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ORIGINAL RESEARCH

Caracterización acústica de las agregaciones de krill (*Euphausia superba*) detectadas automáticamente en el Estrecho de Bransfield e Isla Elefante

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RESUMEN. En el presente estudio se caracterizaron agregaciones de krill (*Euphasia superba*) identificadas en el Estrecho de Bransfield y alrededores de la Isla Elefante. Los datos fueron recolectados con una ecosonda multifrecuencia SIMRAD EK80 durante tres veranos australes: 2018, 2019 y 2020. Para la detección de agregaciones de krill se utilizaron dos frecuencias (38 y 120 kHz) y un algoritmo incluido en un programa destinado para el post procesamiento denominado Echoview versión 9, automatizado con el paquete EchoviewR en R. Se detectaron un total de 22.221 agregaciones. Los descriptores acústicos fueron analizados con la correlación de Pearson. Para la caracterización de agregaciones de krill se aplicó un análisis de componentes principales (PCA), seguidamente de un agrupamiento jerárquico. Para determinar las diferencias temporales de los clústeres fue aplicado un análisis de varianza (ANOVA). Además, a las agregaciones de krill se le asignaron las variables ambientales superficiales para aplicarle un modelo generalizado aditivo (GAM). Utilizando las primeras tres dimensiones del PCA (que explicaron 81% de la variabilidad total) se identificaron tres clústeres. El primer clúster se caracterizó por tener agregaciones de krill con menor altura (2 m), bajos valores en el coeficiente de retrodispersión acústica (7 m^2 mn^{-2}), y estar ubicado a mayor profundidad (81 m). El segundo clúster tuvo las agregaciones más someras (34 m), de menor longitud (75 m) y compacidad (202). Finalmente, el tercer clúster presentó agregaciones de mayor longitud (849 m), volumen (207.412 m^3) y altura (11 m), además de tener elevados valores de retrodispersión acústica (637 m^2 mn^{-2}), oblicuidad (6), compacidad (2.436) y coeficiente de variación (213). Espacialmente, el clúster l se localizó con mayor presencia en los alrededores de la Isla Elefante durante el 2018 y 2019, mientras que para este mismo período los clústeres I y II se ubicaron dispersos en toda la zona de estudio, pero focalizados en el Estrecho de Bransfield. Para 2020 se presentaron anomalías térmicas de + 2 °C aproximadamente y hubo una dispersión de los tres clústeres en toda la zona de estudio, donde se observó que el clúster I se localizó con mayor presencia en el Estrecho de Bransfield. Se encontraron diferencias significativas ($p < 0,05$) entre los clústeres por año. Sin embargo, dichas diferencias no fueron tan marcadas. Mediante un GAM, se estableció que todas las variables para cada clúster fueron significativas ($p < 0,05$). Las agregaciones se mantuvieron en condiciones promedio de temperatura (0,8 °C), salinidad (34,14) y oxígeno disuelto (8,16 ml l^{-1}). A escala interanual, se observó que las características de las agregaciones no cambiaron.

Palabras clave: Hidroacústica, agregaciones, Antártida, ecosonda multifrecuencia SIMRAD EK80, bioestadística.

Acoustic characterization of automatically detected krill (*Euphausia superba*) aggregations in the Bransfield Strait and Elephant Island

