	**0.8**	0.56	-0.6	1			
National MPA km^2	**0.79**	0.35	0.29	**0.68**	0.37	0.36	**0.86**	0.25	0.49	0.58	**0.7**	0.56	-0.5	**0.93**	1		
National Marine area %	**0.76**	0.4	0.3	**0.68**	0.4	0.28	**0.82**	0.34	0.5	0.59	**0.7**	0.49	-0.5	**0.9**	**0.94**	1	
Fisheries Landed Value	0.51	0.32	0.26	0.46	0.24	0.06	**0.66**	0.03	0.37	0.51	**0.6**	0.31	-0.5	**0.62**	0.53	0.45	1

DISCUSSION

The analysis of studies on ecosystem services in Latin America has been attempted before; Laterra *et al.* (2019) proposed a conceptual model to understand the links between the ecosystem services inequalities and the ES supply in Latin America. They found that the well-being of the most affected by those inequalities enhances the vulnerability of their socio-ecological systems.

Perevochtchikova and others (2019) did a systematic review of published papers focusing only on those that follow what they referred to as an integrated approach, i.e., that explicitly deal with the ecological, social, economic, and political dimensions. That way, from 2,520 papers found in two databases (SCOPUS and SCIELO), their analysis was reduced to a small proportion of the literature base (57 papers). They conclude that the most frequently analyzed services were provisioning and regulating ecosystem services related to hydrological and biodiversity. More recently, Balvanera and colleagues (2020) updated the previous synthesis on the state-of-the-art research on ES (Balvanera *et al.,* 2012), highlighting the achievements of a network that periodically organizes International Congresses on Ecosystem Services in the Neotropics. Noteworthy is that those papers are primarily oriented to terrestrial ecosystems and include Latin America and the Caribbean, whereas we focus only on the Pacific coast and climate change.

Our analysis presented here adds to this literature, indicating that the level of research that has taken place on the Pacific coast of Latin America is likely insufficient to support climate adaptation decisions. This is likely to have detrimental policy implications. The economies of the countries within the Pacific coast region rely very heavily on their natural resources, but levels of ecosystem service inequality are high and enhance the vulnerability of socio-ecological systems to climate change (Laterra *et al*., 2019). While socio-ecological resilience reflects long-term historical trends, short-term shocks within this history can cause abrupt change (Whitfield *et al*., 2019). Food provision in the form of seafood is the most critical coastal ecosystem service in the region, providing a significant source of protein, a way of living for marginalized communities, and direct and indirect jobs in artisanal fisheries and small-scale fish farming (Barange *et al*., 2014). Catches in the whole region peaked in 1995 at over 10 million tonnes, with an average of 7.5 million tonnes annually for the last 20 years (FAO, 2018).

Our results suggest that further studies exploring the countries' capacity to cope with climate change's impact on ES at a spatial scale of policy relevance are urgently needed. General Climate Models help describe the potential effects of climate change on a global scale (Frölicher & Laufkötter, 2018), but they are neither accessible to downscale for policymaking nor useful for site-level decisions. Climate change impacts such as sea level rise are of utmost importance for low-lying and heavy-populated regions (Reyer *et al*., 2017). Furthermore, ocean acidification threatens natural systems necessary for ecosystem services, such as coral reefs (Cabral-Tena *et al*., 2018; Norzagaray-Lopez *et al*., 2017) and detrimentally affects mollusc mariculture (Gazeau *et al*., 2013); neither can easily be assessed via global-scale models.

In addition to this region's social and economic importance, the Eastern Pacific is a suitable case study to test the potential impacts of climate change on ecosystem service provision since the region is an eastern boundary upwelling system, bringing nutrients to the surface, creating "blooms" of algae and zooplankton, which feed on those nutrients. These, in turn, provide food for fish, marine mammals, and birds, sustaining one of the largest biomass fisheries, such as the Peruvian anchovy and the California sardine (Sydeman *et al*., 2014). However, upwelling also brings lower pH water to the surface, aggravating the problem of ocean acidification (Manzello, 2010; Agostini *et al*., 2018). In addition, large-scale events such as the Pacific Decadal Oscillation and the El Niño Southern Oscillation (ENSO) synergize with climate change effects shifting the range of commercially important species to higher latitudes and reducing the productivity of fisheries and aquaculture (Sperling *et al*., 2016; Pecl *et al*., 2017). The above makes the Eastern Pacific a natural laboratory to study the impact of climate change on coastal ecosystem services; however, at present, this potential scientific benefit of the region is not being realized due to the research gap documented here and other studies (Muñoz-Sevilla & Le Bail, 2017; Dangles *et al.,* 2016).

In conclusion, the low number of studies from LAC we observed is due to the lack of research capability in the region. The latest figures (June 2018) from the UNESCO Institute of Statistics (UIS, 2018) show that whereas North America and Western Europe have 41% of all world researchers, only 3.8% are in Latin America, spite that these countries make up 8% of the global population, so the research gap we observe is not surprising.

Climate action is one of the United Nations' sustainable development goals that call for urgent action to combat climate change and its impacts. However, with such a limited knowledge of the potential effects of climate change on coastal ecosystem services from the Latin America Pacific and of the community´s dependency on these services, it becomes difficult to establish how achievable this is. For informing decisions and policymaking under a global change scenario, it is pressing to conduct studies along a more significant latitudinal gradient and from different perspectives, as well as strengthen the capacity building of LAC. Therefore, specific and multidisciplinary studies at appropriate scales for policymaking and focused on ecosystem services are much needed in the Pacific coast of Latin America to assess global climate models' limitations and to enable mitigation and adaption to climate change impacts Balvanera *et al.* (2020).

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Spatial and temporal organization of aquatic insect assemblages in two subtropical river drainages

Organización espacial y temporal de ensamblajes de insectos acuáticos en dos cuencas subtropicales

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ABSTRACT

Background. The spatial and temporal changes of assemblages of aquatic insect can be used to detect the anthropic impacts that influence the biological communities. **Goals.** We compared the assemblages of aquatic insect in 1997 and 2014 in two subtropical river drainages, the association with water characteristics, and we discuss their implications for ecosystems conservation. **Methods**. True diversity of the aquatic insect fauna at family level and their community structure for 27 study sites in 1997 and 2014 were assessed. Multivariate analyzes were used to compare aquatic insect assemblages and the abundance of functional feeding groups. **Results**. There were significant differences in the dissolved oxygen (DO) of the water between 1997 and 2014, decreasing its values. Other variables correlated to DO were also modified, with a decrease in pH and an increase in temperature. We found a correlation between reduction of DO and water pH with a decline in the overall abundance of aquatic insects; also, with shifts in the community structure, from the decrease of groups such as some Ephemeroptera and scrapers, to the increase in opportunistic families such as Chironomidae, Culicidae, and other predator families such as Coenagrionidae, Corixidae and Veliidae, and more abundance of collectors. Families such as Heptageniidae and Caenidae decreased in abundance, as well as other benthic groups. **Conclusions**. The assemblages of aquatic insect are useful to indicate a generalized degradation of environmental conditions across localities and time in two subtropical river drainages, related to water quality degradation symptoms such as reduction of pH levels and dissolved oxygen, usually associated with anthropogenic stressors.

Keywords: environmental degradation, functional feeding groups, macroinvertebrates, true diversity, water quality.

RESUMEN

Antecedentes. Los cambios espaciales y temporales de los ensamblajes de insectos acuáticos pueden ser utilizados para detectar los impactos antrópicos que influyen en las comunidades biológicas. **Objetivos.** Comparamos los ensamblajes de insectos acuáticos en 1997 y 2014 en dos cuencas subtropicales, su asociación con las características del agua y discutimos sus implicaciones para la conservación de los ecosistemas**. Métodos**. Se evaluó la diversidad verdadera a nivel de familia, de la fauna de insectos acuáticos en 27 sitios de estudio en 1997 y 2014. Se utilizaron análisis multivariados para comparar los ensamblajes de insectos acuáticos y la abundancia de los grupos funcionales de alimentación. **Resultados**. Se obtuvieron diferencias significativas en el oxígeno disuelto (OD) del agua entre 1997 y 2014, disminuyendo sus valores. También observó una disminución de pH y una tendencia a un incremento de la temperatura. Se identificó una relación entre la disminución de oxígeno y valores menores de pH con una reducción general en la abundancia de insectos acuáticos; asimismo, se observa una relación con cambios en los ensamblajes como lo son una disminución en la representación de grupos como Ephemeroptera y raspadores, el incremento de familias como Chironomidae, Culicidae, Coenagrionidae y Veliidae, y una mayor abundancia de colectores. Familias como Heptageniidae y Caenidae disminuyeron en abundancia, así como otros grupos bentónicos. **Conclusiones**. Los ensamblajes de insectos acuáticos son útiles para indicar una degradación generalizada de las condiciones a través de las localidades y el tiempo en las dos cuencas subtropicales de estudio, con

síntomas de degradación de la calidad del agua como la disminución de los niveles de pH y oxígeno disuelto, generalmente asociados con factores de estrés antropogénicos.

Palabras clave: calidad de agua, degradación ambiental, diversidad verdadera, grupos funcionales de alimentación, macroinvertebrados.

INTRODUCTION

Among the aquatic ecosystems, rivers benefit human communities by providing a supply of water, nutrient retention, removal of toxins, microclimate stability, opportunities for tourism, and are valued by local cultures (Brismar, 2002; Dudgeon, 2019). The main cause of loss of the ecological integrity and deterioration of these ecosystems are human activities (Carpenter *et al.*, 2011; Dudgeon, 2019), while the major threats for freshwater biodiversity are overexploitation, water pollution and flow modification, the invasion of exotic species, land use change, and climate change (Dudgeon, 2019). At the basin scale, land use changes influences in the stream conditions modifying water characteristics, sediment supply and deposition, affecting bank stability, and consequently the aquatic biota (Strayer *et al.*, 2003; Townsend *et al.*, 2003; Allan, 2004).

In order to design adequate proposals for new management and conservation of fluvial ecosystems, it has been proposed to study selected indicator groups and how the ecological elements and processes in these catchments have changed in the long term to reveal their current ecological condition and future threats (Ramírez & Gutiérrez-Fonseca, 2014a). Historical analyzes of changes in aquatic communities offers information about the current conservation status of aquatic ecosystems, in order to infer factors that have impacted these systems and obtain insight into the changing conditions of the surrounding watershed (Karr, 1981; Fausch *et al.*, 1990). Aquatic macroinvertebrates have a range of preferences for environmental conditions, so shift in the assemblages may reflect changes in the aquatic ecosystem and human impacts over time (Li *et al.*, 2012). However, the long-term perspectives and historical comparisons in aquatic macroinvertebrate communities remains often short (Jackson & Füreder, 2006).

Aquatic macroinvertebrates are especially useful to evidence changes in river ecosystems due to anthropic impacts (Barbour *et al.*, 1999; Bonada *et al.*, 2006; Ligeiro *et al.*, 2013). Among them, aquatic insects are generally the most abundant and diverse, as they are one of the most ecologically important groups (Macadam & Stockan, 2015) especially in tropical and subtropical zones (Dudgeon, 2008). They are the main primary consumers and are responsible for transferring the energy of primary productivity to other trophic levels of food chains, and there are elements within this group that are important predators (Hanson *et al.*, 2010; Macadam & Stockan, 2015). Aquatic insects can have highly specific functions in the ecosystem, such as filterers, gatherers, shredders, predators, piercers and scrapers (Merritt *et al.*, 2008; Hanson *et al.*, 2010; Ramírez & Gutierrez-Fonseca, 2014b). Importantly, because aquatic insects deploy a wide array of generalist and specialist feeding strategies, occupy several microhabitats, and have different responses and sensitivities to habitat degradation, they are considered highly useful biological indicators of stream ecological condition (Karr & Chu, 1999).

On the other hand, we must also bear in mind that macroinvertebrate assemblages can not only be affected by pollution or degradation. It has been seen that these assemblages vary due to the flow regime and sediment deposition (Díaz-Rojas *et al.*, 2020). So that high areas of a basin may have greater diversity because the variations of flows given by the slopes allows greater heterogeneity in the landscape than in the low sections where the slope decreases as well as flow (Mesa, 2010). Likewise, when the flow increases in the rainy season, the communities are modified (Quesada-Alvarado *et al.*, 2020). Therefore, it is important to consider the seasonality of the samples, being also throughout the year that differences have been seen in the macroinvertebrate assemblages as the flows and chemical composition of the rivers is modified, for example, by the leachate of the soil in rainy season (Leal-Bastidas *et al.*, 2021).

In the tropics, the influence of hydrological, physical and chemical alterations upon macroinvertebrate communities remains poorly understood (Md Rawi *et al.*, 2014; Ramírez *et al.*, 2015). Several studies on the ecology of aquatic insects in Latin America have been reported, with emphasis on the relationship with abiotic factors (Ramírez & Gutierrez-Fonseca, 2014a). For example, recently, Kohlmann *et al.* (2021) include in their study a analyzes of the relationships between functional feeding groups of aquatic macroinvertebrates with physicochemical such as NO^{3-} K^{+}, biochemical oxigen demand (BDO), oxygen saturation, and pH; Díaz-Rojas *et al.* (2020) relates depth, flow velocity, channel width and roughness of the substrate with macroinvertebrate assemblages composition and functional traits; Quesada-Alvarado *et al.* (2020) describe the relationship between the aquatic macroinvertebrate assemblages with physicochemical and habitat variables, such as NO^{3-}, substrate and flow; and, Mosquera-Restrepo & Peña-Salamanca (2019) explain the relationships between aquatic macroinvertebrates assemblages with dissolved oxygen, BOD, total dissolved solids, and turbidity.

In Mexico, this approach has been used to assess the biotic integrity of rivers in the Río Chiquito basin in the State of Michoacán (Piñón-Flores *et al.*, 2014), variation of macro-invertebrates in the Laguna de Tecocomulco in the State of Hidalgo for one year period (Rico-Sánchez *et al.*, 2014), and impacts of mining activities in three rivers of the Sierra Gorda Biosphere Reserve (Rico-Sánchez *et al.*, 2022). However, these studies involve brief spatial and temporal scale. For these reasons, the present study has the main goal of compare aquatic insect assemblages data of 1997 with data of 2014. Community structure, diversity, functional feeding groups, and the associations with water characteristics were described in two major subtropical river drainages in east-central Mexico, to interpret the ecological impairment indicated by the patterns founded.

MATERIAL AND METHODS

Study area: The study area includes the Pánuco and Lerma-Chapala river drainages, located in east-central Mexico (Fig. 1). It has a subtropical area in the northeast, located in the Eastern Sierra Madre and the Neovolcanic Belt. Central Mexico has the most degraded river drainages in the country (Mercado-Silva *et al.*, 2006). The Lerma-Chapala and Pánuco river drainages are two of the most important basins of this region, and have been highly impacted by loss of vegetation cover (>30%), expansion of cultivated pastures for livestock, increased agricultural activities, combined with expanded industrialization and urbanization

(Cuevas et a*l*., 2010). The Lerma-Chapala river drainage shows an evident problem of physical and chemical anthropogenic transformation, and is considered as the most degraded in Mexico (Cotler-Avalos *et al*., 2004). At the present, the headwaters of both drainages are being considered for special protection status as water reserves in Mexico by the National Commission of Water (Comisión Nacional del Agua, 2011).

A total of 27 sampling sites were selected in permanent rivers and were sampled in 1997 and 2014. We chose some of the main waterways in the following five states (Fig. 1) which included: 1) Aguascalientes: San Pedro River and Calvillo River; 2) Jalisco: Grande River; 3) Guanajuato: Laja River and Apaseo River; 4) Querétaro: Extóraz River, Huimilpan River, Querétaro River, San Juan River, Jalpan River and Santa María River; and, 5) San Luis Potosí: Verde River. The field work was conducted in the dry season (from February to May), when the conditions of habitat and biological community of rivers are more stable (Pérez-Munguía *et al*., 2007; Lyons *et al*., 1995) and the effect of the human activities are more evident (Moncayo-Estrada *et al*., 2015).

Data collection: Water physical and chemical parameters were measured with a multimeter probe (Hach Hydromet Quanta, Loveland, Colorado, USA), and we included pH, dissolved oxygen (mg/L), and temperature (°C). Aquatic insects were sampled using a D-net (300 mm of diameter and 300 µm of mesh size) in all different types of reachable habitat, with a sample effort of 60 minutes per study site. During 1997, aquatic insects were preserved in alcohol in 125 ml jars and brought back to the laboratory and separated from detritus. During 2014, insects were separated *in situ* and were deposited into a plastic vial and preserved in 80% alcohol solution for further transport to the laboratory (Biotic Integrity Lab at Universidad Autónoma de Querétaro). The aquatic insects were identified to the taxonomic level of family using specialized keys (e.g., Arce-Pérez & Roughley, 1999; Merritt *et al*., 2008; Bueno-Soria, 2010; Springer *et al*., 2010). We used the family taxonomic level because it has proven to be a good indicator of the level of ecological disturbance in fluvial ecosystems (cf. Marshall *et al*., 2006; Serrano-Balderas *et al*., 2016; Wright & Ryan, 2016), allows for the categorization of functional traits for the different families in most cases, and highest taxonomic level still providing sufficient resolution regarding biological traits of the organisms, saving time and effort to reach lower taxonomic categories. The functional feeding groups (FFG) were obtained from Ramírez & Gutiérrez-Fonseca (2014b).

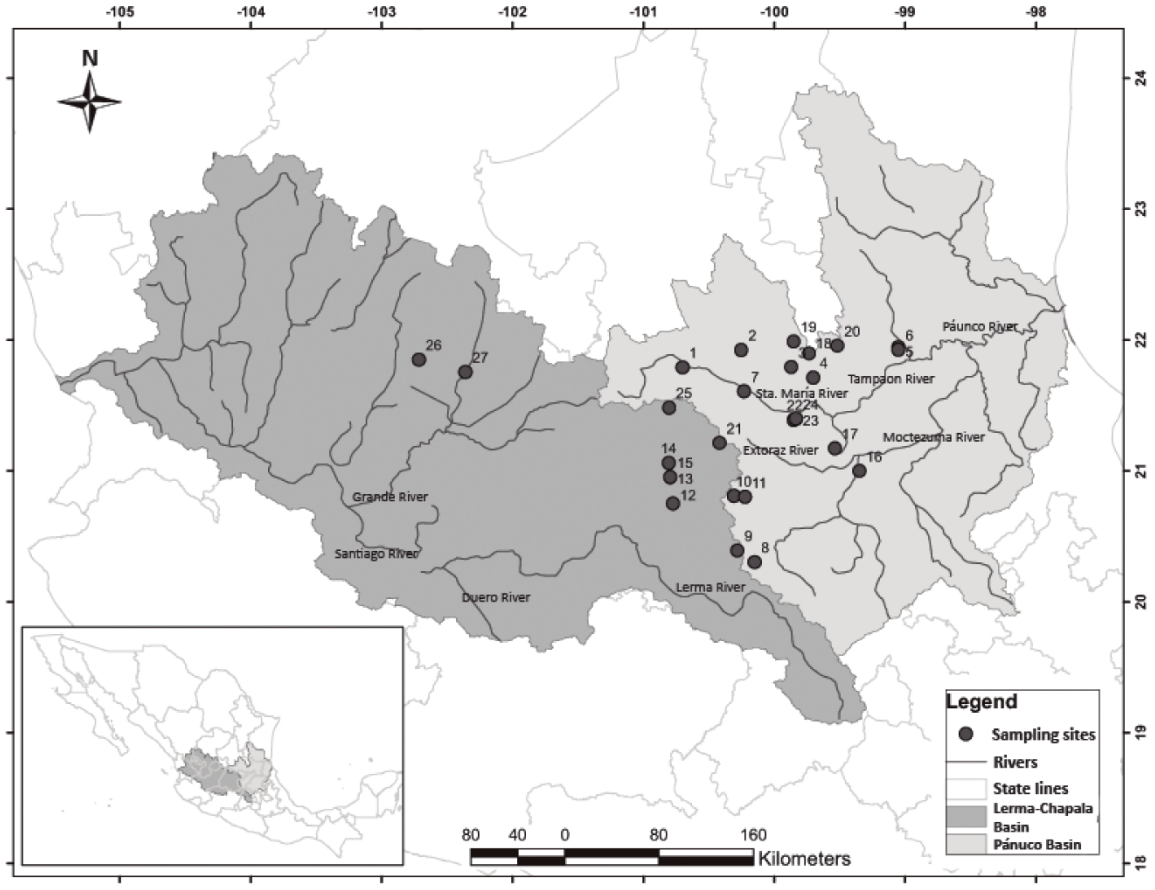

Figure 1. Geographic location of study sites. 1 = Fracción Sánchez, 2 = Planta-La Hacienda, 3 = Puente la Plazuela, 4 = Pinihuan, 5 = Canoas, 6 = Quinta Matilde, 7 = El Realito, 8 = Quiotillos, 9 = El Salto, 10 = Presa del Carmen, 11 = Presa de Rayas, 12 = Comonfort, 13 = La Quemada, 14 = Los Galvanes, 15 = El Xote, 16 = El Oasis, 17 = Chuveje, 18 = Carpintero, 19 = Rascón, 20 = Tamasopo, 21 = Jalpan, 22 = Ayutla, 23 = Santa María (before of Adjuntas), 24 = El Carrizal (Santa Maria after of Adjuntas), 25 = Río Grande, 26 = Calvillo, 27 = Sabinolandia (El Salto de los Salados).

Statistical analysis

Water physicochemistry: Paired T-test were made to assess the difference of the values of each water parameter between two years (1997 and 2014). To analyze and elucidate patterns of all physical and chemical parameters in both years, a principal component analysis (PCA) was conducted, and we normalize all variables using division by their standard deviations because the variables are measured in different units.