ABSTRACT. This study shows the characterization of krill (*Euphausia superba*) aggregations identified in the Bransfield Strait and around of Elephant Island. Data were collected using a multifrequency SIMRAD EK80 echosounder during three austral summers: 2018, 2019 and 2020. For detection of krill aggregations, two frequencies (38 and 120 kHz) and an automated Echoview version 9 algorithm with the EchoviewR package in R were used. A total of 22,221 aggregations were detected. Acoustic descriptors were analyzed with Pearson's correlation. For the characterization of krill aggregations, principal component analysis (PCA) was applied, followed by hierarchical clustering. To determine temporal differences of clusters, an ANOVA was applied. In addition, krill aggregations were assigned to surface environmental variables to apply a generalized additive model (GAM). Three clusters were identified using the first three dimensions of the PCA (which explained 81% of the total variability). The first cluster was characterized by krill aggregations having lower height (2 m), backscattering acoustic energy (7 m^2 mn^{-2}), and being located at a greater depth (81 m). The second cluster had the shallowest swarms (34 m), shortest length (75 m) and compactness (202). Finally, the third cluster had the largest swarms in length (849 m), volume (207,412 m^3) and height (11 m); in addition of having greater acoustic energy (637 m^2 mn^{-2}), obliquity (6), compactness (2,436) and coefficient of variation (213). Spatially, cluster I was located with a greater presence around Elephant Island during 2018 and 2019, while for the same period, clusters I and II were located scattered throughout the study area but focused on the Bransfield Strait. By 2020, thermal anomalies of approximately + 2 °C were presented and a dispersion of the three clusters was noted throughout the study area, where cluster I was located with a greater presence in the Bransfield Strait. Significant differences ($p < 0.05$) were found among the clusters per year. However, such differences were not so marked. Through a GAM, it was determined that all variables for each cluster were significant ($p < 0.05$). Swarms were kept in average conditions of temperature (0.8 °C), salinity (34.14) and dissolved oxygen (8.16 ml l^{-1}). On an interannual scale, it was observed that the characteristics of aggregations remained unchanged.

Key words: Hydroacoustic, aggregations, Antarctica, multifrequency echosounder SIMRAD EK80, biostatistics.

INTRODUCCIÓN

El krill (*Euphausia superba*) es la especie multicelular con mayor biomasa del mundo (Bar-On et al. 2018) encontrándose los mayores stocks en el océano austral (Gascón y Werner 2005) donde se han reportado grandes densidades que han llegado hasta los 2 millones de toneladas en 100 km^2 (Murphy et al. 1988; Nowacek et al. 2011). Además, es una especie clave en el funcionamiento del ecosistema austral (Mac Caulay 1987; Greene et al. 1991; Agnew 1992; Arntz 1997; Alonso et al. 2003; Hewitt et al. 2004; Siegel et al. 2004), siendo el principal alimento de ballenas, focas, pingüinos, aves, entre otros depredadores (Smetacek y Nicol 2005; Trathan y Hill 2016). Debido a su alta abundancia y disponibilidad, desde los años 70 el océano austral se convirtió en una de las pesquerías más importantes (Croxall y Nicols 2004). Sin embargo, el aumento de capturas ha impactado negativamente a sus depredadores, incrementando la competencia entre ellos por las presas (Reid et al. 2004). A causa de la pesca excesiva observada en las subáreas estadísticas FAO 48.1 y 48.2 (Kock 1991), la Comisión para la Conservación de los Recursos Vivos Marinos Antárticos (CCAMLR, por sus siglas en inglés) impuso un plazo para la temporada de pesca desde 1989-1990 (Meyer et al. 2020), que en la actualidad sigue vigente. A pesar de que en las dos últimas décadas las capturas han ido incrementando (Meyer et al. 2020), se ha observado una pequeña recuperación en los niveles de abundancia del krill que posiblemente sean gracias a las medidas de protección tomadas (Barrera-Oro et al. 2017). Esto puede ser corroborado en el subárea 48, en donde las capturas han aumentado y además se encuentran más concentradas en tiempo y espacio (Trathan et al. 2018). Toda esta evidencia ha generado como interrogante conocer el comportamiento y características de las agregaciones de krill en esta zona.

La evaluación poblacional del krill y los métodos de análisis son realizados según las recomendaciones de las reuniones anuales del subgrupo de acústica del CCAMLR, quienes en su esfuerzo por conservar las especies antárticas han incorporado una serie de estudios y metodologías en las cuales se encuentran la estimación de biomasa acústica del método 1 y 2. El método 1 (Hewitt et al. 2004) utiliza un par de frecuencias (Greenlaw y Johnson 1983) y una ecuación de TS (Wiebe et al. 1990; Greene et al. 1991). El método 2 (Greene et al. 1991), utiliza tres pares de frecuencias (Greenlaw y Johnson 1983) y la ecuación simplificada SDWBA (Stochastic Distorted Wave Born Approximation) de TS de Demer y Conti (2006). Los objetivos del componente acústico en estos cruceros científicos fueron estimar la distribución y biomasa del krill utilizando estos dos métodos.