Aquatic insect assemblages: We calculated the true diversity as proposed by Jost (2006, 2007), through assessing of effective numbers of elements at family level, that refers to the numbers of taxa equally probable and necessary to obtain a diversity value (Jost, 2007). This approach is considered logical and works intuitively, unlike other indices such as the Shannon entropy, which measures the uncertainty degree of a species (Jost, 2006). True alpha and gamma diversity of first order was obtained to sensitize the index to the abundant species, because in aquatic insect communities are common to find this pattern of very abundant and rare taxa, which means an inequitable distribution of abundances among all taxa. Jackknife estimator was used because it is appropriate for this group of organisms (Basualdo, 2011; Martínez-Sanz *et al*., 2010). In addition, the true beta diversity was obtained as the effective number of elements in the data set (true gamma diversity) divided by the average number of effective elements of the samples (true alpha diversity); where, one is the minimum number which we can obtained, indicating that all communities are exactly the same, and maximum value are equal to the total of communities (N) (Jost, 2007). We applied paired T test to assess the difference of diversity values between 1997 and 2014, and a similarity percentage analysis (SIMPER) using the Bray-Curtis similarity measure (multiplied with 100), based in abundance per family and FFG was used to identify which taxon discriminates among periods (Clarke, 1993).

Responses of aquatic insect assemblages to water physicochemistry: To assess the effect of water physicochemistry on aquatic insect assemblages we made a non-metric multidimensional scaling (NMDS) based on the Bray-Curtis similarity index, which can be used with cero values in data sets (Bray & Curtis, 1957). NMDS was applied using the abundance per family and per FFG. In the NMDS the environmental variables were associated to the axis and represented with vectors in the plot (Hammer *et al*., 2001). We correlated by the Spearman method the PCA values and NMDS scores to understand the relationships between abiotic variables and aquatic insect assemblages overall, considering the intrinsic relationships, as was used by Escalera-Vázquez & Zambrano (2010) in a study of the effect of variation in abiotic factors on fish assemblages.

The paired T-test and Spearman rank order correlation analysis (Zar, 2014) were made using the statistical software SPSS version 20 (IBM corp., 2011). The true diversity values (Jost, 2006, 2007) were estimated with SPADE software (Chao & Shen, 2010). The multivariate analysis PCA, NMDS and SIMPER (Quinn & Keough, 2002) were obtained using PAST version 3.07 (Hammer *et al*., 2001).

RESULTS

Water physicochemistry: The paired T-test shows a significantly decrease ($p<0.001$) of dissolved oxygen between 1997 and 2014 considering all study sites (i.e., both basins), from 8.2 ± 3.3 mg/L to 3.6 ± 2.2 mg/L. There are no significant differences between temperature (20.3 ± 4.31 to 20.5 ± 4.8 °C) and pH (8.01 ± 0.43 to 7.8 ± 0.35) of both years ($p>0.05$). Nevertheless, the PCA analysis showed a subtle tendency gradient of segregation of data between 1997 and 2014. Three of the main components (PC) had moderately related variables (0.75-0.50). Of these PC1 (eigenvalue=1.76) explained 58.73% of the variance, PC2 (eigenvalue=0.75) explained 25% and PC3 (eigenvalue=0.48) 16.26%. The first component (PC1) showed moderate positively association with the three variables with correlation coefficients of 0.63(pH), 0.58 for dissolved oxygen (DO) and 0.51 for temperature. While the second was strongly positively associated (>0.75) with temperature (0.81) and moderate negatively associated with DO (-0.55) and not so with the pH (-0.15). Whereas the PC3 showed a negative association with pH (-0.76) and positive with DO (0.59) and temperature (0.25). The study sites ordination resulted located diagonally from upper left corner to the lower right corner, following a decrease of dissolved oxygen and pH values, and from the lower left corner to the upper right corner following an increase in temperature. The sites located in the upper left corner zone, comprises mainly the sites sampled in 2014 (Fig. 2).

Aquatic insect assemblages: A total of 71 aquatic insect families were obtained including both drainages. We collected 47 families during 1997 and 61 families during 2014 (Table 1). We found more representativeness of taxa during 2014 and more gamma diversity for both river drainages (Lerma-Chapala, ${}^1D_\alpha = 8.2$ and Pánuco ${}^1D_\alpha = 7.82$ during 1997; and Lerma-Chapala ${}^1D_\alpha = 9.17$ and Pánuco ${}^1D_\alpha = 12.13$ during 2014). The beta diversity was higher in 2014 (${}^1D_\beta = 2.8$) than in 1997 (${}^1D_\beta = 1.49$) on Lerma-Chapala River drainage, and lower in 2014 (${}^1D_\beta = 1.4$) than 1997 (${}^1D_\beta = 1.61$) on Pánuco River drainage. Global gamma and beta diversity was also higher in 2014 (${}^1D_\gamma = 13.34$; ${}^1D_\beta = 2.08$) than 1997 (${}^1D_\gamma = 9.30$; ${}^1D_\beta = 1.81$). However, the Lerma-Chapala river drainage showed higher alpha and beta diversity of families in the 70% of study sites during 1997 (Table 2). These results are consistent with the results of paired T-test that showed not significantly difference of alpha diversity of all sites between years ($p=0.181$).

The SIMPER analysis showed that the main families with contribution for abundance dissimilarity between 1997 and 2014 were Chironomidae (24.8%), Baetidae (16.5%), Coenagrionidae (8.5%), Veliidae (4.9%), Corixidae (4.2%), Culicidae (3.9%), Caenidae (3.7%) and Heptageniidae (1.3%). The abundance of Chironomidae was 61 ± 185 in 1997 and 116 ± 257.7 in 2014; of Baetidae was 60.7 ± 104.9 in 1997 and 38.9 ± 57.2 in 2014; Coenagrionidae showed 2.44 ± 6 in 1997 and 46 ± 129.3 in 2014; Veliidae 2.26 ± 5.1 in 1997 and 22.2 ± 55 in 2014; Corixidae showed 6.48 ± 30.7 of mean abundance in 1997 and 20 ± 51 during 2014; Culicidae 0.5 ± 1.5 in 1997 and 40.7 ± 195.2 in 2014; Caenidae 25.3 ± 99.3 in 1997 and 2.29 ± 13.5 during 2014; and Heptageniidae 3.22 ± 10.15 in 1997 and 0.07 ± 0.38 in 2014.

We found the six FFG: gatherers, filterers, predators, shredders, piercers, and scrapers. The most abundant FFG in both years was the gatherers, follow by predators, and piercers were the rarest (Table 3). SIMPER analysis based on abundance per FFG showed that gatherers contributed with 55.6% to the dissimilitude, with change from 79% of gatherers in 1997 to 44% in 2014; predators contributed with 32% and the quantity of individuals changed from 23 ± 23 in 1997 to 120 ± 188 in 2014; filterers contributed with 8.9% and changed from 8 ± 33 to 48 ± 194 individuals between 1997 and 2014; scrapers contributed with 2% and changed from 3.5 ± 10.1 in 1997 to 0.8 ± 2.4 in 2014; shredders contributed with 0.7% and piercers with 0.5%.

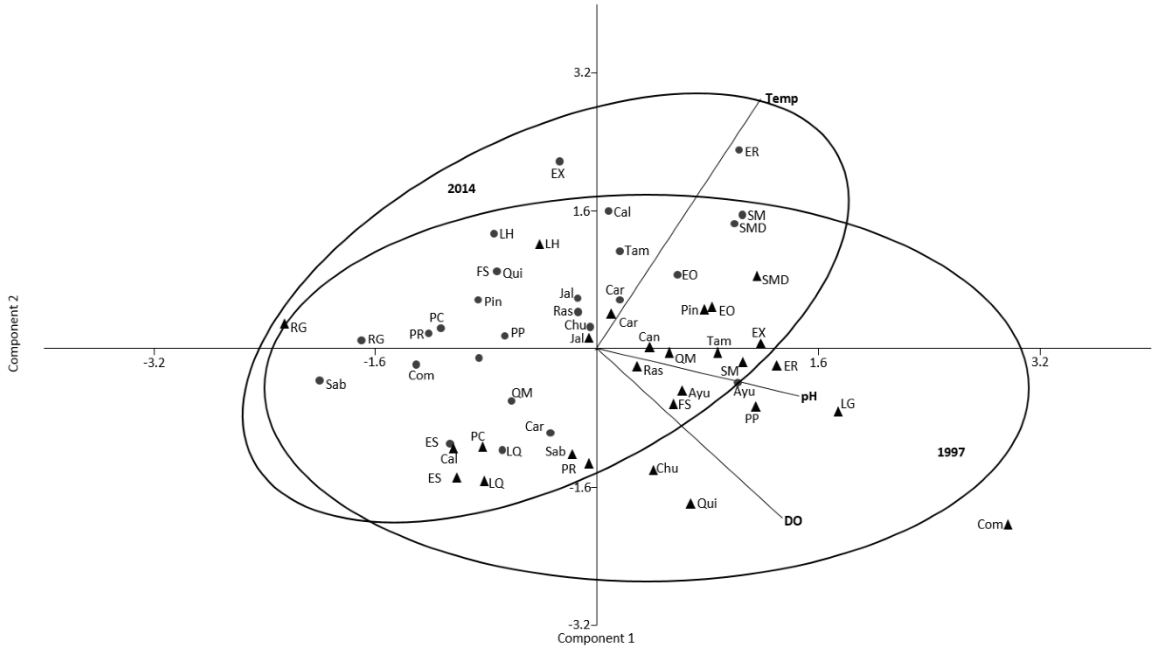

Figure 2. Principal Components Analysis based on physicochemical parameters in rivers of two subtropical river drainages in east-central Mexico (Lerma-Chapala and Pánuco). Data from 1997 (triangles), data from 2014 (circles). Ayutla = Ayu; Calvillo = Cal; Canoas = Can; Carpintero = Car; Chuveje = Chu; Comonfort = Com; El Carrizal (Santa María after of Adjuntas) = SMD; El Oasis = EO; El Realito = ER; El Salto = ES; El Xote = EX; Fracción Sánchez = FS; Jalpan = Jal; La Hacienda = LH; La Quemada = LQ; Los Galvanes = LG; Pinihuan = Pin; Presa de Rayas = PR; Presa del Carmen = PC; Puente la Plazuela = PP; Quinta Matilde = QM; Quiotillos = Qui; Rascón = Ras; Río Grande = RG; Sabinolandia (El Salto de los Salados) = Sab; Santa María (before of Adjuntas) SM; Tamasopo = Tam.

Relationships between aquatic insect assemblages and water characteristics: The NMDS based on number of individuals per site (Fig. 3), showed a pattern where taxa were ordinated in a gradient of dissolved oxygen and pH decrease from the upper left corner to the lower right corner. Additionally, the ordination analysis showed a gradient of decrease in abundance per site in the same direction (from the upper left to the lower right corner). We found some sites with contrasting differences between years, where Comonfort (Com) had 1008 individuals in 1997 and 21 individuals in 2014; Sabinolandia (Sab) changed from 298 individuals in 1997 to 36 in 2014; Fracción Sanchez (FS) increased the number of individuals from 85 to 2491 in 1997 and 2014, respectively; however, ~40% (1017 individuals) belong to Culicidae family and the insect diversity decrease in time (Table 2). The NMDS analysis shows a relationship between low concentrations of dissolved oxygen and lower pH values with fewer number of individuals per site; however, it shows no tendency of grouping by sampling years.

The NMDS based on relative abundance of insects per FFG (Fig. 4) showed that study sites were ordinated on axis one (left to right) in a gradient from low to high number of predators. The second axis (bottom to up) show a gradient of a greater number of filterers, a smaller number of scrapers and gatherers, and lower values of pH and dissolved oxygen. We found important changes in functional feeding groups at some study sites such as Comonfort (Com) where the abundance of gatherers decreased from 82.9% in 1997 to 9.5% in 2014 and the abundance of filterers (from 0.1% to 52.4%) and predators (from 0.2% to 38.1%) increased drastically between 1997 and 2014. At the Ayutla (Ayu) location, the abundance of gatherer decreased from 84.9% to 62.7%, the abundance of filterers and predator increased from 1.9% to 11.6% and from 3.8% to 22.8%, respectively. Fracción Sánchez (FS) showed considerable increase of filterers from 10.6% to 40.8% from 1997 to 2014. This analysis shows a slightly relationship between lower levels of dissolved oxygen and pH with high abundance of filterers and lower abundance of scrapers.

Most of the correlations between the values of PCA with the values from Axis of the NMDS were not significant. The only significant correlation was based on abundance per FFG, using the axis 2 (r_{xy} = -0.28, p = 0.04). The correlation shows that the increase in water temperature and the decrease in dissolved oxygen is related with more abundance of filterers and less abundance of scrapers; however, in this case the correlation coefficient is very low showing that this pattern is not consistent.

DISCUSSION

The rivers in the Lerma-Chapala and Pánuco river drainages showed symptoms of biological and environmental degradation based on differences in aquatic insect diversity and taxa abundance, FFG and water quality parameters such as dissolved oxygen and pH. The aquatic insect structure and the relationship with water physiochemical variables through the space and time were difficult to interpret at the basin scale. However, our analyzes provided general patterns such as the condition of the aquatic insect fauna and water characteristics in two major subtropical river drainages in east-central Mexico.

Table 1. Number of individuals and functional feeding groups per site in rivers in two sub-tropical river drainages in east-central Mexico (Lerma-Chapala River and Pánuco River). Values show the number of individuals. Sampling sites are in parentheses. FFG = Functional feeding group, 1 = El Salto, 2 = Presa del Carmen, 3 = Presa de Rayas, 4 = Comonfort, 5 = La Quemada, 6 = Los Galvanes, 7 = El Xote, 8 = Río Grande, 9 = Calvillo, 10 = Sabinolandia (El Salto de los Salados), 11 = Fracción Sánchez, 12 = La Planta-La Hacienda, 13 = Puente la Plazuela, 14 = Pinihuan, 15 = Canoas, 16 = Quinta Matilde, 17 = El Realito. 18 = Quiotillos, 19 = El Oasis, 20 = Chuvejé, 21 = Carpintero, 22 = Rascón, 23 = Tamasopo, 24 = Jalpan, 25 = Ayutla, 26 = Santa María (above of Adjuntas), 27 = El Carrizal (Sta. Ma. below of Adjuntas).

Family	FFG	1997	2014
Ephemeroptera			
Baetidae	Gatherer	25(1), 26(2), 1(3), 294(4), 135(5), 93(6), 35(9), 35(10), 44(11), 4(12), 5(13), 45(14), 14(17), 182(18), 466(19), 21(20), 19(22), 13(23), 100(24), 18(25), 11(26), 52(27)	7(2), 52(3), 185(5), 29(6), 21(8), 185(9), 1(11), 3(12), 109(13), 127(15), 12(16), 3(17), 99(18), 5(19), 47(20), 6(22), 13(24), 112(25), 12(26), 21(27)
Ephemerellidae	Gatherer	10(11), 66(18)	0
Polymitarcyidae	Gatherer	1(2), 1(3), 1(13), 12(18)	0
Caenidae	Gatherer	519(4), 2(7), 24(10), 52(18), 16(19), 29(20), 6(21), 6(22), 8(23), 13(24), 6(26), 1(27)	70(5), 10(18)
Leptophlebiidae	Gatherer	9(5), 1(6), 16(10), 5(18), 12(19), 63(20), 5(21), 15(26)	9(14), 1(15), 4(16), 15(22), 1(23), 9(24), 5(25), 6(26)
Leptohyphidae	Gatherer	5(26)	1(2), 4(5), 134(13), 6(15), 7(16), 9(18), 10(19), 3(20), 7(22), 1(23), 5(24), 2(27)
Heptageniidae	Scraper	9(4), 1(9), 2(10), 1(19), 1(20), 4(21), 1(22), 2(23), 1(24), 1(25), 12(26), 52(27)	2(20)
Ephemeridae	Gatherer	22(24)	0
Odonata			
Gomphidae	Predator	7(7), 1(10), 1(17), 3(20)	2(6), 16(13), 10(14), 1(15), 1(16), 9(19), 3(21), 6(22), 12(24), 46(25), 5(26), 15(27)
Coenagrionidae	Predator	2(1), 23(2), 1(3), 14(5), 1(7), 18(9), 2(12), 2(14), 1(21), 1(24), 1(26)	20(2), 178(3), 1(4), 88(5), 67(6), 1(7), 1(8), 5(9), 70(12), 20(13), 4(14), 10(15), 11(16), 6(17), 663(18), 21(19), 9(20), 3(22), 2(23), 43(24), 6(25), 5(26), 8(27)
Lestidae	Predator	0	3(3), 3(14), 50(15), 107(16), 20(20)
Platystictidae	Predator	0	2(26)
Macromiidae	Predator	0	1(27)
Libellulidae	Predator	1(10), 4(17), 1(18), 1(19), 4(20), 40(24)	5(2), 2(3), 7(4), 8(7), 3(9), 54(13), 1(14), 6(15), 6(16), 3(18), 3(19), 1(20), 8(24), 9(25), 2(27)
Aeshnidae	Predator	2(1), 5(2), 8(5), 2(6), 14(10), 1(11), 3(12), 17(20), 2(26)	1(1), 4(2), 13(3), 1(16), 14(18), 1(22)
Calopterygidae	Predator	2(1), 1(6), 1(10), 5(18), 3(24)	4(12), 3(14), 1(15), 3(19), 2(20), 3(25), 4(26), 1(27)
Protoneuridae	Predator	3(5), 1(14)	1(9), 3(21)
Plecoptera			
Perlidae	Predator	1(13), 1(14), 2(18)	5(14)
Hemiptera			
Corixidae	Predator	160(4), 1(8), 10(18), 4(25)	1(1), 143(3), 167(5), 71(6), 159(9)
Hebridae	Predator	0	1(5), 1(13), 1(26), 4(27)
Veliidae	Predator	2(1), 1(4), 1(5), 13(10), 5(11), 2(12), 1(13), 5(14), 2(15), 2(16), 24(18), 1(20), 1(21), 1(22)	6(2), 10(5), 7(8), 2(11), 2(12), 212(13), 3(14), 202(15), 3(16), 4(17), 47(18), 42(20), 1(21), 1(23), 43(25), 15(26)

Family	FFG	1997	2014
Mesoveliidae	Predator	0	2(12)
Gerridae	Predator	3(7), 32(10), 10(12), 6(13), 17(15), 7(16), 23(17), 1(18), 10(20), 7(23)	1(3), 4(5), 9(13), 2(15), 7(16), 8(18), 2(21), 5(27)
Belostomatidae	Predator	2(1), 6(3), 1(4), 2(7), 1(16), 2(18), 2(19)	5(2), 5(5), 4(7), 1(8), 17(12), 6(13), 1(15), 3(16), 3(17), 1(20), 9(21)
Naucoridae	Predator	1(6), 4(10), 4(10), 5(11), 17(18), 3(19), 1(22), 5(24)	40(13), 2(21), 3(24), 2(25)
Notonectidae	Predator	4(16), 5(18)	3(5), 1(6), 1(8), 23(9), 2(11), 1(12), 3(15), 21(16), 43(18), 14(20), 21(27)
Saldidae	Predator	1(17)	0
Pleidae	Predator	10(5), 72(6), 3(19)	96(18)
Macroveliidae	Predator	0	47(18)
Nepidae	Predator	0	1(2), 1(5), 1(6), 1(11), 4(12), 11(13), 2(16), 2(18)
Megaloptera			
Corydalidae	Predator	1(10), 1(18), 5(21)	3(14), 3(19), 2(20), 1(23), 1(25), 5(26), 1(27)
Trichoptera			
Hydroptilidae	Piercer	1(7), 1(9), 2(24)	9(5), 2(15), 1(20), 1(24), 3(25), 12(26)
Polycentropodidae	Filterer	0	12(15), 1(16), 2(18), 8(20), 1(21)
Philopotamidae	Filterer	12(10), 1(21)	1(16), 3(24), 25(26), 4(27)
Odontoceridae	Shredder	0	1(20)
Hydrobiosidae	Predator	0	1(14), 2(25), 1(27)
Limnephilidae	Shredder	2(5), 1(10)	0
Calamoceratidae	Shredder	0	3(20), 2(22)
Lepidostomatidae	Shredder	0	3(20)
Leptoceridae	Gatherer	0	1(15), 1(20), 1(24)
Hydropsychidae	Filterer	25(10), 11(24)	1(12), 1(14), 1(19), 2(24), 11(26), 3(27)
Coleoptera			
Gyrinidae	Predator	1(12), 18(14), 7(16), 4(18)	1(5), 1(6), 2(8), 29(16), 1(18), 1(20), 7(24), 3(25)
Dytiscidae	Predator	1(1), 2(2), 1(5), 1(18), 3(20)	4(1), 12(3), 5(5), 13(9), 94(11), 12(13), 31(16), 4(18), 2(19), 5(20), 1(22), 4(25)
Hydrophilidae	Predator	2(6), 1(7), 1(12), 1(17), 3(18)	2(2), 6(3), 16(5), 3(7), 2(8), 82(9), 1(10), 26(11), 1(12), 11(13), 1(15), 6(16), 3(17), 4(18), 2(19), 1(20)
Helophoridae	Gatherer	0	1(11)
Staphylinidae	Gatherer	0	1(5), 1(9), 1(15)
Psephenidae	Scraper	1(18), 5(19), 1(26)	0
Scirtidae	Scraper	1(9)	3(5), 11(12), 2(25)
Dryopidae	Shredder	1(5), 1(22)	1(26)
Elmidae	Gatherer	1(1), 2(4), 2(5), 1(6), 1(9), 19(10), 1(13), 4(18), 9(20), 16(22), 3(23), 154(24), 16(26)	4(13), 3(15), 5(19), 11(20), 1(22), 3(23), 19(24), 9(25), 7(26), 5(27)
Limnichidae	Gatherer	1(20), 3(24)	0

Family	FFG	1997	2014
Lutrochidae	Shredder	0	9(25), 5(26)
Ptiliidae	Scraper	0	1(8), 4(20)
Haliplidae	Shredder	1(5), 6(9)	1(7),1(13), 1(15), 1(20)
Diptera			
Tipulidae	Shredder	0	1(5), 1(27)
Ceratopogonidae	Predator	1(1), 1(2), 11(5), 1(8), 2(13), 3(17), 5(24), 2(25)	1(3), 5(5), 1(6), 1(19), 8(25), 3(26), 3(27)
Chironomidae	Gatherer	21(1), 63(2), 5(3), 19(4), 72(5), 7(6), 4(7), 2(8), 16(9), 92(10), 11(11), 25(12), 33(13), 1(14), 5(15), 5(16), 4(17), 39(18), 40(19), 67(20), 8(21), 11(22), 11(23), 980(24), 27(25), 80(26)	154(1), 128(2), 70(3), 2(4), 341(5), 47(6), 71(7), 171(8), 142(9), 1333(11), 190(12), 11(13), 13(15), 31(16), 50(17), 35(18), 23(19), 10(20), 4(22), 3(23), 42(24), 223(25), 36(27)
Simuliidae	Filterer	1(2), 1(4), 3(5), 1(10), 1(11), 2(20), 160(24), 1(25)	11(2), 7(5), 7(8), 1(14), 1(16), 1(19), 2(22), 9(24), 65(25), 15(26), 8(27)
Syrphidae	Gatherer	0	1(1), 6(10), 1(11)
Dixidae	Gatherer	0	4(5), 8(18), 3(24)
Culicidae	Filterer	2(2), 8(11), 1(12), 1(15), 2(17), 1(18), 1(20)	12(1), 11(4), 5(5), 29(10), 1017(11), 10(13), 15(18), 1(22)
Thaumaleidae	Scraper	1(18)	0
Tabanidae	Predator	1(1), 1(2), 1(10), 1(13), 1(19), 1(24)	2(14), 2(17)
Stratiomyidae	Gatherer	0	1(11), 1(13), 3(25), 1(27)
Muscidae	Predator	0	1(22)
Ephydridae	Gatherer	6(9)	4(9), 12(11)
Psychodidae	Gatherer	2(4), 6(24)	0
Chaoboridae	Predator	2(5), 1(13), 1(20), 5(24)	0
Athericidae	Predator	0	2(20)
Empididae	Predator	1(25)	0
Lepidoptera			
Crambidae	Shredder	3(10), 1(13), 1(15), 1 (18),	1(18), 2(25), 1(26)

It has been shown that the loss of vegetation cover in watershed, mainly riparian vegetation, is a contributing factor in the increase of temperature in freshwater bodies (Allan, 2004; Quinn *et al.*, 1997). The processes of urbanization are related with degradation symptoms such as the reduction of dissolved oxygen in the water (De Jesús-Crespo & Ramírez, 2011; Ometo *et al.*, 2000). These patterns are consistent with the decrease of both dissolved oxygen concentrations and pH values, and an increase in water temperature finding in our study and whit the fact that Cuevas *et al.* (2010) estimates that the Lerma-Chapala and Pánuco River drainages have lost about 30% and 50% of the vegetation cover respectively, due to the expansion of cultivated pastures, increased agriculture, and urbanization.

Biodiversity measurement has been considered has good indicator of ecosystem stability (Maclaurin & Sterelny, 2008). However, some authors argue that alpha diversity often do not present systematic patterns among habitats, which does not always make them as good indicators of the severity of human impacts (cf. Magurran, 2016; Pandolfi & Lovelock, 2014). Another constraint is the fact that all diversity metrics are limited by the ability of researchers to measure them in field, i.e., the community is rarely perfectly measured varying across taxonomic groups, environments, and traits (Jarzyna & Jetz, 2016). The absence of significant differences in the diversity of aquatic insects and the inconsistent patterns in this biological measure in our study, comparing our data from 1997 with data of 2014, are similar to other research, where different gradients of urbanization or river ecosystems degradation were analyzed at the basin scale with no clear responses and patterns in the richness and evenness of aquatic invertebrates (Bonada *et al.*, 2006; Quinn *et al.*, 1997).

The increase in abundance in Chironomidae, Coenagrionidae, Veliidae, Corixidae and Culicidae families, can be related with the environmental degradation. A positive relationship has been reported between the increase of Chironomidae density with land use changes, such as induced grassland and urban sprawl (Jones & Clark, 1987; Quinn *et al.*, 1997). These land use changes are generally associated with an increase in water temperature and sedimentation, and low dissolved oxygen concentrations (Miserendino *et al.*, 2011; Walsh *et al.*, 2005). Chironomids are found in a range of conditions more extensive than any other aquatic insect family; it can exploit an almost complete range of gradient in temperature, pH and oxygen (Ferrington *et al.*, 2008). For this reason, is not surprising that this diverse and opportunistic family showed greater relative abundance in the 2014 when compared to 1997, which was correlated with a decrease of dissolved oxygen and lower values of pH. The larvae of some Odonata are also tolerant and often survive relatively low values of dissolved oxygen and subsist better than many other invertebrates in acidic waters (Suhling *et al.*, 2015), which could explain the increment in individuals of the Coenagrionidae family. Some aquatic Heteroptera, especially Gerromorpha, are good indicators of human disturbance having a high tolerance to eutrophication and acidic waters. Corixidae present a great variation among nutrient and pH tolerance (Lytle, 2015). Accordingly, the increment in these two families of hemipterans, especially Corixidae could be a response of lower pH values. The Culicidae increase in 2014 also can be related with anthropogenic stressors. In this sense, Ribeiro *et al.* (2012) reported that environmental change, such as the increase in agricultural areas, irrigation ponds, and the reduction in vegetation cover, tends to increase the abundance of opportunistic species of Culicidae, mainly those species that are considered vectors of human diseases (Juliano & Lounibos, 2005).

Although Baetidae is an Ephemeroptera family very common and dominant in tropical and subtropical rivers, in this study showed an abundance decrease (together with Heptageniidae and Caenidae families) from the sampling performed in 1997 compared with the year 2014. Baetidae and Heptageniidae families are reported to be sensitive to land use changes, such as urban and cropland increase (Jones & Clark, 1987; Li *et al.*, 2012; Quinn *et al.*, 1997) because many live attached to boulders and feed on the periphyton (Flowers & De la Rosa, 2010). In general, land use changes can result in an increase in fine sediment deposition, reducing available habitat for benthic organisms (Wood & Armitage, 1997) and resulting in a decrease in periphyton (Yamada & Nakamura, 2002) affecting the establishment and development of families such as Heptageniidae.

In terms of the functional feeding groups (FFG), the increase in gatherers, filterers and predators, and the decrease of scrapers are similar to other studies where a reduction in the of river ecosystem integrity is related with agriculture activities and urbanization processes. In this way, Quinn *et al.* (1997) and Friberg *et al.* (2009) registered an increment of filterers densities and Md Rawi *et al.* (2014) document an increase of predators, filterers, and gatherers in association with environmental degradation. This pattern of increase in collectors (filterers and gatherers) can be an indicator of environmental degradation, because filterers have more availability of suspended particles, and gatherers too with the increase in sediment deposition, which implies, in many cases, more fine particulate organic matter as available feeding resources for these groups. The land use change at basin scale, reduction of riparian vegetation cover, wastewater and pollutants discharges, can cause cumulative and additive effects, which impinges on the river community, changing the habitat, water quality and nutrient amount (Allan, 2004). These disturbances provide favorable conditions to some opportunistic groups such as filterers and gatherers. Sedimentation, for example, restricts the suitability for periphyton and biofilm production (Wood & Armitage, 1997; Yamada & Nakamura, 2002) limiting the success of scrapers that feed on it.

The pattern of increase in temperature shown in the PCA (Fig. 2), were not accurately related with the aquatic insect assemblages. It coincides with Friberg *et al.* (2009) and Buendia *et al.* (2014) who found no correlation or strong effect among water temperature and aquatic macroinvertebrate diversity. On the other hand, Jacobsen *et al.* (1997), report a positive relationship between water temperature increase and aquatic invertebrate richness; however, their study focuses on Ecuado-

Table 2. True diversity (number of effective elements) of aquatic insects, alpha (Jack1) beta and gamma (Jack1) of rivers in two sub-tropical river drainages in east-central Mexico (Lerma-Chapala River and Pánuco River) including two years (1997 and 2014). Ayutla = Ayu; Calvillo = Cal; Canoas = Can; Carpintero = Car; Chuveje = Chu; Comonfort = Com; El Carrizal (Sta. Ma. after of adjuntas) = SMD; El Oasis = EO; El Realito = ER; El Salto = ES; El Xote = EX; Fracción Sánchez = FS; Jalpan = Jal; La Hacienda = LH; La Quemada = LQ; Los Galvanes = LG; Pinihuan = Pin; Presa de Rayas = PR; Presa del Carmen = PC; Puente la Plazuela = PP; Quinta Matilde = QM; Quiotillos = Qui; Rascón = Ras; Río Grande = RG; Sabinolandia (El Salto de los Salados) = Sab; Santa María (before of adjuntas) = SM; Tamasopo = Tam. No significant difference of alpha diversity between years were obtained ($p = 0.133$).

Lerma-Chapala			**Pánuco River**		
Study site	1997	2014	Study site	1997	2014
Cal	5.27	1.64	Ayu	3.4	7.4
Com	3.19	3.24	Can	3.2	5.06
ES	5.07	1.63	Car	7.53	6.45
EX	9.24	2.2	Chu	7.57	12.48
LG	2.95	4.44	EO	1.98	9.5
LQ	5.15	6.56	ER	5.6	3.21
PC	4.27	3.59	FS	4.81	2.52
PR	5.8	4.88	Jal	3.66	10.12
RG	4.74	2.34	LH	5.27	3.38
Sab	10.2	1.9	Pin	3.2	11.83
Gamma	8.2	9.17	PP	4.83	7.93
Beta	1.49	2.8	QM	5.8	9.13
			Qui	7.79	4.97
			Ras	5.31	10.41
			SM	4.94	14.2
			SMD	2.12	12
			Tam	5.44	9.54
			Gamma	7.82	12.13
			Beta	1.61	1.4
		1997	2014		
Global *(Both river drainages)*	**Gamma**	9.3	13.34		
	Beta	1.81	2.08		

Table 3. Relative abundance (%) and mean of number of individuals (μ) per functional feeding groups of aquatic insects during 1997 and 2014 in rivers of two Sub-tropical river drainages of east-central Mexico. BRD = both river drainages; SE = standard deviation G = gatherer; Ft = filterers; Pr = predator; Sh = shredders; Pc = piercers; Sc = scrapers. Superscripts *a*, *b* refers to among-year differences (CG, $p = 0.012$; Ft, $p = 0.005$; Sc, $p = 0.026$).

	Year									
	1997					2014				
	Lerma-Chapala		Pánuco		BRD %	Lerma-Chapala		Pánuco		BRD %
	%	M	%	M		%	M	%	μ SD	
CG	74.8	155.2± 250.4	82.2	170.9 ± 318.5	79.4[a]	56.9	169.5± 180.2	36.2	93.3± 98.9	44.6[b]
Ft	2.2	4.5± 11.8	5.4	11.2 ± 41.2	4.2[a]	2.8	8.2± 9.1	4.8	12.3± 18.2	4.0[b]
Pr	13.8	28.7± 27.6	9.6	19.9 ± 19.7	11.1	21.6	64.2± 73.5Ω	57	147± 228.6	42.7
Sh	1.1	2.2± 4.3	0.1	0.1 ± 0.5	0.4	0.2	0.5± 1.1	1.3	3.3± 5.8	0.8
Pc	0.1	0.2± 0.4	0.1	0.1 ± 0.5	0.1	0.3	0.9± 2.8	0.5	1.2± 2.9	0.4
Sc	0.6	1.3± 2.8	2.3	4.9± 12.6	1.7[a]	0.1	0.4± 1.0	0.6	1.5± 3.2	0.4[b]

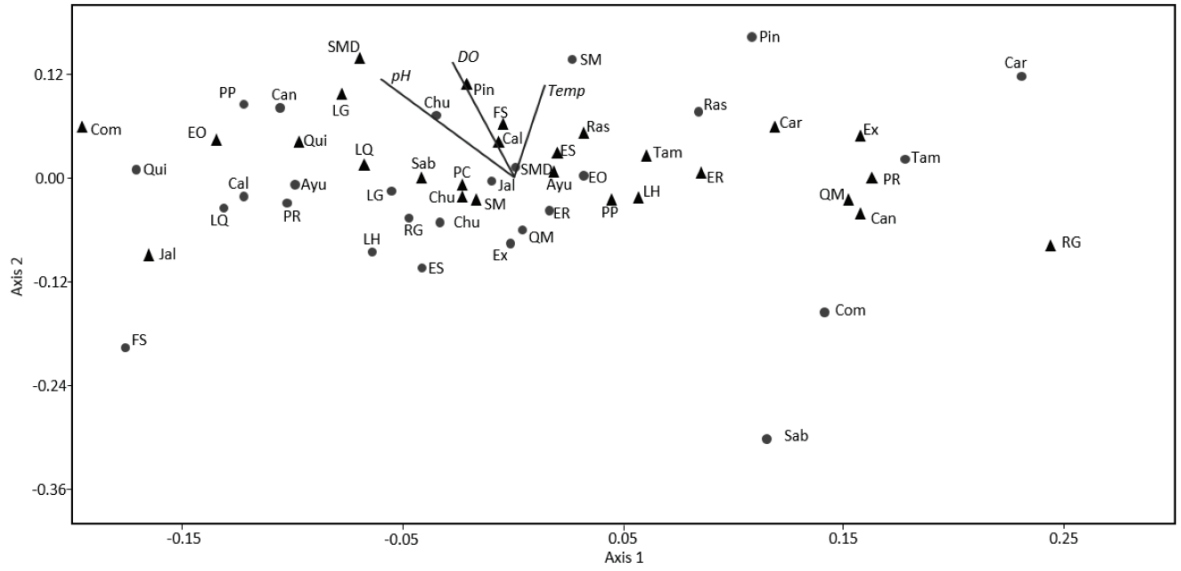

Figure 3. Non metric multidimensional scaling (NMDS) based on the number of individual of aquatic insects per family, in rivers in the Lerma-Chapala and Pánuco River drainages, with data of 1997 (triangles) and 2014 (circles). Stress: 0.19. Temp = temperature, DO = dissolved oxygen. Ayutla = Ayu; Calvillo = Cal; Canoas = Can; Carpintero = Car; Chuveje = Chu; Comonfort = Com; El Carrizal (Santa María after of Adjuntas) = SMD; El Oasis = EO; El Realito = ER; El Salto = ES; El Xote = EX; Fracción Sánchez = FS; Jalpan = Jal; La Hacienda = LH; La Quemada = LQ; Los Galvanes = LG; Pinihuan = Pin; Presa de Rayas = PR; Presa del Carmen = PC; Puente la Plazuela = PP; Quinta Matilde = QM; Quiotillos = Qui; Rascón = Ras; Río Grande = RG; Sabinolandia (El Salto de los Salados) = Sab; Santa María (before of Adjuntas) SM; Tamasopo = Tam.

rian mountain streams and temperate lowland streams, and refers to a lower range of temperatures, contrasting with those of this study. Li *et al.* (2012) report that an increase in water temperature was correlated with the scarcity of Ephemeroptera, Plecoptera and Trichoptera. We also found a correlation among lower pH levels and lower dissolved oxygen concentrations with decrease in aquatic insect abundance, more presence of filterers and a decrement on scrapers. These results appear to differ with Townsend *et al.* (1983) who found more abundance of filter feeders in sites with higher levels of pH in temperate streams that are commonly acid, and they attribute this pattern to the greater range of resources in fewer acid streams.

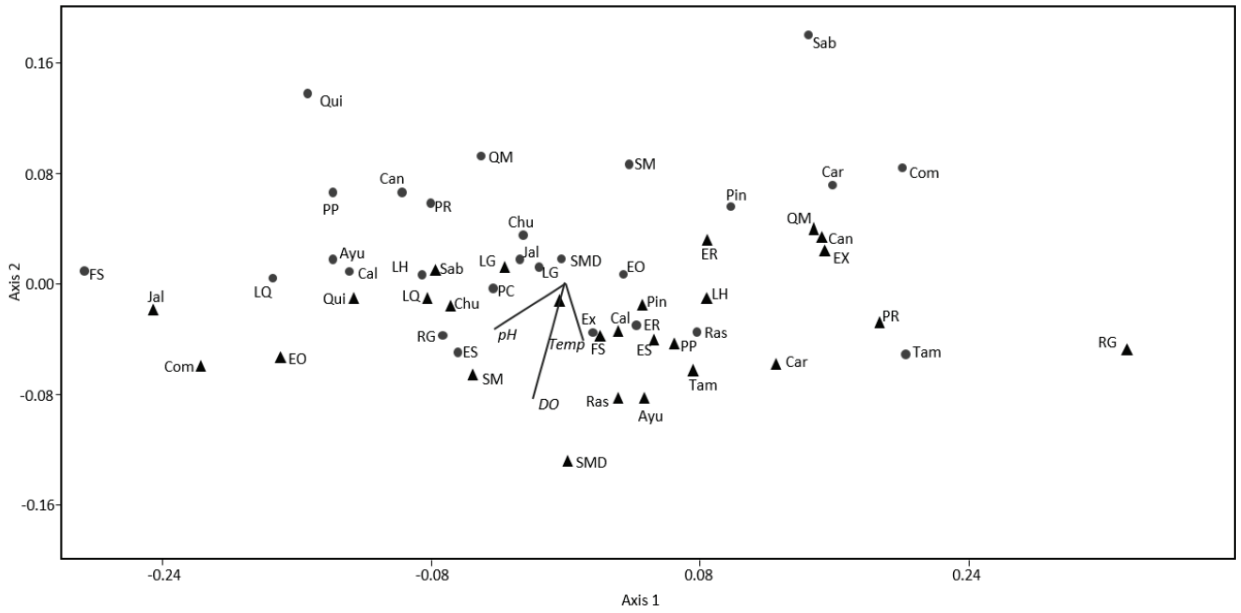

Figure 4. Non metric multidimensional scaling (NMDS) based on relative abundance of aquatic insects per functional feeding group in rivers of two Sub-tropical river drainages including data from 1997 (triangles) and 2014 (circles) Stress: 0.09. Temp = temperature, DO = dissolved oxygen. Ayutla = Ayu; Calvillo = Cal; Canoas = Can; Carpintero = Car; Chuveje = Chu; Comonfort = Com; El Carrizal (Santa María after of Adjuntas) = SMD; El Oasis = EO; El Realito = ER; El Salto = ES; El Xote = EX; Fracción Sánchez = FS; Jalpan = Jal; La Hacienda = LH; La Quemada = LQ; Los Galvanes = LG; Pinihuan = Pin; Presa de Rayas = PR; Presa del Carmen = PC; Puente la Plazuela = PP; Quinta Matilde = QM; Quiotillos = Qui; Rascón = Ras; Río Grande = RG; Sabinolandia (El Salto de los Salados) = Sab; Santa María (before of Adjuntas) SM; Tamasopo = Tam.

The negative relationship among dissolved oxygen and aquatic insect abundance found in this study, coincides with other studies where it is suggested that the availability of dissolved oxygen restricts macroinvertebrate diversity (Jacobsen *et al.*, 1997; García-Alzate *et al.*, 2010; Md Rawi *et al.*, 2014). Moreover, it has reported that with a decrease in dissolved oxygen there is an increase in insect predators (Md Rawi *et al.*, 2014), which is in accordance with our data increment of the mean abundance of Coenagrionidae, Veliidae and Corixidae, a predator's groups. Only one location (Fracción Sánchez) showed a substantial increase in the number of individuals, explained by the addition of 1017 individuals (>40% of abundance) of the family Culicidae, but a decrease in insect diversity from 1997 to 2014. The positive relationship between reduced water dissolved oxygen and an increase in the filterers, is mainly explained by the great abundance of individuals of Culicidae (categorized as filterers) in 2014, whose members are independent of water dissolved oxygen as they can obtain this resource directly from the atmosphere (Clements, 1992; Wallace & Walker, 2008).

Across the ecosystems of the world, freshwaters are the most endangered (Nel *et al.*, 2009), and subtropical streams and rivers are especially threatened because are greatly diverse ecosystems usually more than temperate waters (Dudgeon, 2008). Additionally, some pressures are increasing in developing countries (Strayer & Dudgeon, 2010), most located within tropical and subtropical zones (cf. Sachs, 2001). The rivers of the Lerma-Chapala and Pánuco River drainages have been affected by the combined and cumulative negative effects of human activities (Cuevas *et al.*, 2010).

The results of this study show symptoms of both chemical and biological degradation of these subtropical rivers. The evidence is supported by an increase in water temperature, and a decrease in dissolved oxygen concentrations and lower pH water levels along the space and time. For the aquatic insects, there was an increase in opportunistic and tolerant taxa with a corresponding decrease in sensitive groups. Also, the patterns in the FFG included an increase in the collectors (filterers and gatherers) and a decrease in scrapers. These symptoms reflected loss of river functional processes including energy transformation, nutrient turnover, storage and processing of organic matter, retention and cycling of nutrients, and transportation and deposition of sediments in both river drainages, with the most severe changes occurring in the Lerma-Chapala. In this basin the degradation condition was evident, because the anthropic impacts, including loss of habitat, contamination of waters, increase in sediment deposition and loss of riparian vegetation cover caused by human population growth and agricultural and livestock activities has been noticeably greater than in the Pánuco River basin (Cotler-Avalos *et al.*, 2004; Cuevas *et al.*, 2010).

Our data reflected the generalized degradation of rivers of two subtropical river drainages in east-central Mexico, which continues unabated and it is evidence of a deficiency in ecosystems conservation strategies in the country. This degradation it's a risk for the support ecological systems and the public health, because generates the conditions for the proliferation of mosquitoes, capable of transmitting viral infectious diseases such as Dengue (Secretaría de Salud, 2001; Instituto de Diagnóstico y Referencia Epidemiológicos, 2016), Chikungunya (Staples & Fischer, 2014) and Zika (Secretaría de Salud, 2016), which have occurred in Mexico and other tropical and subtropical zones.

This is the first analysis in Mexico that explores the relationship between aquatic insect assemblages and water quality variables,

using this information to indicate degradation levels in two major river drainages through a long-term time scale comparison. This research contributes to the understanding of the trends in the responses of the aquatic biota related to water parameters, providing a framework for the application of historical comparison studies for evaluating the ecological conditions in rivers and to interpret the surrounding landscape impairment in other similar subtropical zones.