En los diferentes estudios se ha podido observar que el krill forma diferentes tipos de agregaciones (Kalinowski y Witek 1985) espacio-temporales (Murphy et al. 1988). Este tipo de comportamiento agregativo está influenciado por factores físicos como el oxígeno disuelto (Brierley y Cox 2010), corrientes, turbulencia, *eddies* (Pinel-Alloul 1995), entre otros. Además, otros factores tales como reproducción, alimentación, beneficios energéticos y evasión de los depredadores (Ritz 2000). El krill logra escapar de sus depredadores migrando verticalmente hacia el fondo durante el día donde es menos visibles (Ritz 1994). Asimismo, aprovecha las capas de hielo para ocultarse (Reiss et al. 2017). Estos comportamientos han sido estudiados ampliamente con redes de arrastre y métodos acústicos (Tarling et al. 2002), encontrándose que el krill tiene una gran capacidad de natación y mantiene agregaciones a mesoescala (Zhou et al. 2004).

El presente trabajo tiene como objetivos caracterizar las variaciones y distribución espacial de las agregaciones de krill en la subárea antártica FAO 48.1, sobre la base de los registros acústicos detectados automáticamente en el estrecho de Bransfield y alrededores de la Isla Elefante durante tres campañas científicas antárticas estivales (2018, 2019 y 2020).

MATERIALES Y MÉTODOS

Área de estudio

La zona de estudio se ubicó en el estrecho de Bransfield y alrededores de la Isla Elefante que corresponden a la subárea estadística FAO 48.1 (Figura 1). Se realizaron tres campañas antárticas estivales (ANTAR). El diseño de muestreo acús-

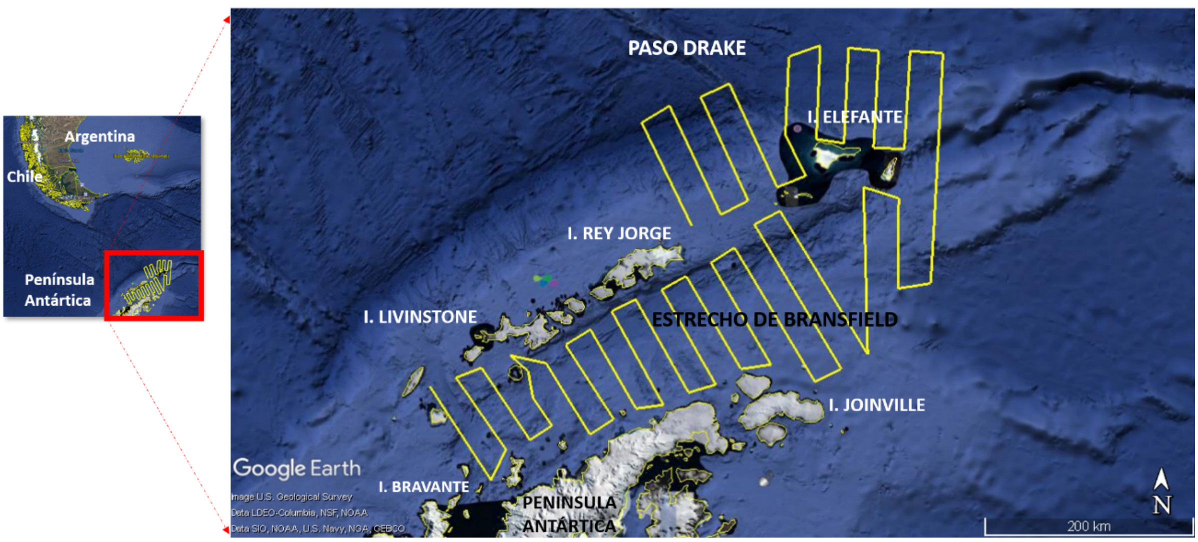

Figura 1. Área de estudio y trayecto planificado (líneas amarillas) para la campaña ANTAR XXVI (fuente: Google Earth).
Figure 1. Study area and planned path (yellow lines) for the ANTAR XXVI survey (source: Google Earth).

tico fue de tipo sistemático estratificado con transectas paralelas con longitudes entre 40 y 120 mn de largo y una separación de 15 mn (Simmonds y MacLennan 2005; AMRL 2011; Cossio y Reiss 2011). La prospección acústica comprendió un promedio de 24 transectas en 13 días efectivos de operación en el mar, principalmente en la primera quincena de enero de cada año.