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Growth parameters and activity of xenobiotic-metabolizing enzymes of juvenile *Litopenaeus vannamei* fed diets containing aflatoxins and an aflatoxin binder

Parámetros de crecimiento y actividad de enzimas metabolizadoras de xenobióticos de juveniles de *Litopenaeus vannamei* alimentados con dietas conteniendo aflatoxinas y un secuestrante de aflatoxinas

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ABSTRACT

Background. Shrimp farms are increasingly vulnerable to aflatoxin (AF) negative effects as frequent inclusion of plant-based ingredients is used in the diets. **Goals.** Evaluate the impact of a bentonite-based AF binder on growth parameters, alkaline phosphatase (ALP) and glutathione s-transferase (GST) activities of juvenile *Litopenaeus vannamei* shrimp fed AF contaminated diets for 42 days. **Methods**. Juvenile shrimps were randomly assigned to the following study groups, including a non-contaminated diet (NCD), an AF contaminated diet (ACD), and ACD supplemented groups with either 1, 1.5 or 2 g/kg feed of Mycofix Plus (MyP), a clay-based mycotoxin binder that contains bentonite, enzymes, algae, and plants extracts. **Results.** Shrimp from ACD had a significantly lower mean weight, growth rate, feed intake and nitrogen retention percentage when compared to shrimp from the NCD group. All MyP treatments resulted in similar mean weight, growth rate and nitrogen retention efficiency to NCD animals, at the same time, only the ACD + 1 and ACD + 1.5 MyP had ALP and GST activities similar than that of shrimp fed NCD. Interestingly, shrimp from ACD + 2 MyP had high ALP and GST activities, even higher than the ACD animals. **Conclusions**. MyP protected shrimp from adverse AFs effects, however, at the highest dose, it increased metabolic enzyme activities, likely due to other bioactive compounds present in MyP.

Keywords: Aflatoxins, binder, growth, mycotoxin, shrimp

RESUMEN

Antecedentes. Las granjas camaroneras son cada vez más vulnerables a los efectos negativos de las aflatoxinas (AF), ya que se utilizan con frecuencia ingredientes de origen vegetal en las dietas. **Objetivos**. Evaluar los efectos de un secuestrante de AF a base de bentonita sobre los parámetros de crecimiento y la actividad de las enzimas fosfatasa alcalina (ALP) y glutatión S-transferasa (GST) en juveniles de camarón *Litopenaeus vannamei* alimentados con dietas contaminadas con AF durante 42 días. **Metodos.** Los camarones se asignaron aleatoriamente a los siguientes grupos de estudio, que incluyeron una dieta no contaminada (NCD), una dieta contaminada con AF (ACD) y grupos suplementados con ACD y conteniendo 1, 1.5 o 2 g/kg de alimento de Mycofix Plus (MyP), un aglutinante de micotoxinas a base de arcilla que contiene bentonita, enzimas, algas y extractos de plantas. **Resultados**. Los camarones de ACD tuvieron un peso medio, una tasa de crecimiento, un consumo de alimento y un porcentaje de retención de nitrógeno significativamente más bajos en comparación con los camarones del grupo NCD. Todos los tratamientos MyP dieron como resultado un peso medio, una tasa de crecimiento y una eficiencia de retención de nitrógeno similares a los camarones alimentados con NCD, mientras que solo ACD + 1 y ACD + 1.5 MyP tuvieron actividades ALP y GST similares a las de los camarones alimentados con NCD. Curiosamente, los camarones de ACD + 2 MyP tenían altas actividades de ALP y GST, incluso más altas que los animales ACD. **Conclusiones**. MyP protegió a los camarones de los efectos adversos de las AF; sin embargo, en la dosis más alta, aumentaron las actividades de las enzimas metabólicas probablemente debido a otros compuestos bioactivos presentes en MyP.

Palabras claves: aflatoxinas, secuestrante, crecimiento, micotoxinas, camarón

INTRODUCTION

The high plant feedstuff inclusion levels influence the existence of mycotoxicosis in aquaculture production in finished feeds (Gonçalves *et al.,* 2020). *Aspergillus* spp*., Penicillium* spp., and *Fusarium* spp. are the main fungal species responsible for most mycotoxins present in meals (Kabak, 2009; Freire & Da-Rocha, 2017). Recent reports of a mycotoxin survey revealed the presence of aflatoxin (AF), zearalenone, T-2 toxin, and deoxynivalenol in numerous feedstuff samples (21,709) collected from 79 countries, from which it was concluded that 6.5 samples out of 10 had at least one of the mentioned mycotoxins above the threshold levels, and 87% of the test samples contained ten or more mycotoxins and metabolites, being AF the most found (Biomin, 2021a). The incidence of mycotoxins is said to be influenced by harvest and storage conditions. For instance, hot and humid environments are the main factors that promote fungal contamination and toxin production (Saad, 2016). Particularly for *Aspergillus* species, temperature between 25 and 35ºC are known to promote AF development (Daou *et al*., 2021). These conditions can occur in most aquaculture facilities. Several studies have demonstrated up to 24 different types of mycotoxins (including metabolites) in raw ingredients and complete fish feeds (Koletsi *et al*., 2021). Deoxynivalenol, AF, zearalenone, ochratoxin, fumonisin B1, fumonisin B2, fusaric acid, ergotamine, and deoxynivalenol-3-glucoside were the most predominant mycotoxins (Gonçalves *et al.*, 2017; Koletsi *et al*., 2021).

The effects of feeding mycotoxin contaminated diets to fish and shrimp has resulted in significant reduction in feed consumption and growth rate, immune suppression, liver lesions, alterations of phase I xenobiotic biotransformation enzymes such as cytochrome P450 (CYP450) and alkaline phosphatase (ALP), phase II enzymes such as gluthatione S-tranferase (GST) and mortality (Santacroce *et al*., 2008; Ghaednia *et al*., 2013; Mahfouz & Sherif, 2015; Pérez-Acosta *et al*., 2016; Zeng *et al*., 2016; Tapia-Salazar *et al*., 2017; Yu *et al*., 2018). From all mycotoxins, aflatoxins (AFs) are particularly important due to their toxicity and carcinogenic effects (IARC, 2012). Aflatoxin B1 is considered one of the most toxic compounds for terrestrial and aquatic species (Mohamed *et al*., 2017). Different strategies had been developed to reduce the toxic effect of mycotoxin consumption, such as physical decontamination, chemical decontamination, and biological decontamination (Daou *et al*., 2021). Earlier studies showed that the use of AF binders such as mixtures of aluminosilicate minerals and *Saccharomyces cerevisiae* (*S. cerevisiae*) yeast cell walls; mixtures of glucomannans from cell walls of *S. cerevisiae* with *Chlorella vulgaris* cell walls; as well as mixtures of bentonite (a montmorillonite rich mineral), *Trichosporon mycotoxinivorans* yeast and algae extracts included at levels that the manufacturing industry recommends can improve but not revert toxic effects of diets contaminated with 75 µg/kg AFs on shrimp (Tapia-Salazar *et al*., 2017). Tapia-Salazar *et al*. (2017) observed that feeding diets containing AF + 2.5 g/kg MyP resulted in a similar feed intake to the CD diet. The inclusion levels for MyP used for these authors were selected from terrestrial studies due to the lack of information on aquatic organisms. Therefore, more information related to the effectivity of Mycofix Plus® (MyP), to reduce aflatoxicosis at lower inclusion levels in shrimp is required. The objective of the present study was to evaluate the protective effects of different levels of MyP on white shrimp *Litopenaeus vannamei* (*L. vannamei*) fed 200 µg/kg AF-contaminated diet in terms of growth rate, survival, feed conversion ratio and the enzymatic activity of ALP and GST after 42 feeding days.

MATERIALS AND METHODS

Preparation of contaminated corn: The AF contamination of corn was performed at the Nutek S.A. de C.V. company facilities in Tehuacan, Puebla, Mexico. The contamination protocol followed methods previously described by Tapia-Salazar *et al*. (2012). Spores from *Aspergillus parasiticus* were inoculated onto white corn grains inside an Erlenmeyer flask and then placed in an incubator at 25°C for 7 days. Contaminated corn was then sterilized for 30 min at 120°C, left to dry for 5 days in a biosafety recirculation hood, then milled with a grinder and passed through a steel sieve with an opening size of 850 µm (ASTM mesh #20). Total AFs were measured with high-performance liquid chromatography (HPLC) following the official AOAC method 994.08 (AOAC, 2007).

Preparation of experimental diets: To assure optimal growth of juvenile shrimp, a non-contaminated control diet (NCD) containing 40% crude protein and 7% lipid content was prepared with wheat flour (43%), soybean meal (8%) and shrimp meal (4%) as main ingredients. The AF contaminated diet (ACD) was prepared by adding 10.9% of contaminated cornmeal in substitution for the wheat flour. The AF content in ACD (200 µg/kg) was based on a previous study where a significant reduction in shrimp growth, feed intake, and survival was observed (García-Pérez *et al*., 2020). Three more experimental diets were prepared with ACD complemented with 1, 1.5 or 2 g of MyP per kg of diet. Briefly, the ingredients for diet preparation were ground in a Cyclotec™ 1093 sample mill (Foss-Tecator, Denmark) to obtain an average particle size of 500 µm, after milling, the ingredients were mixed with soy lecithin and fish oil for 10 min in a Kitchen Aid mixer and then warm water (30%) was added to be mixed for 15 more min. Finally, the dough was processed through a grinder (fitted with a metal screen with 1.6 mm hole diameter) at a 40 min/kg passage rate at 75ºC. The pellets were then dried in a convection oven at 100ºC for 8 min and were left to cool overnight before placing on Ziploc bags. The chemical composition of experimental diets (Table 1) was calculated using previously reported methods (Cruz-Suárez *et al*., 2009). Total AFs in the diet were measured by a fluorometer method using AflaTest® immunoaffinity columns (VICAM, Milford, MA). Columns can detect the four most common AFs expected to be found in feed, such as AFB1, AFB2, AFG1, and AFG2. Proximate analysis on diets was done to determine dry matter (%) and water absorption (%) following methods previously reported (García-Pérez *et al*., 2013).

Animal study conditions: Juvenile shrimp of *L. vannamei* species were maintained in a closed recirculation system containing artificial seawater (Fritz®, Dallas, TX, USA) using a 350 mL/min flow-through rate. Each tank was equipped with an air-water lift system for internal recirculation. All tanks were interconnected to achieve the same conditions simultaneously. The study did not require ethical approval to develop research with invertebrates as it is unnecessary by the Mexican regulations. However, studies were developed adhering to maintenance and euthanasia protocols reported for decapods (Approved protocols for decapods, cephalopods, and fish, 2018; Leary *et al.,* 2020). Water temperature and salinity were measured daily while pH, ammonium, nitrates, and nitrites, were measured weekly.

Shrimp source and feeding protocol: For this study, juvenile shrimp were obtained from Municipio de Rosario, Sinaloa, Mexico, through a donation by a local farm. Upon arrival, shrimp were acclimated for 4 days in 500 L tanks. After acclimation, shrimp were individually weighed (75 mg average initial weigh) and allocated to 15 tanks (12 shrimp

Table 1. Composition and proximate analysis of experimental diets for juvenile white shrimp

	NCD	ACD	ACD+ 1 MyP	ACD+ 1.5 MyP	ACD+ 2 MyP
Formula (g/kg)					
Wheat meal	431.26	431.47	435.18	434.59	434.1
Fish meal	380.18	379.97	387.87	387.99	388.06
Soybean meal	80	80	67.4	67.45	67.34
Constant ingredients†	97.6	97.6	97.6	97.6	97.6
Non-contaminated corn	10.953	---	---	---	---
Contaminated corn	---	10.953	10.953	10.953	10.953
MyP	---	---	1	1.5	2
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Chemical composition (% dry matter)					
Moisture					
Protein	42.6 ± 0.3	42.5 ± 0.09	42.1 ± 0.2	42.2 ± 0.2	42.2 ± 0.3
Crude lipids	7.3 ± 0.3	7.3 ± 0.4	7.2 ± 0.3	7.4 ± 0.3	7.3 ± 0.4
Fiber	2.9 ± 0.02	2.9 ± 0.1	2.6 ± 0.03	2.5 ± 0.09	2.8 ± 0.2
Ash	10.4 ± 0.01	10.6 ± 0.1	10.4 ± 0.02	10.5 ± 0.1	10.9 ± 0.02

†Constant ingredients (g/kg): shrimp meal 40, fish oil 20, soy lecithin 20, alginic acid 10, vitamin mixture 3.5, mineral mixture 2.5, antioxidant 0.5, mold inhibitor 0.5, cholesterol 0.2, vitamin C 0.2 and vitamin E 0.2

Vitamin mixture composition: retinol, 4000 IU/g; thiamin, 24 g/kg; riboflavin, 16 g/kg; DL Ca pantothenate, 30 g/kg; pyridoxine, 30 g/kg; cyanocobalamin, 80 mg/kg; ascorbic acid, 60 g/kg; menadione, 16 g/kg; cholecalciferol, 3200 IU/g; tocopherol, 60 g/kg; biotin, 400 mg/kg; niacin, 20 mg/kg; folic acid, 4 g/kg.

Mineral mixture composition: Co, 2 g/kg; Mn, 16 g/kg; Zn, 40 g/kg; Cu, 20 g/kg; Fe, 1 mg/kg; Se, 100 mg/kg; I, 2 g/kg.

per tank). Five study groups were formed as follows: NCD, ACD, ACD + 1, 1.5, or 2 g of MyP per kg of diet, and each group had three replicates. Replacement animals were kept in a tank and when necessary, dead shrimp were replaced over the first three days after distributing the animals in each study group. Shrimp were kept on 12/12 light/dark cycle conditions for 42 feeding days. The initial feeding ratio was based on a 20% total biomass found in each tank. Feeding protocol and calculation of feeding rate were based on previous research (Tapia-Salazar *et al*., 2012). Shrimp were fed 3 times a day (8:00 am, 12:00 pm and 5:00 pm); siphoned leftover feed from the tank before the new fresh feed was provided. Pelleted feed was broken down into small crumbs to ensure meal was available to all shrimp.

Growth parameters: During the experiment, shrimp from each experimental group were individually weighed at 0, 14, 28, and 42 days using a digital scale. The amount of feed provided per tank was adjusted every two weeks after shrimp weight. Before weighing, excess water in shrimp was removed with a cotton cloth. Survival and feed consumption were recorded daily; appropriate feed adjustments were done considering the remaining shrimp and leftover feed in each tank, every day. Growth rate, feed consumption, feed conversion ratio, and survival rate were calculated by using formulas described by García-Pérez *et al*. (2013). A sample of shrimp (10 g) from the replacement tank was taken at time 0 and at the end of the experiment to measure water and nitrogen content in shrimp tissue. Shrimp samples for nitrogen content were freeze-dried and then ground in a mill. Calculation of nitrogen retention efficiency was done according to the following formula:

$$\frac{AFW\ (g)\ \times\ FCP\ (\%) - AIW\ (g) \times\ ICPC\ (\%)}{CCP\ (g)} \times 100$$

Were:

AFW: Average final weight

FCP: Final crude protein in carcass

AIW: Average initial weight

ICPC: Initial crude protein in carcass

CCP: Consumed crude protein

Enzymatic activity biomarkers: At the end of the experiment, hepatopancreas samples of three shrimp from each tank were taken to measure ALP and GST activities. Hepatopancreas samples were homogenized in double distilled water at 4 ºC in a 1:10 proportion (sample weight:water, m/v) with a mortar and pestle for 4 minutes. Then homogenized samples were centrifuged at 2000 g for 15 minutes at 4 ºC, and the supernatant was aliquoted into 0.1 mL Eppendorf tubes and stored at -70 ºC until use. Protein content in tissue extracts was quantified using Bradford method and bovine serum albumin (BSA) as a calibration curve (Bradford, 1976). ALP activity was determined by

using p-nitrophenyl phosphate (substrate). The reaction was performed using 200 µL of diethanolamine buffer (1.0 M) with 50 mM $MgCl_2$ (pH 9.8), then 10 µL of the enzymatic extract and 10 µL of the substrate were added at a final concentration of 0.4 mM. Absorbance was immediately registered at 405 nm in 120-second intervals for up to 10 minutes in an EPOCH microplate reader (Biotek, Vermont, USA). For each sample, three analytical replications were conducted. The sample was replaced with a buffer in control wells. The linearity of the reaction was verified, and the enzymatic activity was expressed as µmol/min/mg protein using p-nitrophenol molar extinction coefficient of 18.5 mM/cm (Mazorra *et al*., 2002). GST activity was analyzed using the Habig *et al*., (1974) method adapted to microplates. A volume of 300 µL of a substrate mixture containing reduced L-glutathione (200 mM) and 1-chloro-2, 4-dinitrobenzene (CDNB; 100 mM) in Dulbecco's phosphate-buffered saline (2.7 mM KCl, 1.5 mM KH_2PO_4, 136.9 mM NaCl and 8.9 mM $Na_2HPO_4 \bullet 7H_2O$, pH 7.2) along with 10 µL of hepatopancreas extract were added into each well. Absorbance was immediately read at 340 nm every minute for 10 minutes. GST activity was expressed as µmol/min/mg protein, using a molar extinction coefficient of 9.6 mM/cm for CDNB (Brodeur *et al*., 2011).

Statistical analysis: Statistical analyses were done using SSPS software (version 16.0, SPSS Inc., Chicago, Illinois). Average body weight per tank was used to calculate the growth rate and feed conversion ratio. Normality of data was not verified as it is not required for sample size ≥ 25 (Van den Berg, 2022). Homoscedasticity (homogeneity of variances) was verified with Levene´s test. The test variables (growth parameters and enzymatic activity) were analyzed with a one-way ANOVA test and then followed by Tukey´s multiple comparisons tests to detect significant differences among experimental groups ($p < 0.05$).

RESULTS

Experimental diets: Chemical composition of the experimental diets was similar among treatments (Table 1). The concentration of total AFs in ACD was 200 µg/kg while NCD did not show detectable levels of AFs (Limit of detection=1 ng AF/kg diet). Values of dry matter loss and water absorption capacity of diets are presented in Table 2. One-way ANOVA showed that MyP addition at 1.5 and 2 g/kg had a higher reduction of water absorption ($p = 0.001$) while NCD had a significantly lower dry matter loss when compared to ACD and ACD + 1.5 diets ($p < 0.001$) but not significantly different from ACD + 1 MyP or ACD + 2 MyP.

Animal study conditions and growth parameters: Water condition parameters (mean ± standard deviation) were maintained during the study with the following values recorded for salinity 35 ± 3 g/L, temperature 30 ± 2 ºC, pH 8.1 ± 0.1, ammonium 0 mg/L, nitrites 0.2 mg/L, and nitrates 40 ± 15 mg/L. Shrimp final growth parameters such as weight, feed intake, growth rate, feed conversion ratio, survival (%) and nitrogen retention efficiency (%) are presented in Table 3. When compared to NCD, one-way ANOVA showed that shrimp consuming ACD had a significant reduction in growth, feed intake and nitrogen retention efficiency ($p < 0.05$). Interestingly, shrimp from all MyP groups had mean weight, growth rate and nitrogen retention efficiency similar to animals from NCD. Particularly, animals feed with ACD + 2 MyP showed no differences ($p < 0.05$) in mean weights, feed intake, growth rate, or nitrogen retention efficiency when compared to animals from NCD group. However, animals with the low and medium dose of MyP (i.e., 1 and 1.5 g of MyP) did not reach significance to be different from animals on ACD. No significant differences were observed for feed conversion ratio or survival. An apparent improvement in feed intake was noted due to MyP addition to ACD, however, only the 2 g/ kg inclusion of MyP showed a significant difference among study groups, resulting on a higher average feed intake of 3.8 g compared to the 2.9 g observed in ACD group. Regarding the growth rate, shrimp that consumed ACD + 2 MyP had higher growth compared to the shrimp fed with ACD (2980% vs 2185% respectively), and it was similar to the growth rate of shrimp consuming NCD (2964%). Nitrogen retention efficiency was lower for the shrimp consuming ACD (21.7%) than that for shrimp of NCD and ACD+2 MyP groups (35.6% and 35.01% respectively), animals from these treatment groups had the highest nitrogen retention values (p=0.007).

Enzymatic activity: Shrimp fed with ACD had a higher ALP and GST activity than organisms fed with NCD (Figure 1). Regarding ALP, the shrimp from ACD + 1 MyP and ACD + 1.5 MyP groups had the lowest enzymatic activity values and were similar to the shrimp from the NCD group. On the contrary, the shrimp from the ACD + 2 MyP group showed the highest enzyme activity value, and this difference was significant when compared to all other treatment groups ($p < 0.05$). GST activity

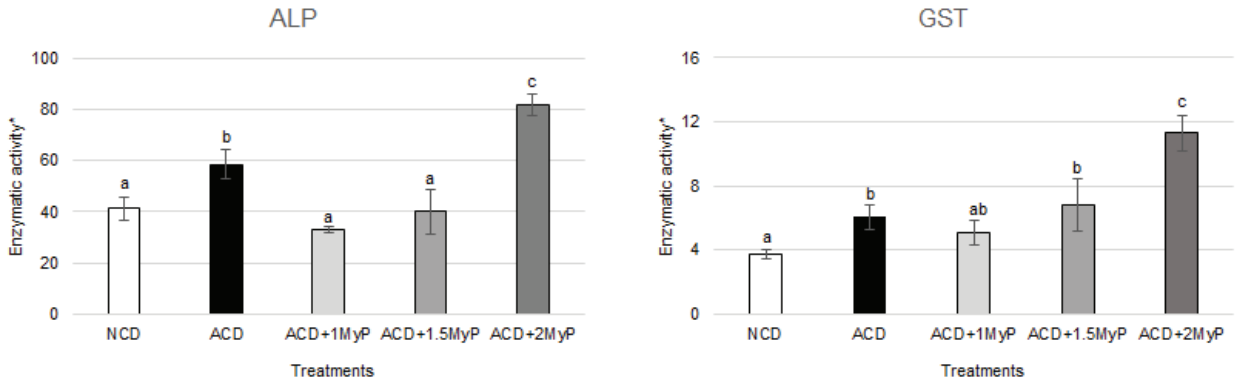

Figure 1. ALP and GST enzymatic activities from juvenile of shrimp *L. vannamei* consuming either NCD, ACD or ACD supplemented with MyP at 1, 1.5 or 2 g/kg feed.

did not result in significant differences among the ACD + 1 MyP and ACD + 1.5 MyP groups, however, shrimp belonging to the ACD + 2 MyP group had the highest enzyme activity among all treatment groups, including the ACD group ($p < 0.05$). Overall, a trend of increased activity was observed for both enzymes as higher concentrations of MyP were present in the diets.

DISCUSSION

The current study showed that ACD consumption by shrimp for a 42-day period resulted in significant reductions in growth parameters such as mean weight, feed intake, growth rate, and nitrogen retention efficiency. These results are consistent with those published in previous studies where consumption of AFs resulted in evident adverse effects in shrimp of the same species and age, and under similar experimental conditions (Tapia-Salazar *et al.*, 2012; García-Perez *et al.*, 2020), and in shrimp of similar species such as *Penaeus monodon* (Bautista *et al.*, 1994; Gopinath & Paul-Raj, 2009). The recurring dietary exposure to AFs exerts a substantial impact on shrimp farming, causing subclinical symptoms that subsequently result in impaired health status, followed by decreased production efficiency (Wang *et al.*, 2018). In livestock production, the addition of mycotoxin binders or AFs adsorbents to the diets is an economical strategy to prevent the negative effects caused by the presence of these toxins in feed (Farooqui *et al.*, 2019). According to Neeratanaphan & Tengjaroenkul (2018), the use of Thai bentonite has been shown to improve weight gain, survival rate, red blood cell counts and alanine aminotransferase enzyme in white shrimp feed diet supplemented with 0.5% bentonite when compared to those fed toxin contaminated diet only (150 ppb AFB1). Most mineral-based binders claim to be effective for AF sorption and this has been supported by *in vitro* and *in vivo* studies where montmorillonites (Ramos & Hernández, 1996), and zeolites (Piva *et al.*, 1995) have shown a high *in vitro* binding affinity for AFs, and hydrated sodium calcium aluminosilicates (HSCAS) (Döll *et al.*, 2005) and bentonites (Schell *et al.*, 1993) have proven to reduce the harmful effects of this toxin on performance parameters in other species such as pigs. Some mycotoxin binders are also formulated with a variety of other ingredients including antioxidants, probiotics, yeast, or plant materials that may improve their effectiveness (Tapia-Salazar *et al.*, 2017). That is the case of MyP which is also described as a mycotoxin deactivator (Hanif *et al.*, 2008). Since the adsorption of AF to mineral binders is essentially a surface phenomenon, its effectiveness depends on several physical characteristics such as pore size and distribution, total charge, and charge distribution (Di-Gregorio *et al.*, 2014). In the present study, shrimp that were fed diets supplemented with MyP had increased mean weight, feed intake, growth rate and nitrogen retention efficiency, while the shrimp that consumed the ACD + 2MyP presented significant differences when compared with the shrimp fed with ACD. Regarding cost-benefits, by using our FCR data, an approximate price of 32 Mexican pesos (Mxp) for a regular 1 kg of shrimp diet, and 0.22 Mxp as the approximate cost of 1 g of MyP, the cost of production of 1 kg of shrimp with a regular shrimp diet contaminated with AF (200 µg/kg) and complemented with 1, 1.5 or 2 g/kg MyP has an average estimate of 85 Mxp, similar to the cost of production using non-contaminated diet (83 Mxp), and contrarily to what is expected for shrimp only ingesting AF contaminated diet (98.24 Mxp).

Table 2. Dry matter loss (DML) percentage and water absorption capacity (WA) of experimental diets.

Experimental diet	DML (%)	WA (%)
NCD	16.1 ± 0.01^{a}	182 ± 3.6^{c}
ACD	18.1 ± 0.7^{bc}	164 ± 20^{bc}
ACD+1 MyP	17.3 ± 0.1^{ab}	141 ± 6^{b}
ACD+1.5 MyP	19.3 ± 0.3^{c}	104 ± 4^{a}
ACD+2 MyP	16.9 ± 1.1^{ab}	109 ± 10^{a}
SEM	0.32	8.45
Sig.	0.001	0.001

Data presented are the means ± standard deviations. Different letters in the same column indicate significant differences among groups with Tukey mean comparisons ($p < 0.05$).

The use of mycotoxin binder or deactivator products has been successfully used in terrestrial animals to prevent the effect of consuming AFs (Hussain *et al.*, 2017; Kehinde *et al.*, 2018; Nazarizadeh & Pourreza, 2019; Saleemi *et al.*, 2020), however, in aquaculture, few studies have used mixed-nature adsorbents that contain ingredients that are known to modulate xenobiotic biotransformation enzymes. Agouz & Anwer (2011) found an increase in growth and survival in common carp (*Cyrpinus carpio*) supplemented with a synthetic probiotic-immunostimulant product (Biogen®) and a commercial smectite clay composed of

Table 3. Growth parameters of white shrimp fed non-contaminated diet (NCD) or aflatoxin-contaminated diet (ACD) supplemented with MyP

Experimental diet	Mean weight (g)	Feed intake (g/shrimp)	Growth rate (%)	Feed conversion ratio	Survival (%)	Nitrogen retention efficiency (%)
NCD	2.34 ± 0.17^{b}	4.07 ± 0.2^{c}	2964 ± 234^{b}	2.59 ± 0.1	97.2 ± 4.8	35.6 ± 3.6^{b}
ACD	1.73 ± 0.1^{a}	2.99 ± 0.1^{a}	2185 ± 137^{a}	3.07 ± 0.1	97.2 ± 4.8	21.7 ± 2.3^{a}
ACD+1 MyP	1.92 ± 0.2^{ab}	3.3 ± 0.2^{ab}	2432 ± 274^{ab}	2.88 ± 0.3	100 ± 0	29.4 ± 3.3^{ab}
ACD+1.5 MyP	2.02 ± 0.3^{ab}	3.24 ± 0.2^{ab}	2500 ± 489^{ab}	2.76 ± 0.6	94.4 ± 4.9	29.5 ± 2.9^{ab}
ACD+2 MyP	2.33 ± 0.8^{b}	3.84 ± 0.07^{bc}	2980 ± 137^{b}	2.3 ± 0.1	91.6 ± 8.4	35.01 ± 1.6^{b}
SEM	0.078	0.211	104	0.101	1.37	1.62
Sig.	0.023	0.008	0.025	0.123	0.415	0.007

Data presented are the means ± standard deviations. Different letters in the same column indicate significant differences with Tukey mean comparisons ($p < 0.05$)

an activated, broad-spectrum HSCAS (Myco-Ad®) in a diet contaminated with 22 ppb AF and 15 ppb ochratoxin. Other studies showed that shrimp ingesting a diet with 75 µg AF/kg and supplemented with 2.5 g/kg MyP, improved the growth rate, although not significantly (Tapia-Salazar *et al*., 2017). The reasons for the differences found between the Tapia-Salazar *et al*. (2017) study and our current study are likely related to the different AFs contamination levels (75 µg/kg AFs vs 200 µg/kg), dietary inclusion amounts of MyP product (2.5 g/kg diet vs 1, 1.5 and 2 g/kg diet), as well as the average initial weights of the organisms used (210 mg vs 76 mg).

Regarding enzymatic activity, the present study found an increase in ALP and GST activities in shrimp fed with ACD when compared to those fed with NCD. The increase in enzymatic activity due to the consumption of AF is consistent with previous results observed in the aquatic organisms (Mahfouz & Sherif, 2015; Manal, 2016). ALP is a hepatopancreatic enzyme involved in detoxification, elevated ALP activity is found in the hepatopancreas when this organ needs to metabolize large amounts of xenobiotics (Boonyaratpalin *et al*., 2001). Previous works (García-Pérez *et al*., 2020) showed that ALP activity can increase in shrimp consuming aflatoxins (200 ppb) when compared to animals consuming non-contaminated diet (58.4 µmol/min/mg protein vs 41.3 µmol/min/mg protein, respectively). Contrarily, some studies show a reduction in hepatopancreas ALP activity in enzyme extracts incubated with AFB1 (Perez-Acosta *et al*., 2016), however, *in vitro* data may not reflect what occurs *in vivo*. Increased GST activity has also been shown in shrimp exposed to AFB1 at levels as low as 15 ppb (Zhao *et al*., 2017). Clearly, increased activity of both enzymes can be beneficial to shrimp that are exposed to AFB1-contaminated diets.

Interestingly, in the current study, shrimp from ACD + 1 MyP and ACD + MyP 1.5 did not show significant differences in ALP activity when compared to animals from NCD group. Thus, highlighting the beneficial effects of dietary inclusion of MyP at either 1 or 1.5 g/kg inclusion. MyP is a clay-based feed additive frequently used as an AF adsorbent. The mineral components belong to the aluminosilicate group, specifically, the bentonite type (rich in montmorillonite), according to the manufacturer. Bentonites have a high capacity for AF adsorption (Thieu & Pettersson, 2008) and the mechanisms of toxin adsorption have been described as chemisorption on mineral surfaces (Grant & Phillips, 1998) or physisorption with the interlayer cations (Deng *et al*., 2010). Upon binding, a complex toxin-mineral is formed thus reducing AF bioavailability from the gastrointestinal tract (Ramos & Hernández, 1997), which may prevent hepatopancreas damage and the consequent changes in ALP and GST enzyme activity.

Among treatments, the shrimp belonging to the ACD + 2 MyP group had the highest values of both enzymes, even higher than shrimp fed ACD. It is known that MyP contains a proprietary blend of plant and algae extracts said to support liver function (Biomin, 2021b). For instance, Abdel-Rahim *et al*., (2021) reported that when feeding juvenile *Litopenaeus vannamei* with 500 mg/kg of *Sargassum polycystum* algae supplemented feed, the ALP activity showed a higher mean of 8.80 U/L compared to the 6.33 U/L mean registered for control animals (no-algae supplemented feed). The source of algae in MyP is not known (undisclosed for patent protection) but seaweed and macroalgae are a rich source of bioactive compounds (Thanigaivel *et al*., 2014), and some have hepatoprotective activity (Schleder *et al*., 2018). Hence, we proposed that the inclusion of MyP at 1 and 1.5 g/kg inclusions were able to maintain ALP and GST enzymatic activities similar to those values observed for control animals. However, at a higher inclusion of MyP (2 g / kg), other bioactive compounds present in this product may stimulate hepatopancreas xenobiotic metabolic functions resulting on increased ALP and GST enzyme activities.

CONCLUSION

Feeding MyP to juvenile *L. vannamei* shrimp ingesting 200 µg/kg of AF was able to reduce the negative effects exerted by the toxin on growth parameters and on enzymatic activities. Overall health and performance of all shrimp ingesting MyP were similar that animals fed NCD. This was especially observed in animals with the high dose of MyP (2 g/kg). Additionally, the high dose of MyP (2 g/kg) resulted in enzymatic ALP and GST activities higher than all other groups, including control animals and animals fed ACD suggesting a further hepatopancreas xenobiotic-metabolizing enzymes stimulation caused by other bioactive compounds in MyP. Further studies can be directed to identify the specific bioactive compounds on MyP and to delineate if there is an additive or synergistic effect on MyP ingredients and AF on ALP and GST enzymes.

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Diversity of archaea in tropical and subtropical estuarine-lagoon ecosystems. A synthesis

Diversidad de arqueas en ecosistemas estuarino-lagunares tropicales y subtropicales. Una síntesis

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ABSTRACT

Background. Tropical and subtropical estuarine ecosystems are among the most productive ecosystems on the planet, their seasonal fluctuations, their permanent or ephemeral connection with the ocean and freshwater discharges, generate a high biodiversity that provides numerous ecosystem services. In these ecosystems, biodiversity research has focused on macro-organisms and less attention has been paid to prokaryotes, particularly the archaea group. **Goal.** Based on a bibliographic review of the Archaea Domain in estuaries, coastal lagoons and mangroves located in tropical and subtropical zones, to provide a synthesis of the factors that influence the presence and distribution of archaea in these ecosystems and the role they play in biogeochemical cycles. **Methods**. A search was made of the articles published with the keywords Archaea + tropical coastal ecosystems and Archaea + subtropical coastal ecosystems. **Results.** The analysis of the environmental sequences obtained, from molecular techniques, in studies of the diversity of prokaryotes in coastal lagoons, estuaries and tropical and subtropical mangroves, have revealed a high diversity of archaea belonging mainly to methanogens and anaerobic methanotrophs (Phyla Euryarchaeota), ammonium-oxidizing archaea (Thaumarchaeota) and representatives of the Superphyllum Asgard. These groups can potentially participate in the carbon, nitrogen, and sulfur cycles, in aerobic or anaerobic conditions, with heterotrophic or autotrophic metabolisms, and their abundance and distribution are related to the physicochemical conditions of the ecosystems. **Conclusions**. The diversity of Archaea in tropical and subtropical coastal ecosystems is greater than previously recorded. These microorganisms play a vital role in various biogeochemical cycles as well as climate change.

Keywords: archaea biodiversity, coastal ecosystems, tropical and subtropical zones

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RESUMEN

Antecedentes. Los ecosistemas estuarinos tropicales y subtropicales se encuentran entre los ecosistemas más productivos del planeta, sus fluctuaciones estacionales, su conexión permanente o efímera con el océano y las descargas de agua dulce, generan una alta biodiversidad que proporciona numerosos servicios ecosistémicos. En estos ecosistemas, la investigación de la biodiversidad se ha centrado en los macroorganismos y se ha prestado menor atención a los procariontes, particularmente el grupo de las arqueas. **Objetivo**. A partir de una revisión bibliográfica del Dominio Archaea en estuarios, lagunas costeras y manglares ubicados en zonas tropicales y subtropicales, proporcionar una síntesis de los factores que influyen en la presencia y distribución de las arqueas en estos ecosistemas y el papel que desempeñan en los ciclos biogeoquímicos. **Métodos**. Se efectuó una búsqueda de los artículos publicados con las palabras clave Archaea + ecosistemas costeros tropicales y Archaea + ecosistemas costeros subtropicales. **Resultados**. El análisis de las secuencias ambientales obtenidas, a partir de técnicas moleculares, en los estudios de diversidad de los procariontes en lagunas costeras, estuarios y manglares tropicales y subtropicales, han revelado una alta diversidad de arqueas pertenecientes principalmente a metanógenos y metanotrofos anaeróbicos (Phyla Euryarchaeota), arqueas oxidantes del amonio (Thaumarchaeota) y representantes del Superphyllum Asgard. Estos grupos pueden potencialmente participar en los ciclos del carbono, nitrógeno y azufre, en condiciones aerobias o anaerobias, con metabolismos heterótrofos o autótrofos y su abundancia y distribución están

relacionadas con las condiciones fisicoquímicas de los ecosistemas. **Conclusiones**. La diversidad de Archaea en ecosistemas costeros tropicales y subtropicales es mayor de la registrada previamente. Estos microorganismos desempeñan un papel vital en diversos ciclos biogeoquímicos así como en el cambio climático.

Palabras clave: Biodiversidad de arqueas, ecosistemas costeros, zonas tropicales y subtropicales.

ECOSISTEMAS COSTEROS TROPICALES

The tropical zone is located between 24° north and south latitudes, delimited by the Tropics of Cancer and Capricorn, respectively. It is one of the largest areas on the planet, while the subtropical region is located between the tropical and temperate zones, between 24°and 40° north and south latitudes. These regions are characterized by fewer temperature fluctuations and constant rainfall, mainly near the equator. The tropics account for 40% of the planet´s total surface area and harbor a greater diversity than temperate and cold latitudes, which is distributed in terrestrial, marine and coastal ecosystems (Willing *et al.*, 2003).

Tropical coastal areas have diverse ecosystems, including coastal lagoons, estuaries, and mangroves. These ecosystems are extremely complex, they are transition zones between freshwater terrestrial drainage and the coastal marine zone; this mixture of water with different salinity levels creates brackish conditions (Pérez-Ruzafa *et al.*, 2011). Despite of this common characteristic, each one has specific properties that distinguish them from each other.

Estuaries are more common on the coasts of temperate climates, where freshwater inflow is sufficient to keep the sea mouth open (Harris, 2008). The main axis of an estuary is perpendicular to the coastline; morphologically, it is funnel-shaped and river inflow flows directly into the ocean up to the effective limit of tidal influence, diluting seawater and forming a longitudinal salinity gradient (Perillo, 1995). In addition to the salinity gradient, other chemical gradients are usually present, including a decrease in the content of organic matter and nitrogenous compounds (mainly ammonium and nitrates) from the river input area to the sea mouth, while oxygen content increases in the same direction (Webster *et al.*, 2015).

Coastal lagoons are generally shallow bodies (most are less than 5 m deep), temporarily or permanently open to the sea, characterized by the presence of a sandy barrier that separates the lagoon from the ocean and connection to the sea is maintained through the mouth or tidal channels (De Wit *et al.*, 2001). The main axis of the lagoon is parallel to the coast and most lagoons are connected to a freshwater continental basin through river inputs, some permanent and some temporary. In tropical latitudes, these inputs can fluctuate considerably, with minimum volumes during the dry season and maximum volumes during the rainy season, due to increased precipitation. In contrast to estuaries, coastal lagoons are common in tropical and subtropical coasts, where precipitation patterns are highly seasonal, resulting in significant fluctuations in river discharge and associated hydrological gradients, which are usually more complex than those in estuaries and which influence on ecosystem functioning (Barnes, 2001; Harris, 2008).

Mangrove forests are coastal wetlands constituted by woody trees and affected by tidal and freshwater influences. They are ecosystems adjacent to coastal lagoons and estuaries. Mangrove roots are adapted to flooded and anoxic soils that favor the development of anaerobic metabolisms, including methanogenesis (Taketai *et al.*, 2010). Mangrove forests support an important food web based on detritus, they are ecologically important, protect the coastline and act as sediment and nutrients traps (Holguin *et al.*, 2001).

Coastal lagoons, estuaries and mangroves are areas of high biodiversity and productivity due to high nutrient content, which are ecologically viable for fisheries; provide different ecosystem services such as nutrient retention, flood control and sediment stabilization, as well as social benefits (Knoppers, 1994; Cloern *et al.*, 2014; Pérez-Ruzafa *et al.,* 2019). Despite their importance, estuarine ecosystems are among the most vulnerable environments to climate change and anthropogenic activities (urbanization, industrialization, tourism, agriculture, livestock, fishing) that introduce various pollutants and lead to eutrophication issues and oxygen deficits (Esteves *et al.*, 2008; Howarth *et al.*, 2011).

In estuarine-coastal lagoon and mangrove ecosystems, biodiversity research has been centered on macroorganisms and less attention has been paid on microorganisms, particularly on the group of prokaryotes and much less on archaeal groups. The goal of this contribution was synthesized, as far as possible, the knowledge of the Archaea Domain, their diversity, metabolism, and distribution in tropical and subtropical coastal ecosystems.

METHODS

A descriptive review of the information available in Academic Google and Scopus databases was carried out. In the first phase of the search, general keywords were used: archaea+tropical coastal zones, archaea+subtropical coastal zones, archaea+tropical or subtropical coastal lagoons, archaea+tropical or subtropical estuaries, archaea+mangroves. In the second phase, the name of the Superphyla was included as keyword and other specific keywords were used.

From the available information, articles that were published from 2010 to date were selected, however, some articles with a publication time greater than ten years (2000-2010) were considered due they contained information on the archaea identified in coastal ecosystems, and which mainly included the group of methanogens related to the objective of this work. In some cases, secondary references from these contributions were consulted to clarify concepts or to be precise in questions do not solve yet.

As a result of the review, 68 articles were selected, 14 included characteristics of archaea and perspectives related to their evolution, diversity, and ecology. 20 publications contained information on the distribution of archaea in tropical estuarine environments and their relationship with environmental characteristics. Finally, 34 articles included joint information on diversity, distribution, and ecological processes (participation in biogeochemical cycles).

ARCHAEA. CHARACTERISTICS AND CLASSIFICATION

Estuarine ecosystems and mangroves are important reservoirs of organic matter of both autochthonous origin (produced by the ecosystem itself) and allochthonous origin (derived from terrestrial runoff and entering through river inflow, as well as from adjacent vegetation). This or-

ganic matter, along with nutrients, favor the development of a complex microbiota, bacteria, and archaea, that contribute to maintain the health of the ecosystem (Danovaro & Pusceddu, 2007).

Archaea are microscopic, single-celled, prokaryotic organisms originated approximately 2.6-2.8 G years ago. One of the accepted phylogenetic hypotheses mentions that Archaea, together with Eukarya, originated from a last common ancestor, possibly hyperthermophilic, more recent than LUCA (Last Universal Common Ancestor), for which these two domains are considered sister lineages (Woese *et al*., 1990; Gribaldo & Brochier-Armanet, 2006). Their name derives from the Greek " ", which means "ancient things", since they have so far been one of the oldest molecular structures ever studied. Archaea have unique characteristics that differentiate them from the Bacteria Domain, such as the lack of peptidoglycans in their cell wall, making them resistant to lysozymes and penicillin; they also have a cell membrane composed of a monolayer of lipids with ether bonds, instead of ester bonds (present in bacteria and eukaryotes), which give them greater thermal resistance. Their DNA replication mechanism is like Eukarya Domain, with several RNA polymerases as well as characteristic tRNAs and rRNAs (Woese *et al.,* 1978).

Study of the Archaea Domain was initially limited because most of these microorganisms cannot be cultured in the laboratory and were considered a unique group in the microbial biosphere, since the first ones were identified in extremophilic environments (hyperthermophile, hypersaline or acidophilic). However, technological advances of culture-independent techniques, have made it possible to detect them in a wide variety of moderate environments, making clear that archaea are widely distributed throughout the biosphere and that they possess great metabolic versatility (Bhattacharyya *et al*., 2015).

Archaea was established as the third Domain in the phylogenetic tree of life in 1977, based on the work of Woese and Fox, who analyzed 16S rRNA sequences. Initially, this domain was divided into two major phyla: Crenarchaeota (hyperthermophiles) and Euryarchaeota (mesophiles, methanogens, and halophiles); since then, the taxonomy and phylogeny of Archaea has changed significantly, largely due to the use of culture-independent molecular techniques, which have advanced significantly in the last ten years, mainly with the application of metagenomics.

The recent development of whole metagenome shotgun from environmental communities, has contributed to the discovery of new genes, enzymes, and metabolic pathways, providing insight into the structure and function of different communities of the Archaea domain in different habitats (Hernández De Lira *et al*., 2014; Cortés-López *et al*., 2020). The analysis of metagenomic data from many archaeal lineages in environmental samples, in addition to the analysis of transcriptomes and the development of different computational tools, (bioinformatics) has changed the classification of the Archaea Domain, leading to proposals for new clades at the level of phyla, classes, and orders, as well as the designation of new names for them (Adam *et al*., 2017; Baker *et al*., 2020). The current taxonomic classification of archaea recognizes three Superphylum: Asgard, DPANN and TACK, and the Phyllum Euryarchaeota. The names of Superphylum DPANN and TACK are formed using the initials of each of the phyla that were initially included; however, in Baker's *et al.* (2020) more phyla have been added to each one (Table S1). The recognized superphyla have been described based on ribosomal protein phylogenies as well as on the review of concatenated protein phylogenies (Seitz *et al*., 2016; Baker *et al*., 2020).