Datos acústicos

Los datos acústicos fueron recolectados utilizando una ecosonda científica multifrecuencia EK80 (SIMRAD) con cinco frecuencias nominales de 18, 38, 70, 120 y 200 kHz y transductores de tipo de haz dividido: ES18-11, ES38-7, ES70-7C, ES120-7C y ES200-7C kHz, instalados en un blíster retráctil localizado en la quilla de la embarcación BAP "Carrasco" a 5,95 m de la superficie del mar. Estos datos acústicos estuvieron referenciados con la hora, fecha y posición geográfica.

La calibración de la ecosonda se realizó previo al inicio del muestreo acústico, siguiendo las recomendaciones técnicas estándares de Foote (1990) y Demer et al. (2015). La misma se realizó frente a la Bahía Almirantazgo de la Isla Rey Jorge-Islas Shetland del Sur (latitud: 062° 05,62′ S, longitud: 058° 27,15′ W, profundidad promedio de inmersión esfera: 33 m). El blanco de referencia utilizado para calibrar las cinco frecuencias fue una esfera de carburo de tungsteno (WC) de 38,1 mm de diámetro.

La trasmisión de los pulsos sonoros fue de manera simultánea y en el modo de onda continua (CW) en todas las frecuencias. Se utilizó el programa EK80 (versión 1.10.3 en la campaña ANTAR XXV y 1.12.2 en las campañas ANTAR XXVI y XXVII). La duración de pulso fue de 0.512 ms y el rango de grabación para todas las frecuencias fue hasta los 500 m de profundidad. La Unidad Básica de Muestreo (UBM; Simmonds y MacLennan 2005) fue de 1 mn. La emisión de los pulsos de sonido fue máxima. Cuando se observó desincronización de la ecosonda (aparición de falsos ecos), el intervalo de disparo de la ecosonda fue cambiado a 500 ms. La velocidad de navegación nominal para la adquisición de datos acústicos fue de 10 nudos (kn, 18 km h^{-1}). El almacenamiento de los datos fue en formato propietario *.raw de SIMRAD.

Datos biológicos

Se ejecutaron muestreos de pesca utilizando una red IKMT (Isaac-Kidd Midwater Trawls) cuyas dimensiones fueron 1,8 m de abertura vertical y 2,54 m^2 de área total de boca. El tamaño de malla fue de 505 µm, y un flujómetro (General Oceanics Modelo 2030 RC) que permitió cuantificar el volumen de agua filtrada. Las estaciones de lances programados fueron ejecutados a la profundidad donde se registraban los ecotrazos de krill detectados y visualizados en la pantalla de la ecosonda. La velocidad de arrastre promedio fue de 2,3 kn y el tiempo promedio de arrastre efectivo fue aproximadamente 20 min. Los lances fueron agrupados por área geográfica (Tabla 1).

Datos oceanográficos

Se efectuaron estaciones oceanográficas utilizando el CTDO Sea Bird Electronics 9 plus, el cual permitió la cuantificación de los parámetros físicos. La profundidad máxima muestreada fue de 1.000 m. Se obtuvieron registros continuos de alta frecuencia (cada metro) de la presión (profundidad), conductividad (salinidad), temperatura y oxígeno de la columna de agua. El tiempo que tomó la ejecución de las estaciones oceanográficas (lanzamiento de roseta con CTDO) fue aproximadamente de una hora en promedio.

Procesamiento de los datos acústicos

Para cada ecograma se realizó un procesamiento eliminando señales no biológicas e interferencias, como así también se corrigieron las líneas de

Tabla 1. Rango de tallas del krill recolectados con la IKMT y sus respectivos rangos de ΔSv para cada par de frecuencias utilizados en el método 1. Estrecho de Bransfield (EB), Norte de la Isla Joinville (JV) e Isla Elefante (IE).
Table 1. Size range of krill collected with the IKMT and their respective ΔSv ranges for each pair of frequencies used in method 1. Bransfield Strait (EB), North Joinville Island (JV) and Elephant Island (IE).