Superphylum Asgard (Phyla Lokiarchaeota, Thorarchaeota, Odinarchaeota, and Heimdallarchaeota). Asgard was recently described from the analysis of genomes available in public databases (NCBI, MG-RAST, GenBank), the phyla that are part of this group have been reported mainly in the sediment from aquatic environments (MacLeod *et al*, 2019). Asgard is apparently monophyletic and is considered key to understanding the origin of the Eukarya Domain (Seitz *et al*., 2019; Cai *et al*., 2020), as several of its members have genes encoding numerous eukaryotic signature proteins (ESPs) such as those related to cytoskeleton formation; they also have mitochondrial and plastid sequences present in eukaryotes (Petitjean *et al*., 2015; Baker *et al*., 2020) as well as some similarities to the genetic structure of eukaryotes (introns, histones, and RNA polymerases).

Superphylum DPANN (Phyla Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeota, Altiarchaea, Pacearchaeota, Woesearchaeota). DPANN was proposed by Rinke *et al*. (2013), includes ultra-small archaea (0.1-0.6 µm) with reduced genomes (490 kb-1.2 Mb) (Baker *et al*., 2010; Adam *et al*., 2017) and limited metabolic capacity because they lack several essential genes that are necessary for most biosynthetic pathways. It has been suggested that they depend on the metabolism of other archaea, with some of them considered symbionts of the Thermoplasmatales (Baker *et al*., 2010) or parasitic ectosymbionts of the Thermoprotei class (genus *Ignicoccus*) (Huber *et al*., 2002; Rinke *et al*., 2013).

Superphylum TACK (Phyla Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota) was proposed by Guy & Ettema (2011). The genetic diversity of this phylum found in environmental samples from uncultured communities revealed the existence of mesophilic groups in addition to the commonly reported acidophilic and thermophilic groups, such as Crenarchaeota. Geoarchaeota was later proposed as a phylum in the Superphylum TACK (Baker *et al*., 2020).

Phylum Euryarchaeota, is not classified as a Superphylum, it consists mainly of methanogenic and halophilic archaea, as well as different kinds of archaea from extremophilic environments. The methanogen group has been one of the most studied.

DISTRIBUTION AND DIVERSITY OF ARCHAEA IN TROPICAL AND SUBTROPICAL COASTAL ECOSYSTEMS

Archaea were thought to be a minor microbial component of coastal zones; however, recent studies have shown that they account for a significant component of these ecosystems. Archaea from different phyla have been found in different coastal ecosystems, nevertheless, their function and distribution in tropical coastal ecosystems are poorly understood (Cadena *et al*. 2019).

Table 1 shows the groups in the Archaea Domain that have been identified in estuaries, coastal lagoons, and mangroves areas from tropical and subtropical latitudes, mainly in Brazil, China, and India, with few studies in Australia, Thailand, and Mexico. Particularly in Mexico, research on archaea has focused on the study of methanogens and methane production.

Table 1. Diversity of the Archaea Domain reported from tropical and subtropical coastal ecosystems.

Ecosystem	Location	Identified groups of Archaea	Reference
Tropical estuary (surface water)	Guanabara Bay, Brazil	Euryarchaeota (Thermoplasmatales, Methanosarcinales, Methanococcoides, *Methanoplanus petrolearia* (Olliver *et al.*, 1998) Göker et al. 2015)	Vieira *et al.*, 2007
		Crenarchaeota, *Candidaytus Nitrosopumilus maritimus*	
Subtropical estuary (sediment)	Estuary of Pearl River, China	Lokiarchaeota (DSAG), Bathyarchaeota (MCG), Thorarchaeota (MBG-B), Euryarchaeota (Methanomicrobiales, Methanosarcinales, Halobacteriales), Hadasarchaea (SAGMEG)	Jiang *et al.*, 2011
Tropical estuary (surface and bottom water)	Cochin estuary, India	Crenarchaeota	Vipindas *et al.*, 2015
Tropical estuary (sediment)	Mandovi estuary, India	Euryarchaeota (Methanomicrobiota, Methanococci, Methanopyri, Halobacteria)	Khandeparker *et al.*, 2017
		Crenarchaeota, Thermoprotei, Methanosarcinales,Thermoplasmatales	
Subtropical estuary (sediment)	Estuary of Pearl River, China	Thaumarchaeota, Bathyarchaeota, Methanomicrobia, Parvarchaeota, Aenigmarchaeota	Liu *et al.*, 2014, Liu X. *et al.*, 2018
Coastal lagoon	Celestún Lagoon, Yucatan, Mexico	Methanosaetaceae, Verstraetearchaeota	Cadena *et al.*, 2019
Burnett River Estuary (pore and surface water)	Australia	Methanobacteriales, Methanocellales, Methanococcales, Methanomassiliicoccales, Methanomicrobiales	Euler *et al*, 2020
		Methanosarcinales	
Mangrove (sediment)	Dar-es-Salaam, Tanzania	Methanococcoides strain	Lyimo *et al.*, 2009
Mangrove (sediment)	Dar-es-Salaam, Tanzania	Euryarchaeota (MBG-D, Methanosarcinales, Methanomicrobiales, Methanobacteriales, halophilic cluster,	Lyimo *et al.*, 2009
		Crenarchaeota (MBG-B, Crenarchaea)	
Mangrove (sediment)	Sao Paulo, Brasil	*Methanopyrus kandlery* (Kurr *et al.*, 1992)	Taketai *et al*, 2010
		Género *Methanococcus, Methanosarcina* cluster	
Mangrove	Buri, Thailand	Candidatus *Nitrosopumilus*	Yasawong *et al.*, 2013
		Euryarchaeota (Methanomicrobiales, Methanosarcinales, Thermoplasmatales)	
Mangrove (water and sediment)	Parnaioca river, Brazil	Thaumarchaeota, Crenarchaeota, Euryarchaeota	Silveira *et al.*, 2013
Mangrove (sediment)	Chol Buri mangrove, Thailand	Thaumarchaeota (Candidatus *Nitrosopumilus maritimus*), Euryarchaeota (Methanobacteriales, Methanosarcinales, Methanomicrobiales, Thermoplasmatales, *Candidatus Nitrososphera*)	Yasawong *et al.*, 2013
Mangrove (sediment)	Sundarbans, India	Euryarchaeota (Thermoplasmatales, Marine Group II, Halobacteria, Methanomicrobia, Methanobacteria)	Batthacharyya *et al.*, 2015
		Thaumarchaeota (Marine Group I)	
Mangrove (sediment)	Southeastern China	Euryarchaeota,Thaumarchaeota, Lokiarchaeota, Bathyarchaeota, Nitrospirae	Zhang *et al.*, 2019
Mangrove (sediment)	Southeastern China	Woesearchaeota, Thaumarchaeota, Bathyarchaeota, Euryarchaetoa, Asgard, Aenigmarchaeota, Altiarchaeota	Zhang *et al.*, 2021

In addition to the identified phyla, a significant number of new genomes from uncultured and unclassified lineages have also been reported in these ecosystems (Adam *et al*., 2017; X. Liu *et al*., 2018; Baker *et al*., 2020).

From an ecological point of view, communities have a series of characteristics, including biodiversity, abundance, and distribution, which together determine their organization. The organization of the community depends on different factors, such as its response to changes in physicochemical conditions (environmental gradients), and biological relationships (such as competition, predation, parasitism, symbiosis, or mutualism); as well as the performance of the community itself (null model).

Most studies of the archaea in tropical estuarine ecosystems, as well as in mangrove forests, have established that their organization seems to be closely related with their tolerance and response to the physicochemical gradients existing in these environments. Inputs of organic matter and nutrients, as well as temperature, salinity, pH, and oxygen content, are the environmental variables that have been commonly associated with the structure of archaea (Purdy *et al*., 2002; Hugoni *et al*., 2015; Zou *et al*., 2017; Zhang *et al*., 2021).

The influence of chemical gradients (mainly salinity and oxygen) existing in estuaries and coastal lagoons, apparently it could depend on the physiological versatility of the different phyla, since some archaea groups proliferate at low salinities in subtropical estuaries, while others require higher salinity (Xie *et al*., 2014); the same situation occurs with oxygen, according to the review carried out by Zou *et al.* (2020). The structure of the archaeal community may also be the result of their adaptation to anthropogenic pollution, having reported a high archaeaplankton diversity (termoplasmatales and methanogenic groups) in the polluted region of Guanabara Bay (Brazil) while in pristine areas the diversity was lower (Vieira *et al*., 2007). A greater diversity of archaea, mainly from the group of methanogens, was reported in mangrove sediments contaminated with hydrocarbons and heavy metals (Yasawong *et al*., 2013; Hu *et al*., 2016).

It has been determined that there are differences in the diversity of archaea between the water column and the sediment, with the lowest diversity in the former. The phyla Euryarchaeota and Thaumarchaeota (lineage *Nitrosopumilales*), one of the most abundant chemoautotrophs in the picoplankton, predominate in the water column, showing that light is an important variable related to their abundance and distribution. Apparently, the abundance of Thaumarchaeota decreases as the amount of light increases in the water column (Battacharyya *et al*., 2015; Li *et al*., 2015; Zou *et al*., 2020).

As a result of the accumulation of organic matter and its aerobic degradation, oxygen is depleted within the first few centimeters of estuarine sediments, creating anoxic conditions (Böttcher *et al*., 2000) and microniches favorable to the development of a greater number of archaeal species. As a result, a stratification of the archaeal community has been observed, in some cases diversity is less in the upper strata, which increases at greater depth. Bathyarchaeota, Lokiarchaeota and Woesearchaeota are predominant in the surface layer, reporting that these groups are more abundant in oxygen-limited zones (Castelle *et al*., 2015). Thorarchaeota and Halobacteriales were reported in the transition zone between sulfate reduction and methanogenesis, while Methanomicrobiales, Methanosarcinales and ANME-2 were found at greater depth (Jiang *et al*., 2011).

Distinctive phyla Bathyarchaeota, Thaumarchaeota, Woesearchaeota and Euryarchaeota have also been found in mangrove sediments. Zhang *et al.* (2021) determined that the diversity of the archaeal community was greater in the first 10 cm of the sediment and decreased at greater depth, reporting a stratification of the identified phyla: Woesearchaeota (the most diverse) and Thaumarchaeota were predominant from 0 to 10 cm; in contrast, Bathyarchaeota (more abundant), Euryarchaeota and Asgard archaea were predominant in the strata of 10-30 cm depth. Aenigmarchaeota and Altiarchaeota were detected as rare taxa.

It seems that the dynamics of Archaea communities in these sediments also depend on environmental factors, including temperature, salinity, pH and the concentration of ammonium, nitrate, and organic carbon, that influence each of the Archaea phyla differently. Thaumarchaeota was negatively related to salinity, pH, and total carbon concentration, and positively related to the amount of nitrite (Zhang *et al*., 2019). Euryarchaeota, the most abundant group and whose sequences, for the most part, are related to methanogens, has been directly related to organic matter content and negative oxidation-reduction potentials (-150 mV) (Lyimo *et al*., 2002; Taketai *et al*., 2010) and its abundance is influenced by temperature (23-35°C), and pH (6.5-8.3) (Lyimo *et al*., 2009; Yasawong *et al*., 2013; Euler *et al*., 2020). Other studies have shown a higher diversity of methanogens in the upper layer of the sediment compared to the deep layer, being related with high levels of nutrients, while it decreases with high concentrations of heavy metals (Jing *et al*., 2016).

In Indian mangroves, Thermoplasmatales was the most abundant euryarchaeal group (18.75-23.3%), followed by Halobacteriales (Bhattacharyya *et al*., 2015). Thermoplasmatales is an order with acidophilic members that can grow both with or without oxygen and were predominant in surface sediments, relating their presence to high concentrations of organic matter and hydrocarbon pollution; most of the sequences were affiliated to the genus *Halogranum*. In contrast, methanogens of the classes Methanomicrobia and Methanobacteria grew in pristine conditions.

An important aspect of community ecology is the latitudinal distribution of biodiversity, and it has been shown that biodiversity is high in tropical regions in almost all taxonomic groups. X. Liu *et al.* (2018) analyzed 4,000 16S rRNA gene sequences from 24 estuaries of different latitudes, seven of them located in tropical and subtropical areas of Brazil, India, and China (estuaries of the Cunha, Zuari, Urucu, Jiulong, Mandovi, Santos-Sao Vicente and Pearl rivers), and determined that Archaea diversity seems to follow the same global distribution pattern as other taxonomic groups, i.e., their diversity is great in estuaries of low latitudes and decreases towards middle and high latitudes. This same study reported that the phylum Thaumarchaeota was the predominant group (approximately 40% of the total), followed by Bathyarchaeota and Euryarchaeota, both accounting for 25% each; Woesearchaeota accounted for 6%, and the rest of the phyla made up the remaining 4%. The few temperature fluctuations in tropical areas, as well as a big influence of anthropogenic activities in coastal areas, mainly the discharge of domestic and industrial wastewater introducing terrestrial microbial populations, and high concentrations of ammonium and organic matter are factors that explain the diversity of archaea reported in tropical latitudes (Vieira *et al*., 2007).

ARCHAEA AND BIOGEOCHEMICAL CYCLES

The complexity of estuarine ecosystems favors the presence of specific ecological niches for the resident microbiota that participates in several biogeochemical cycles (Euler *et al.*, 2020). Mainly on their potential metabolism, inferred from the genetic information of the different Archaea phyla, as well as the information obtained experimentally using culture-dependent techniques, it has been established that archaea, along with bacteria, play an active role in the cycles of matter and energy, both in the water column and in the sediment, and the coexistence of archaea with other microbial groups could give rise to different ecological relationships in the microbial web (Offre *et al.*, 2013; Zou *et al.*, 2020). The main cycles in which archaea could play a key role in coastal ecosystems are the carbon, nitrogen, and sulfur cycles.

1. Carbon cycle

Estuarine ecosystems receive organic matter of allochthonous and autochthonous origin, this organic matter is the key component of the biogeochemical carbon cycle and most of the Archaea phyla identified in coastal ecosystems of tropical and subtropical latitudes have the potential to participate in this cycle, either with heterotrophic or autotrophic metabolism, under aerobic or anaerobic conditions (Fig. 1).

Most of the archaea of Superphylum Asgard could be obligate anaerobes and heterotrophs, capable of degrading different organic compounds, including amino acids, peptides and fatty acids produced by the hydrolytic activity of other microorganisms. Thorarchaeota, identified in the sulfatereduction-methanogenesis transition zone in mangrove sediments, could grow from both organic and inorganic carbon compounds (mixotrophy), and would be able to fix CO_2 through autotrophic acetogenesis (Y. Liu *et al.*, 2018; MacLeod *et al.*, 2019). Additionally, Heimdallarchaeota would be capable of phototrophic metabolism at low oxygen conditions.

Asgard archaea also present the genes of the Wood-Ljungdahl pathway, meaning that these microorganisms can use H_2 as an electron donor and CO_2 as an acceptor in the reductive acetyl-coenzime A (acetyl-CoA) pathway during biosynthesis (MacLeod *et al.*, 2019). Superphyllum Asgard could also be important in the degradation of toxic organic compounds, Lokiarchaeota is potentially capable of metabolizing halogenated organic compounds, with hydrogen-dependent growth (Sousa *et al.*, 2016; Adam *et al.*, 2017; Manoharan *et al.*, 2019). Helarchaeota could degrade alkane hydrocarbons (ethane and butane) under the anaerobic conditions reported in estuarine sediments (Seitz *et al.*, 2019); while Thorarchaeota, Heimdallarchaeota and Lokiarchaeota, by presenting malonyl-CoA and benzoyl-CoA pathways, have the potential to utilize aliphatic and aromatic hydrocarbons (Firrincieli *et al.*, 2021).

In the Superphylum DPANN, Woesearchaeota has been identified mainly in sediments, and its genomic information suggests that it could play a role in acetogenesis and hydrogenesis; and the group could have a syntrophic relationship with methanogens (X. Liu *et al.*, 2018).

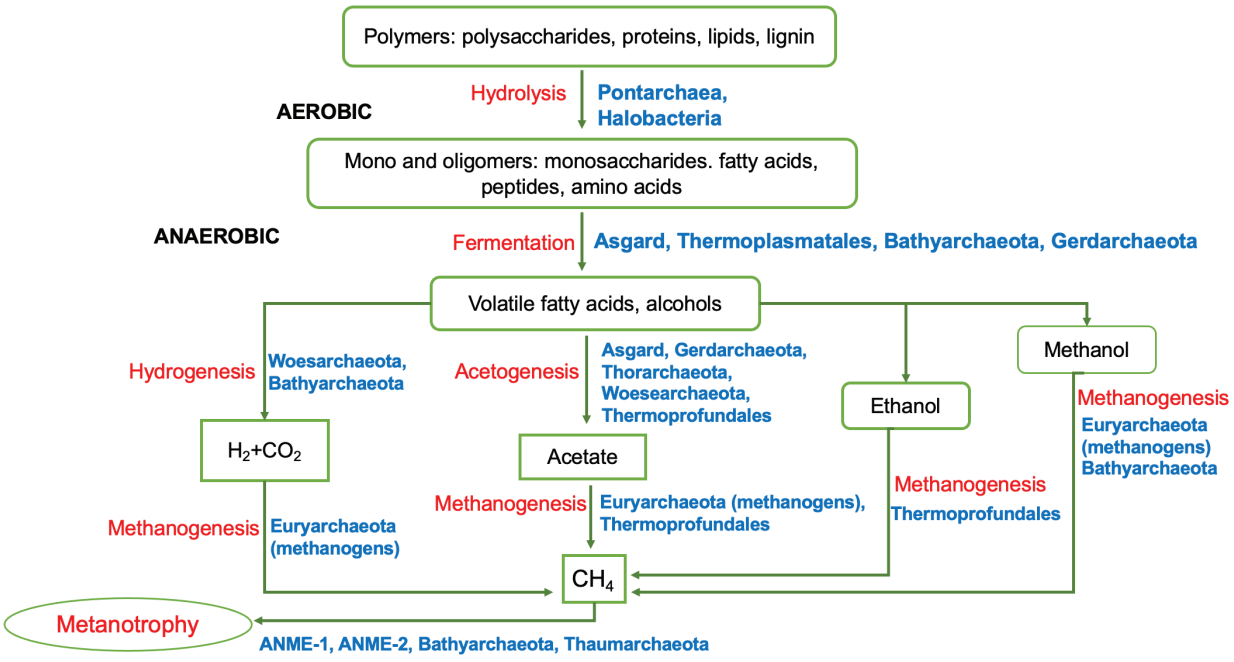

Figure 1. Schematic representation of the participation of archaea during the degradation of organic carbon. The name of the group of archaea that participates in the metabolic pathway is indicated in blue. The name of the main metabolic process is indicated in red: the decomposition of polymers is carried out with water as one of the reagents (Hydrolysis), the monomers obtained are oxidized in the absence of oxygen during Fermentation, releasing different products such as CO_2, hydrogen (Hydrogenesis), acetate (acetogenesis) and alcohols (ethanol, methanol). These products will be used by different groups of archaea to form methane (Methanogenesis). Methane represents the substrate in Methanotrophy.

Phylum Euryarchaeota is abundant both in the water column and in sediments. The members of this phylum can develop with or without oxygen and may play a central role in the sedimentary carbon cycle (Zou *et al.*, 2020). In aerobic conditions, groups MG-II and MG-III, which were proposed to be grouped as Pontarchaea (Adam *et al.*, 2017), are capable of degrading carbohydrates, lipids and proteins and could be related to the degradation of high molecular weight organic compounds (Rinke *et al.*, 2019; Tully, 2019), while Halobacteria are most active in the degradation of organic matter in hypersaline conditions and its presence in mangrove sediments is correlated with high amounts of organic matter produced during algal blooms, Halobacteria can grow aerobically as well as anaerobically (Bhattacharyya *et al.*, 2015; Webster *et al.*, 2015).

Metatranscriptomic analysis suggests that Marine Benthic Group D (MBG-D, or Thermoprofundales) under anoxic conditions may be mixotrophic, having genes for autotrophic pathways, as well as to produce acetate and ethanol via fermentation, though it may also be involved in acetate utilization (Lazar *et al.*, 2017; Zou *et al.*, 2020). It has been suggested, based on a co-occurrence analysis, that the order Thermoprofundales could potentially interact with the phyla Lokiarchaeota and Hadesarchaeota (Zhou *et al.*, 2019). Thermoplasmatales could degrade long-chain fatty acids and reduce sulfite.

One of the most studied groups in the phylum Euryarchaeota, related to the anaerobic carbon cycle, is the methanogens. Organic matter in brackish ecosystems is mineralized mainly in the sediment through anaerobic processes, and methanogenic archaea (MA), together with sulfate-reducing bacteria (SRB), play a key role in the later stages of anaerobic mineralization (Holguin *et al.*, 2001; Yasawong *et al.*, 2013). Due to the salinity gradient present in estuaries and coastal lagoons, sulfate reduction is predominant in marine zones, while methanogenesis is predominant in freshwater (Fukui *et al.*, 1997); in this area, the highest abundance of MA has been recorded, as well as the highest production and emission of methane (Lyimo *et al.*, 2002; Torres-Alvarado *et al.*, 2013, 2016). The key factor that regulates this difference is the sulfate concentration (Takii & Fukui, 1991; Purdy *et al.*, 2001, 2002).

MA can use different substrates, such as acetate and methylated compounds, as a source of carbon, or they can grow autotrophically from H_2/CO_2 (Thauer *et al.*, 2008). Methanobacteriales, the most abundant order (57.7% of the total methanogenic community), with clones affiliated to the genera *Methanobacterium, Methanothermobacter, Methanobrevibacter* and *Methanoculleus*, can use H_2/CO_2 to produce CH_4. Methanosarcinales with clones related to the genera *Methanolobus, Methanomethylovorans,* and *Methanococcoides*, can utilize methylated compounds, as well as *Methanosaeta sp.*, an acetate obligate. Methanomicrobiales the least abundant, accounting for 11.5% of the total community, with most of the sequences affiliated with the genus *Methanoculleus,* is a methylotrophic methanogen (Yasawong *et al.*, 2013).

Acetate and H_2/CO_2 are also important substrates for SRB, resulting in competition between SRB and MA for available hydrogen and acetate, sulfate reduction in brackish ecosystems is thermodynamically favored because it produces more energy per mole of hydrogen ($\Delta G°$ of 98.8 kJ/mol) or acetate ($\Delta G°$ of -43.8 kJ/mol). In comparison, methane

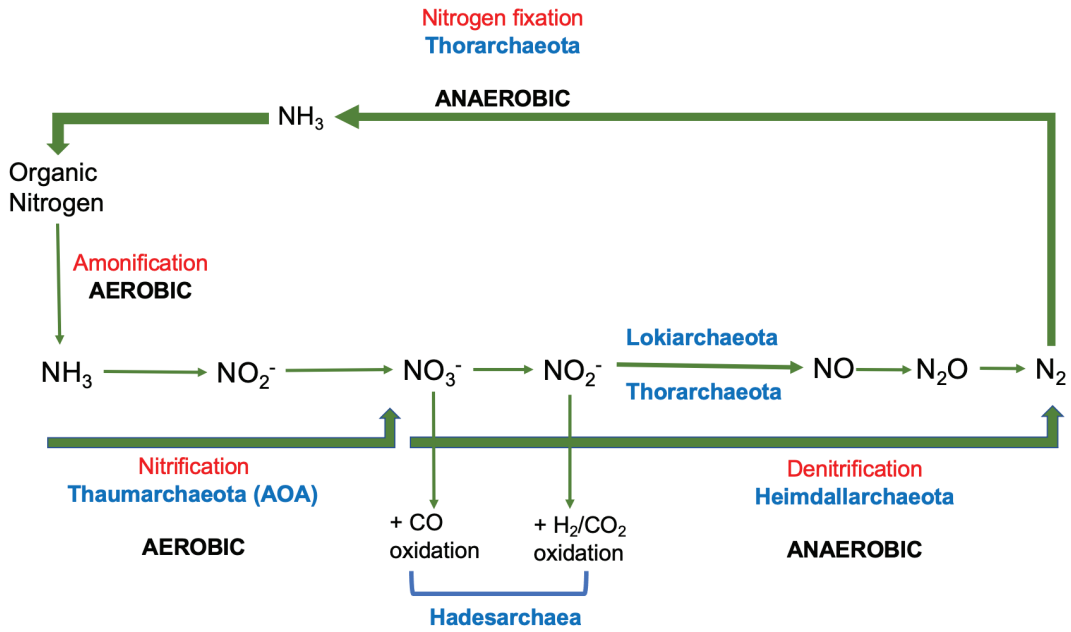

Figure 2. Schematic representation of the participation of archaea in nitrogen cycle. The name of the group of archaea that participates in the metabolic pathway is indicated in blue. The name of the main metabolic process is indicated in red: the decomposition of organic nitrogen involves aerobic and anaerobic processes. In the presence of oxygen, organic N is transformed into ammonium (ammonification), which is subsequently converted into nitrates, releasing nitrites as an intermediate product (nitrification). Under anaerobic conditions, nitrates are transformed into molecular nitrogen. This compound is assimilated in the absence of oxygen to form organic molecules.

formation produces a ΔG° of -74.8 kJ/mol and -19.9 kJ/mol with hydrogen and acetate, respectively (Howarth, 1993; Canfield *et al.*, 2005). The coexistence of SRB and MA in estuarine and mangrove sediments is possible due the latter use non-competitive substrates such as methanol or mono-, di- or trimethylamines; this would explain why methylotrophic methanogens are the major component in these sediments (Lyimo *et al.*, 2002). In addition, pectin is released in the early stages of tree lignin degradation and quickly hydrolyzed to produce methanol in mangrove sediments, which favors the growth of methylotrophic methanogens. It has also been reported that the species *Methanosarcina semesiae* Lyimo can utilize dimethyl sulfide (Lyimo *et al.*, 2000).

Methanogenesis results in the formation of methane (CH_4) and CO_2, which are important gases involved in the greenhouse effect related to global climate change (Karl &Tilbrook, 1994). Estuaries, coastal lagoons, and mangroves are the main marine environments that emit methane to the atmosphere (Kreuzwieser *et al.*, 2003).

Methane can also be used as an energy source by methanotrophic or methane-oxidizing bacteria (MOB) and by nitrifying bacteria in the oxic-anoxic interface of the sediments, both microbial groups are strict aerobes (Higgins *et al.*, 1981; Lidstrom, 2001) and can consume approximately 90% of the methane produced (Carini *et al.*, 2003).

Methane can also be oxidized under anaerobic conditions by some methanogenic archaea, which can use it as a source of reduced carbon (Blair & Aller, 1995). Anaerobic methane oxidation is carried out by at least two phylogenetically distinct groups of archaea, ANME-1 and ANME-2, that generally form consortia with SRB, mainly of the class Deltaproteobacteria, and with denitrifying or nitrate-reducing bacteria, the consortium metabolism involves a syntrophic relationship based on interspecific electron transfer. The archaea seem to oxidize methane, and the resulting compounds are utilized by the bacterial groups (Orphan *et al.*, 2001; Valenzuela *et al.*, 2017). The phyla Batyarchaeota and Thaumarchaeota could also utilize methane (Valenzuela *et al.*, 2017; Baker *et al.*, 2020). Methane oxidation via aerobic and anaerobic processes is biogeochemically important because it helps reduce methane emission into the atmosphere.

Several of the phyla included in Superphylum TACK are heterotrophs, while others have a chemolithoautotrophic metabolism (Baker *et al.*, 2020). Species of the phylum Bathyarchaeota could be key microorganisms in the carbon cycle in estuarine sediments due to the abundance of organic matter, and based on their genomics, it has been suggested that they could be involved in the degradation of carbohydrates, fatty acids and proteins; some representatives of this group apparently grow under both aerobic and anaerobic conditions (Adam *et al.*, 2017); the latter are characteristic of estuarine sediments where such archaea could play an important role in fermentation processes, mainly in acetogenesis from H_2+CO_2, as well as in the methane cycle, since they can be involved in methyl-dependent methanogenesis or in dissimilatory oxidation of methane. It has been suggested this phylum could also play a role in the degradation of recalcitrant organic compounds such as lignin, abundant in mangrove sediments. Some representatives of this group could have bacteriochlorophyll, suggesting they could carry

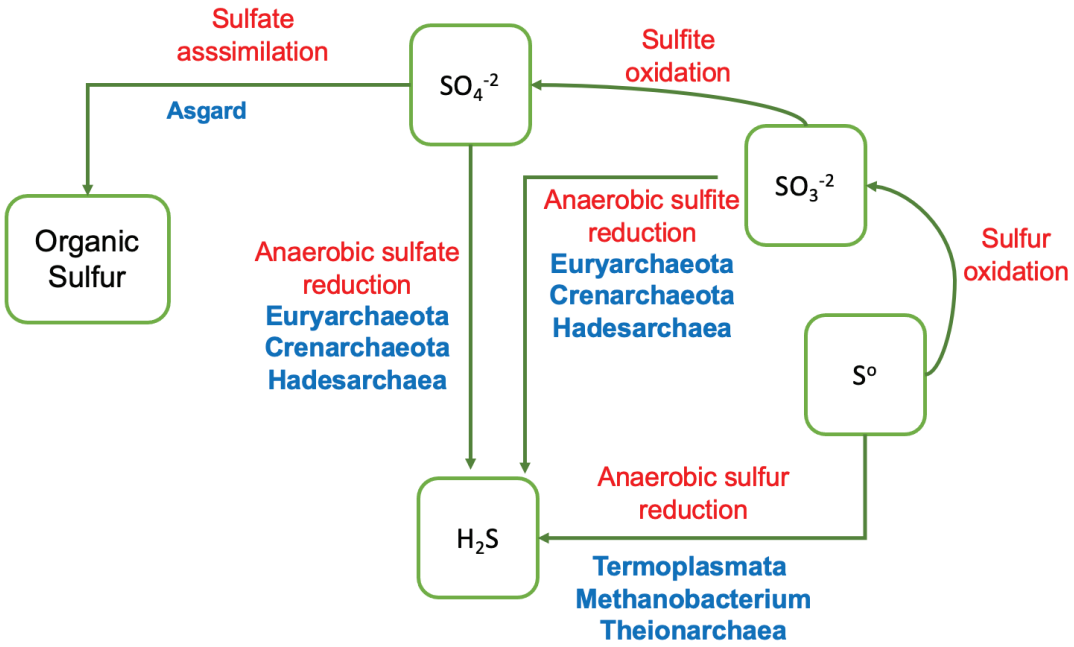

Figure 3. Schematic representation of the participation of archaea in sulfur cycle. The name of the group of archaea that participates in the metabolic pathway is indicated in blue. The name of the main metabolic process is indicated in red: sulfates represent the central compound of the sulfur cycle, in the absence of oxygen, they can be used as electron acceptors during sulfate reduction, producing sulfide (sulfidogenesis). The reduction of elemental sulfur and sulfites also generate sulfide. Sulfates can also be transformed into organic compounds through an assimilation process.

out photosynthesis (Jiang *et al*., 2011; Kubo *et al*., 2012; Evans *et al*., 2015; Lazar *et al*., 2016; Yu *et al*., 2018; Zhou *et al*., 2018; Baker *et al*., 2020; Zou *et al*., 2020). The abundance of Bathyarchaeota is positively related with high concentrations of nutrients and organic matter in the sediments, and to salinity, existing freshwater, and saline groups (Zou *et al*., 2020).

A second important phylum is Gerdarchaeota, with members apparently facultative, capable of utilizing organic carbon compounds under aerobic or anaerobic conditions; in anoxic sediments, they could be capable of carrying out a fermentation process, producing acetate (acetogenesis), though they could also grow lithoautotrophically with H_2 and CO_2 (Cai *et al*., 2020).

2. Nitrogen cycle

The nitrogen cycle involves compounds with different oxidation-reduction states. The cycle between the different forms of nitrogen is the base for several microbial metabolisms, both aerobic and anaerobic. Results of metagenomic and metatranscriptomic studies suggest that different phyla of archaea could be participating mainly in the fixation of molecular nitrogen into ammonium, as well as in the nitrification, denitrification, and disassimilatory reduction of nitrate (Fig. 2).

Nitrification is an aerobic metabolism, where ammonium (NH_3) is transformed to nitrates (NO_3^-) via nitrites (NO_2^-) involving ammonium oxidizing archaea (AOA) and ammonium oxidizing bacteria (AOB) (Abell *et al*., 2010). This superphylum TACK is one of the most important in the nitrogen cycle, mainly Thaumarchaeota, an AOA that participate in the first phase of nitrification; among the AOA, the Nitrosopumilales group (*Nitrosopumilus*, *Nitrosoarchaeum,* and *Cenarchaeum* genera) is dominant in water column, and *Nitrososphaerales* in sediments (Bhattacharyya *et al*., 2015; Adam *et al*., 2017). In estuaries of subtropical zones, AOA are important oxidizing ammonia, having quantified $2.27x10^5$ genes/g sediment in the Tobarí Bay (Beman, 2014), while in a tropical estuary of India (Cochin estuary), AOA were more abundant at the surface (0.56-6.3 x 10^3 cells/ml) of the water column compared to the bottom (0.32-2.9 x 10^3 cells/ml), and the greatest diversity was determined in the mesohaline region (Vipindas *et al*., 2015). The presence of AOA was related to temperature and oxygen concentration, it has been established that nitrification in estuarine ecosystems in tropical areas decreases with low oxygen concentrations and the presence of H_2S (Corredor *et al*., 1999); however, it seems that some AOA could carry out autotrophic ammonia oxidation at low oxygen concentrations such as those found in the first strata of the sediment (Silveira *et al*., 2013; Yasawong *et al*., 2013).

These archaea involved in the nitrification process could be sharing a niche in the water column with nitrifying bacteria (*Nitrosomonas sp*.). A relatively high nitrification rate by AOA was reported in the Cochin estuary before the monsoon season, however with the influence of the monsoon and limnetic characteristics, nitrification by bacteria was more important (Vipindas *et al*., 2015). An important aspect of AOA is that their presence is favored in polluted, eutrophic estuaries because they have genes associated with the transport and regulation of heavy metals (Zou *et al*., 2019).

Nitrification contributes to the reduction of nitrogen from anthropogenic activities and, being an oxygen-consuming process, it could participate in the formation of hypoxic and anoxic conditions in coastal ecosystems (Caffrey *et al*., 2007). Nitrification is a key process in the nitrogen cycle, because is coupled with anaerobic processes that oxidize ammonia, including denitrification, and dissimilatory nitrate reduction. During denitrification, nitrate and nitrite are reduced to molecular nitrogen, producing nitrous oxide (N_2O) as an intermediate product. This metabolism is carried out under anaerobic conditions by denitrifying bacteria and archaea mainly in estuarine sediments (Francis *et al*., 2007).

It has been suggested that Asgard in estuarine sediments have the potential to participate in denitrification and dissimilatory nitrate reduction, having been reported that Heimdallarchaeota possesses the genes for the enzymes nitrate reductase and nitrite reductase, while the presence of nitrite reductase has also been established in Lokiarchaeota and Thorarchaeota (Seitz *et al*., 2019). In the Phylum Euryarchaea, Hadesarchaea (SAGME) seems to be heterotrophic and presents genetic information for carbon monoxide oxidation, coupling it to nitrate reduction; and it can couple H_2/CO_2 oxidation to nitrite reduction (Lazar *et al*., 2017). The activity of different phyla during denitrification could also include the production of nitrous oxide (N_2O), a greenhouse gas (Santoro *et al*., 2011).

The information for nitrogenase has only been reported in the Phylum Thorarchaeota, proposing that this group could carry out an assimilation of molecular nitrogen into ammonium (MacLeod *et al*., 2019).

3. Sulfur cycle

In the sulfur cycle, there are oxidation and reduction reactions that transform sulfur from its most oxidized form (sulfate) to its most reduce form (sulfide); these transformations are regulated by different microorganisms, bacteria, and archaea. Archaea can use many sulfur compounds as electron donors or acceptors during sulfur metabolism (Offre *et al*., 2013). Major processes are found in archaea include sulfur oxidation, anaerobic sulfate or sulfite reduction and assimilatory metabolism (Fig. 3).

Thermoplasmata can carry out anaerobic reduction of elemental sulfur using H_2 as electron donor (Liu *et al*., 2012); the ability to produce H_2S from elemental sulfur, has also been observed in methanogenic archaea such as Methanobacterium (Offre *et al*., 2013). The ability to use sulfate or sulfite during anaerobic respiration (sulfate reduction/ sulfite reduction), producing sulfide, has been demonstrated in some members belong to Euryarchaeota and Crenarchaeota. Hadesarchaea has also the potential to carry out sulfidogenesis (Lazar *et al*., 2017).

It is suggested that all the phyla of the superphylum Asgard possessing the genetic information for the sulfate adenylyl transferase and phospho-adenosine-sulfate-reductase, could have the capacity to carry out an assimilatory sulfate reduction, reducing sulfate and incorporate it as a sulfur source for biosynthesis of proteins (MacLeod *et al*., 2019). Thorarchaeota, have elemental sulfur and thiosulfate reduction genes suggesting they participate in intermediate sulfur cycling (Seitz *et al*., 2016). Theionarchaea could be associated with the reduction of sulfur compounds (Lazar *et al*., 2017).

CONCLUSIONS

In the last ten years knowledge of the Archaea domain has increased, metagenomic and metatranscriptomic studies are providing important information on metabolic pathways and physiology of these microorganisms in natural ecosystems. Metagenomics and, in general, “omics”

technologies, represent an opportunity in studies of prokaryotic diversity and ecology, and for maximum exploitation of the data generated by these techniques, it is extremely important to have bioinformatics and computational biology tools.

From the development and application of independent cultivation techniques, it is a fact that in coastal ecosystems the presence, distribution and diversity of Archaea is evident; however, it is necessary to carry out a greater number of studies in tropical coastal lagoons and estuaries to have an extensive inventory of the biodiversity of the Archaea Domain. The discovery of new environmental sequences has been changing our understanding of their diversity, distribution, and metabolic functions, and it is clear there are still more archaeal species to be discovered, as well as their role in ecosystems.

An important aspect to consider in future research is the study of possible ecological relationships between archaea and the other important group of prokaryotes, bacteria. Ecological relationships influence the organization of communities and metabolic pathways in biochemical cycles, as well as its relationship with another group of important microorganisms in these ecosystems, bacteria.

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Zhang, C-J., J. Pan, C-H. Duan, Y-M. Wang, Y. Liu, J. Sun, H-C. Zhou, X. Song & M. Li. 2019. Prokaryotic diversity in mangrove sediments across southeastern China fundamentally differs from that in other biomes. *mSystems*4: e00442-19. DOI:10.1128/mSystems.00442-19

Zhang, C-J., Y.L. Chen, Y.H. Sun, J. Pan, M-W. Cai & M. Li. 2021. Diversity, metabolism, and cultivation of archaea in mangrove ecosystems. *Marine Life Science & Technology* 3:252–262. DOI:10.1007/s42995-020-00081-9

Zhou, Z., Y. Liu, K.G. Lloyd, J. Pa, Y. Yang, J-D. Gu & M. Li. 2019. Genomic and transcriptomic insights into the ecology and metabolism of benthic archaeal cosmopolitan, Thermoprofundales (MBG-D archaea). *ISME Journal* 13:885-901. DOI:10.1038/s41396-018-0321-8

Zhou, Z., J. Pan, F. Wang, J-D. Gu & M. Li. 2018. Bathyarchaeota: globally distributed metabolic generalists in anoxic environments. *FEMS Microbiology Reviews* 42:639-55. DOI:10.1093/femsre/fuy023

Zou, Z., H. Meng, Y. Liu, J. D. Gu & M. Li. 2017. Stratified Bacterial and Archaeal community in mangrove and intertidal wetland mudflats revealed by High Throughput 16S rRNA Gene Sequencing. *Frontiers in Microbiology* 8:2148. DOI:10.3389/fmicb.2017.02148

Zou, D., Y. Li, S.J. Kao, H. Liu & M. Li. 2019. Genomic adaptation to eutrophication of ammonia-oxidizing archaea in the Pearl River estuary. *Environmental Microbiology* 21:2320-2332. DOI:10.1111/1462-2920.14613

Zou, D., H. Liu & M. Li. 2020. Community, distribution, and ecological roles of estuarine Archaea. *Frontiers in Microbiology* 11:2060. DOI:10.3389/fmicb.2020.02060

INSTRUCCIONES PARA AUTORES

HIDROBIOLÓGICA es una publicación **cuatrimestral** del Departamento de Hidrobiología de la División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana Iztapalapa (UAM-I), que difunde trabajos originales e inéditos de investigación sobre temas relacionados con el ambiente acuático. No se aceptarán trabajos previamente publicados en obras con ISSN o ISBN (proceedings, resúmenes en extenso, libros, etc.). Las contribuciones podrán ser en español o en inglés y en ambos casos contendrán un resumen en español y un abstract en inglés.

HIDROBIOLÓGICA publica cuatro tipos de documentos**: artículos, notas científicas, artículos de revisión y monografías taxonómicas**. A criterio del Comité Editorial, se podrán publicar números especiales sobre tópicos o temas específicos. No se aceptarán trabajos preliminares o inconclusos, ni aquellos que pudiendo integrarse como una unidad, sean presentados por separado en forma de pequeñas contribuciones o notas seriadas.

La revista cubre un perfil amplio con las siguientes cuatro áreas generales en la que participan diversos editores asociados:

1 Área Morfología, Sistemática y Filogenia

2 Área Ambiental

3 Área Manejo de Recursos Acuáticos

4 Área de Ecología

Los trabajos recibidos serán objeto de arbitraje guiado por miembros del Comité Editorial y por los asesores del Consejo Editorial. En esta evaluación se considerarán:

1) Originalidad y rigor científico.

2) Contribución al avance en las diversas áreas del conocimiento hidrobiológico.

3) Presentación, en lo referente a coherencia, continuidad y consistencia.

4) Empleo apropiado de tablas, figuras y fotografías en relación con el texto.

Los manuscritos y figuras que no se ajusten a las siguientes instrucciones serán devueltos sin evaluación a los autores para que procedan a su adecuación.

Todo trabajo recibido por los Editores merecerá un acuse de recibo inmediato. En el caso de contribuciones firmadas por diversos autores, la correspondencia necesaria durante el proceso editorial se establecerá con el primer autor, salvo indicación distinta, indicada en el manuscrito.

Una vez que los manuscritos hayan sido revisados y se haya comprobado que cumplen cabalmente con las normas editoriales, serán introducidos en el sistema OJS (Open Journal System) de Hidrobiológica para iniciar su proceso de evaluación.

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c) Artículo de revisión

d) Monografía taxonómica

Las contribuciones deberán ser enviadas a través del portal Open Journal System (OJS) de HIDROBIOLÓGICA, enviando además un aviso a las siguientes direcciones de correo electrónico.

Dirección de la revista *Hidrobiológica*: rehb@xanum.uam.mx

Dirección de apoyo a la revista: enlacerevistahidrobiologica@gmail.com

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Los autores deberán ajustar la estructura de su manuscrito dependiendo de la modalidad del trabajo.

Los archivos de texto (manuscrito, pies de figuras, tablas) se enviarán en formato Word y las figuras (fotografías, mapas, composiciones) en formato jpg o tif con buena calidad, mayor o igual a 300 ppp (puntos por pulgada).

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Los manuscritos se presentarán en **tamaño carta**, escritos a **doble espacio**, sin sangría y utilizando el tipo **Univers condensada** o **Arial 12** puntos en todo el trabajo.

Los **márgenes del texto tendrán 3 cm** de cada lado. Los manuscritos elaborados en procesadores de palabras: Word o formato RTF, **no estarán justificados**, sino alineados a la izquierda y **sin espacio interpárrafo**. La versión final de los manuscritos aceptados deberá acompañarse de la actualización electrónica correspondiente.

Título

Será breve y se presentará tanto en inglés como en español. Sin dejar de ser explícito con respecto al tema de trabajo, no deberá exceder de 20 palabras y deberá estar escrito con mayúsculas y minúsculas. Se

propondrá también un título resumido en el idioma en que esté escrito el manuscrito, no mayor de seis palabras. Ambos se presentarán en una página aparte, en la cual se hará constar también el nombre completo de los autores iniciando con el nombre de pila, indicando claramente la forma en la que aparecerán los créditos y su dirección institucional completa, incluyendo exclusivamente el correo electrónico del autor designado para la correspondencia.

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Laboratorio de Biología Acuática, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo. Avenida Francisco J. Mújica s/n, Ciudad Universitaria, col. Felícitas del Río, Morelia, Michoacán, 58040. México

Resumen

En un sólo párrafo y con una extensión de mínimo 250 a máximo 280 palabras, se escribirá en una página por separado y será acompañado de su traducción al inglés (Abstract). Ambos deben estructurarse utilizando los siguientes rubros; **Antecedentes**., **Objetivos**., **Métodos**., **Resultados**., **Conclusiones**.

Palabras clave

Los autores propondrán un máximo de cinco palabras clave, tanto en español, como en inglés (Keywords), y deberán estar ordenadas alfabéticamente.

Texto

Dividido en secciones: **INTRODUCCIÓN**, **MATERIALES Y MÉTODOS, RESULTADOS**, **DISCUSIÓN**, **AGRADECIMIENTOS**, **REFERENCIAS**, **TABLAS** (con título en la parte superior), **LISTADO DE PIES DE FIGURA, COPIAS DE LAS FIGURAS** en Word ordenadas consecutivamente con su pie de figura en la parte inferior. Los títulos de las secciones se ubicarán en el centro de la página, claramente diferenciados del texto y escritas con mayúsculas y en negritas. Evitar en lo posible el uso de subtítulos y en caso necesario, emplear negritas nuevamente y de forma continua con el texto. Los objetivos y las conclusiones deberán incluirse en la introducción y en la discusión, respectivamente. **En ningún caso se usarán sangrías**.

Las páginas deberán ir debidamente foliadas con números consecutivos y arábigos. Para facilitar el arbitraje y la redacción de los comentarios por parte de los revisores, **se recomienda que los renglones de todo el texto, estén numerados consecutivamente desde el inicio hasta el final del manuscrito**.

En símbolos y unidades se empleará el sistema métrico decimal.

Los nombres latinos de especies biológicas se escribirán en cursivas y cuando se citen por primera vez en el texto, incluirán la autoridad nomenclatural, sin abreviaturas. Para este punto revisar las siguientes ligas:

http://www.marinespecies.org/aphia.php?p=search

http://www.algaebase.org/search/species/

http://ucjeps.berkeley.edu/cgi-bin/get_bpu_from_number.pl?lookfor=11802511802611802711802 8

Las citas en el texto que incluyan dos autores deberán incorporar el símbolo **&** y para las de tres o más autores se usará *et al.,* (en cursivas)

Taxa nuevos

La descripción de taxa nuevos para la ciencia deberán ajustarse a los Códigos Internacionales de Nomenclatura.

Referencias bibliográficas

Los criterios que deberán prevalecer en esta sección serán:

a) Orden alfabético del apellido del primer autor

b) Citas de trabajos del mismo autor primeramente se organizarán en orden cronológico las que tengan un sólo autor, seguidas por las publicadas con dos autores en orden alfabético de acuerdo al apellido del segundo autor y cronológico si ambos apellidos coinciden.

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Un ejemplo de las citas más comunes se presenta a manera de ayuda:

Publicaciones periódicas

Ahmad, V. U. & M. S. Ali. 1991. Pinnatifinone, a new halogenated chamigrene from the red alga *Laurencia pinnatifida* (Lamour). *Scientia Pharmaceutica* 59 (2): 243-246.

Ahmad, V. U., M. S. Ali & S. Bano. **1990a**. Marine natural products. XII: laurol, a new metabolite from the red alga *Laurencia pinnatifida* (Lamour). *Scientia Pharmaceutica* 58 (2): 299-301.

Ahmad, V. U., S. Bano, W. Shaikh, S. Uddin & M. Shameel. **1990b**. Isolation and structure determination of 1,1,6,6-tetrachloro, 3,4-diphenyl hexane from brown alga *Dictyota dichotoma. Pakistan Journal of Scientific and Industrial Research* 33 (3): 428-430. (Nótese que ésta y la cita anterior deberán ser mencionadas en el texto como Ahmad *et al.*, 1990a y Ahmad *et al.*, 1990b).

Ahmad, V. U., M. S. Ali, S. Bano & M. Shameel. 1991. Pinnatifolide, a new metabolite from red alga *Laurencia pinnatifida* Lamour. *Pakistan Journal of Scientific and Industrial Research* 34 (1): 161-162.

Libros

Lind, O. T. 1985. *Handbook of common methods in limnology*. Kendall-Hunt Publishing Company, Dubuque. 199 p.

Eaton, A. D., L.S. Clesceri & A. E. greenberg (eds.). 1995. *Standard methods for the examination of water and wastewater*. 19th ed. American Public Health Associaton (APHA). Washington, D. C. Folio variado.

Capítulos de libro

Litter, M. M. & D. S. Litter. 1998. Structure and role of algae in tropical reef communities. *In*: Lembi, C. A. & J. R. Waaland (eds.). *Algae and human affairs*. Cambridge University Press, pp. 29-56.

Suárez-Morales, E. & M. Elías-Gutiérrez. 1992. Cladóceros (Crustacea: Branchiopoda) de la reserva de la biosfera de Sian Ka´an, Quintana Roo y zonas adyacentes. *In*: Navarro, D. & E. Suárez-Morales (eds.). *Diversidad biológica en la reserva de la biosfera de Sian Ka´an, Quintana Roo. Vol. 2*. Centro de Investigaciones de Quintana Roo. Chetumal, pp. 145-161.

Tesis

Ibáñez-Aguirre, A. L. 1995. Algunos aspectos de la dinámica de poblaciones de *Mugil cephalus* (Linneo, 1758) y *M. curema* (Valenciennes, 1836) (Pisces: Mugilidae) en la Laguna de Tamiahua, Veracruz. Tesis de Doctorado en Ciencias (Biología), Facultad de Ciencias, UNAM. CDMX, México. 216 p.

Otros

CNA (Comisión Nacional del Agua). 2003. *Ley Federal de Derechos Normas Aplicables en materia de Aguas Nacionales y sus Bienes Públicos Inherentes 2003.* Diario Oficial de la Federación. México, D.F. Enero 2: 173-191.

Systematics Agenda. 2000. 1994. *Systematics Agenda 2000: Charting the Biosphere.* Technical Report. New york. 34 p.

Las citas a los documentos que se encuentran en la web deben hacerse de la siguiente manera: se mencionarán el autor (o en su caso la organización responsable de la publicación del documento, como por ejemplo FAO, WHO, FDA etc.), la fecha de consulta y el título, seguidos por:

En español: disponible en línea en: http://www.fao.org/fishery/ culturedspecies/Litopenaeus_vannamei/en (consultado el 19 febrero 2010).

En inglés: Available online at: http://www.fao.org/fishery/culturedspecies/Litopenaeus_vannamei/en (downloaded February 19, 2010).

Ejemplos:

FAO (Food and Agriculture Organization). 2004. El estado mundial de la pesca y la acuicultura-2004 (SOFIA). Disponible en línea en: http://www.fao.org/docrep/007/y5600e/y5600e00.htm (consultado el 19 febrero 2010).

WHO (World Health Organization). 2004. Vitamin and mineral requirements in human nutrition. 2nd ed. World Health Organization, Geneva. Available online at: http://whqlibdoc.who.int/publications/2004/9241546123.pdf (downloaded February 19, 2010).

Cuando se trate de artículos, libros etc. disponibles en las dos maneras, se dará primero la cita completa y posteriormente la página web, de acuerdo al siguiente ejemplo:

SAGARPA-CONAPESCA. 2006. *Anuario estadístico de acuacultura y pesca 2006*. Secretaría de Agricultura, Ganadería y Desarrollo Rural, Pesca y Alimentación. Comisión Nacional de Pesca. Mazatlán. 219 p. También disponible en la página web (si el escrito es en inglés, usar: also available at:) http://www.conapesca. sagarpa.gob.mx/wb/cona/cona_anuario_estadistico_de_pesca

Adición del DOI a las Referencias

Los libros y publicaciones periódicas colocados en las referencias bibliográficas que posean DOI (Digital Object Identifier), agregarlo al final de la referencia correspondiente, como se muestra a continuación:

Calor, A. 2009. Considerações Acerca da Filogenia de Trichoptera Kirby 1813: da Análise dos Dados para as Hipóteses ou dos Cenários para os Dados. *Entomobrasilis* 2 (1): 01-10. DOI:10.12741/ebrasilis.v2i1.24

Tablas

Se presentarán a doble espacio, **orientadas verticalmente (a menos que la tabla contenga varias columnas)**, numeradas consecutivamente con números arábigos, con **un breve título en la parte superior y referidas al texto**. Deberán escribirse con letras y números en tipo Univers condensada o Arial 10 puntos, con mayúsculas y minúsculas; si son necesarias notas aclaratorias, éstas se pondrán en la parte inferior de la figura, con tamaño de fuente 8. **Se evitarán las líneas verticales** y **horizontales así como el uso de columnas que implique el empleo de tabuladores.**

Figuras

Las figuras deben ser originales, en caso de que algunas de ellas que forman parte del manuscrito hayan sido publicadas previamente, el autor estará obligado a solicitar los permisos correspondientes e indicar la referencia y cita correspondiente de donde son tomadas. En caso de que las figuras se modifiquen, indicarlo con la leyenda "Fig. modificada de (...)".

Además de las figuras incorporadas en el texto en Word, éstas se enviarán en archivos separados en alta resolución, cada uno identificado por el autor y con la numeración correspondiente a la figura. Serán numeradas consecutivamente con números arábigos y referidas al texto en forma secuencial. Las leyendas deberán escribirse con mayúsculas y minúsculas. El tamaño máximo para una figura o grupo de figuras será de 17 cm de longitud y 13 cm de ancho; el mínimo permitido será de 8 X 8 cm. Letras y números tendrán como máximo 10 puntos y como mínimo 8. Las figuras a escala deberán acompañarse de una escala gráfica. Todos los términos, símbolos y abreviaturas serán los empleados en el texto. **Es indispensable que las figuras o dibujos se envíen como archivos TIFF o JPG, con una definición mínima de 300 ppp., por ejemplo: Figura_1_Meave_dinos.jpg**

Fotografías

Sólo las estrictamente indispensables y con buen contraste. Cuando se realicen composiciones se dejará un pequeño espacio entre foto y foto. Las dimensiones máximas y mínimas se apegarán a las mencionadas en el inciso de figuras. Los números y letras no serán mayores de 10 puntos ni menores de 8 puntos. Las fotografías deben ser enviadas por separado y con buena calidad. Se numerarán como figuras en orden consecutivo a su referencia en el texto. **Se aceptarán figuras, o fotografías a color, cuando su uso sea indispensable y su costo será cubierto por los autores al momento de pagar los gastos de publicación.**

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Para la elaboración de notas, los autores deberán seguir el formato: **TÍTULO** en el idioma del trabajo, **TÍTULO** traducido al inglés o al español, **AUTORES, INSTITUCIONES DE ADSCRIPCIÓN, RESUMEN, ABSTRACT (resumen en inglés), Palabras clave** y **Keywords, AGRADECIMIENTOS** y **REFERENCIAS.** Éstas se apegarán a las normas editoriales de los artículos de investigación, **aunque sin apartados en el cuerpo de la nota**. Se ajustará el texto a un mínimo de cinco cuartillas y un máximo de siete, a doble espacio. Se recomienda la presentación de una sola tabla o figura.

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Este tipo de artículo podrá llevar el mismo formato que los artículos científicos o al menos los encabezados de **INTRODUCCIÓN**, **DISCUSIÓN** y **REFERENCIAS**, incluyendo en ellos los subtemas que los autores consideren pertinentes.

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HIDROBIOLÓGICA is a peer-reviewed research journal published every four months by the Departamento de Hidrobiología de la División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana Iztapalapa (UAM-I). The journal publishes original papers related to aquatic environments. Data previously published in works with ISSN or ISBN (proceedings, extensive abstracts, books, etc.) will not be accepted. Contributions can be in Spanish or English. In both cases an abstract in Spanish and English must be included.

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Various associate editors participate with the Journal to cover a wide variety of topics within the following four general areas:

1 Morphology, Systematics, and Phylogenics

2 Environment

3 Aquatic Resources Management

4 Ecology

All articles received **will go through a review process guided by a member** of the Editorial Committee or an Editorial Board Advisor. This evaluation will consider:

1) Originality and scientific rigor

2) Advances in knowledge of the different areas of hydrobiology

3) Coherence, continuity, and consistency of presentation

4) Appropriate use of tables, figures, and photographs in the text

Manuscripts and figures that do not comply with the following instructions will be returned to the authors without evaluation so that appropriate changes can be made.

All manuscripts received by the Editors will be immediately acknowledged. Correspondence during the editorial process will be directed to the first author unless otherwise indicated in the manuscript.

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a) Scientific article

b) Scientific note

c) Review article

d) Taxonomical monograph

Contributions should be submitted through the portal Open Journal System (OJS) of HIDROBIOLÓGICA, also sending a notice to:

Hidrobiológica: rehb@xanum.uam.mx

Assistant editor: enlacerevistahidrobiologica@gmail.com

Editor-in-chief: rta@xanum.uam.mx

AUTHOR GUIDELINES

Authors must adjust the structure of their paper to the type of manuscript being submitted.

Text files (manuscript, figure legends, tables) must be presented in Word format, while figures (photographs, maps, compositions) should be in good-quality **JPG** or **TIFF** format, equal or higher than 300 dpi.

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All manuscripts must be submitted in **letter format**, single column, **double spaced**, without tabs, in **Universe Condensed** or **Arial 12** font.

Texts will have **3 cm margins on each side**. Manuscripts written in Word or RTF word processors **should not be justified,** but rather aligned to the left with **no space between paragraphs**. The final version of accepted manuscripts must be accompanied by relevant electronic updates.

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The title should be concise, no longer than 20 words, and indicative of the nature of the paper. It must be written in English and Spanish in capital and lower-case letters. A short title of up to six words should also be provided in the same language as the rest of the manuscript. Both titles must be submitted on a separate sheet that includes authors' names. These should include the last name and one first name of each author spelt in full, clearly indicating the order in which credits must appear and their institutional address, including the email of the corresponding author.

For postal addresses, follow the pattern:

(Laboratory/Area/Department), (Faculty/Institute/Center), (University/Campus). (Address with street and number), (City, State or Province), (ZIP Code). (Country)

For puntuation, follow the example:

Laboratorio de Biología Acuática, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo. Avenida Francisco J. Mújica s/n, Ciudad Universitaria, col. Felícitas del Río, Morelia, Michoacán, 58040. México

Abstract

We require a one-paragraph abstract in English and a one-paragraph "resumen" in Spanish, each with a **maximum length of 250 words**. Both must be submitted on a separate sheet. **Background**., **Goals**., **Methods**., **Results**., **Conclusions**.

Keywords

Appropriate **keywords** (4-5) should be provided in English and Spanish in alphabetical order.

Main text

The following sections must be included in a bold center title: **INTRODUCTION**. This should state the investigated problem, the aim of the work, and previous relevant work with appropriate references. The **MATERIALS AND METHODS** used should be stated clearly in sufficient detail to permit others to repeat the research, if so desired. **RESULTS** should be presented concisely, with tables or illustrations for clarity. **DISCUSSION** should cover the significance of the findings without repetition of material in the Introduction and Results sections. This section must contain the conclusions of the work, **ACKNOWLEDGEMENTS**, and **REFERENCES.** It is very important that references be checked carefully. Subheadings should be avoided but if necessary they must be in bold and continuously written within the paragraph.

Pages should be numbered consecutively with Arabic numerals. **Please number all lines in the manuscript consecutively** in order to facilitate the review process by allowing reviewers to pinpoint specific references more easily.

The decimal metric system should be used for symbols and units.

Latin names of **biological species should be written in italics**. The first mention of a species in the text should include the **nomenclature authors without abbreviation**. In the case of **animal species, the year of publication of the description** should be indicated.

References in the text that include two authors should incorporate the ampersand (**&**) symbol, whereas three or more authors should include *et al.* (in italics).

New taxa

Description should follow the international code of nomenclature.

References

References should adhere to the following guidelines:

a) In alphabetical order by the first author's last name.

b) Citations of works by the same author in chronological order, then those published by two authors in the same order (alphabetically by the second author's last name and chronologically in case of coincidence).

c) References in the text with **et al.** should be ordered chronologically.

Authors' names should be written in capital and lower-case letters, not in capital letters exclusively. Initials of the first author will follow the last name. For additional authors, initials will precede the last name. In the case of two or more initials these should be separated by a period and a space. **Journal titles should not be abbreviated**. **Book and journal titles should be in italics.** The total number of cited authors in the text should coincide with the total number of references. Citations of the same author, published in the same year, or with the same co-authors (last name followed by *et al.*), should use the small letters (a, b, c) within the text and in the reference section. Never use indentations.

Examples of the most common citations are presented below:

Periodical publishing

Ahmad, V. U. & M. S. Ali. 1991. Pinnatifinone, a new halogenated chamigrene from the red alga *Laurencia pinnatifida* (Lamour). *Scientia Pharmaceutica* 59 (2): 243-246.

Ahmad, V. U., M. S. Ali & S. Bano. **1990a**. Marine natural products. XII: laurol, a new Metabolite from the red alga *Lauren-cia pinnatifida* (Lamour). *Scientia Pharmaceutica* 58 (2): 299-301.

Ahmad, V. U., S. Bano, W. Shaikh, S. Uddin & M. Shameel. **1990b**. Isolation and structure determination of 1,1,6,6-tetrachloro, 3,4-diphenyl hexane from brown alga *Dictyota dichotoma. Pakistan Journal of Scientific and Industrial Research* 33 (3): 428-430. (Please note that this citation and the one before, should be mentioned in the text as Ahmad *et al.* 1990a and Ahmad *et al.* 1990b).

Ahmad, V. U., M. S. Ali, S. Bano & M. Shameel. 1991. Pinnatifolide, a new metabolite from red alga *Laurencia pinnatifida* Lamour. *Pakistan Journal of Scientific and Industrial Research* 34 (1): 161-162. 4

Books

Lind, O. T. 1985. *Handbook of common methods in limnology*. Ken-dall-Hunt Publishing Company, Dubuque. 199 p.

Eaton, A. D., L. S. Clesceri & A. E. Greenberg (eds.). 1995. *Standard methods for the examination of water and wastewater*. 19th ed. American Public Health Associaton (APHA). Maryland. Varied folio.

Book chapter

Litter, M. M. & D. S. Litter. 1998. Structure and role of algae in tropical reef communities. *In*: Lembi, C. A. & J. R. Waaland (eds.). *Algae and human affairs*. Cambridge University Press, pp. 29-56.

Suárez-Morales, E. & M. Elías-Gutiérrez. 1992. Cladóceros (Crustacea: Branchiopoda) de la reserva de la biosfera de Sian Ka´an, Quintana Roo y zonas adyacentes. *In*: Navarro, D. y E. Suárez-Morales (eds.). *Diversidad biológica en la reserva de la biosfera de Sian Ka´an, Quintana Roo. Vol. 2*. Centro de Investigaciones de Quintana Roo. Chetumal, pp. 145-161.

Thesis

Ibáñez-Aguirre, A. L. 1995. Algunos aspectos de la dinámica de poblaciones de *Mugil cephalus* (Linneo, 1758) y *M. curema* (Valenciennes, 1836) (Pisces: Mugilidae) en la Laguna de Tamiahua, Veracruz. Tesis de Doctorado en Ciencias (Biología), Facultad de Ciencias, UNAM. CDMX, México. 216 p.

Others

CNA (Comisión Nacional del Agua). 2003. *Ley Federal de Derechos Normas Aplicables en materia de Aguas Nacionales y sus Bienes Públicos Inherentes 2003.* Diario Oficial de la Federación. CDMX, México. Enero 2: 173-191.

Systematics Agenda 2000. 1994. *Systematics Agenda 2000: Charting the Biosphere.* Technical Report. New York. 34 p.

Online citations

References to online-only journals and books should include the author (or the responsible agency, i.e., FAO, FDA, WHO, etc.), title, website, and date of access, followed by:

In Spanish: Disponible en línea: http://www.fao.org/fishery/ culturedspecies/Litopenaeus_vannamei/en (consultado el 19 febrero 2010).

In English: Available online at: http://www.fao.org/fishery/ culturedspecies/Litopenaeus_vannamei/en (downloaded February 19, 2010).

Examples:

FAO. 2004. El estado mundial de la pesca y la acuicultura 2004 (SOFIA). Available on line at: http://www.fao.org/docrep/007/y5600e/y5600e00.htm (downloand february 19, 2010).

WHO. 2004. Vitamin and mineral requirements in human nutrition. 2nd ed. World Health Organization, Geneva. Available online at: http://whqlibdoc.who.int/publications/2004/9241546123.pdf (downloaded February 19, 2010).

Citations available in both printed and online sources should be cited as follows: First with a complete citation and then the website address, as in the following example:

SAGARPA-CONAPESCA. 2006. *Anuario estadístico de acuacultura y pesca 2006*. Secretaría de Agricultura, Ganadería y Desarrollo Rural, Pesca y Alimentación. Comisión Nacional de Pesca. Mazatlán. 219 p. Also available at: http://www.conapesca. sagarpa.gob.mx/wb/cona/cona_anuario_estadistico_de_pesca

Addition of DOI to References

The references of books and articles that have DOI (digital object identifier), it must be added at the end, as shown below:

Calor, A. 2009. Considerações About da Filogenia de Trichoptera Kirby 1813: da Análise dos Dados for Hipóteses ace or dos Cenários for Dados. *Entomobrasilis* 2 (1): 01-10. DOI:10.12741 / ebrasilis.v2i1.24

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Tabular material must be clearly set out with the number of columns in each table kept to a minimum and **vertically oriented** using double spacing **without tabs**, Universe Condensed or Arial 10 font. Tables, numbered consecutively with Arabic numerals, must be typed on separate sheets, leaving sufficient space around the copy for printer's instructions. Tables must have **concise headings at the top** that enable comprehension without reference to the main text. Please ensure that the data in columns are consistent in the number of significant figures. Footnotes should be kept to a minimum and indicated by asterisks and daggers (*, †) at the bottom of the table with type 8. **Vertical and horizontal lines should be avoided**.

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