ANTAR Nº	Clúster	Rango de talla (mm)	Transecta (Nº lances)	Rango de talla (mm)	Rango de ΔSv (dB) para cada par de frecuencia (kHz) 120-38	200-120	200-38	Área geográfica
XXV (verano austral 2018)	A	13-57	1 al 13 (31)	10-60	2,5 a 17,7	-0,5 a 6,8	2,0 a 24,5	EB
	B	19-58	14 al 17 (12)	20-60	2,5 a 14,7	-0,5 a 2,1	2,0 a 16,8	JV
	C	13-59	18 al 23 (28)	10-60	2,5 a 17,7	-0,5 a 6,8	2,0 a 24,5	IE
XXVI (verano austral 2019)	A	12-50	1 al 11 (13)	10-50	4,6 a 17,7	-0,5 a 6,8	4,1 a 24,5	EB
	B	12-62	12 al 18 (18)	10-60	2,5 a 17,7	-0,5 a 6,8	2,0 a 24,5	JV
	C	12-62	19 al 28 (18)	10-60	2,5 a 17,7	-0,5 a 6,8	2,0 a 24,5	IE
XXVII (verano austral 2020)	A	14-60	3 al 11 (19)	10-60	2,5 a 17,7	-0,5 a 6,8	2,0 a 24,5	EB
	B	17-54	12 al 18 (13)	20-60	2,5 a 14,7	-0,5 a 2,1	2,0 a 16,8	JV
	C	17-61	19 al 23 (10)	20-60	2,5 a 14,7	-0,5 a 2,1	2,0 a 16,8	IE

fondo. Las señales indeseadas fueron extraídas aplicando la variable *background noise removal* (De Robertis y Higginbottom 2007). Además, se realizó un remuestreo cada dos pings utilizando la media y aplicando la variable *resample by number of pings*. Los pares de frecuencias aplicados fueron de acuerdo con el método 1 de Greene et al. (1991) y el método 2 de Conti y Demer (2006), usando la variable *minus*. Se utilizó la variable *range bitmap* para limitar los rangos ΔSv de acuerdo con cada par de frecuencia. Cada combinación de frecuencias fue usada para extraer los datos sobre el ecograma de frecuencia nominal. El par de frecuencia de $Sv_{200\text{-}38\ kHz}$ se aplicó para la profundidad 0 a 100 m, mientras que la de $Sv_{120\text{-}38\ kHz}$ de 100 a 250 m.

Para la detección de las agregaciones de krill se utilizaron el par de frecuencias $Sv_{120\text{-}38\ kHz}$ en el ecograma sintético aplicando la automatización con la función EVSchoolDetect de EchoviewR (Harrison et al. 2015). Posteriormente, se realizó la exportación de las planillas de cálculos de las celdas y regiones en formato *.csv (valores separados por comas). De estas planillas de celdas, se utilizaron las variables descritas en la Tabla 2.

Análisis de los datos

Caracterización de las agregaciones del krill

Para evaluar la correlación y excluir las variables que tengan características similares se realizó un análisis de correlación de Pearson. Para la caracterización de las agregaciones de krill fueron usadas las variables morfométricas: profundidad, altura, longitud, volumen, compacidad; variables estadísticas de las agregaciones: oblicuidad y coeficiente de variación; y variables energéticas: NASC (Tabla 2). Luego, con el objetivo de reducir la dimensionalidad de las variables se aplicó un PCA. Posteriormente, se retuvieron aquellos componentes principales que explican alrededor de 80% de la variabilidad de los datos. Finalmente, usando las cargas de los componentes retenidos se procedió a realizar un

Tabla 2. Descriptores acústicos usados para la caracterización de las agregaciones de krill.
Table 2. Acoustic descriptors used for the characterization of krill aggregations.

Nombre	Unidad	Descripción
Coeficiente dispersión de área náutica (NASC)	m^2 mn^{-2}	Es una medida de dispersión de área en lugar de dispersión de volumen. Se calcula al integrar una región, celda o selección.
Profundidad	m	Distancia lineal y vertical promedio desde la superficie hasta la agregación o cardumen.
Altura	m	Una medida de la extensión vertical de una agregación o cardumen, límite superior menos límite inferior.
Longitud	m	Longitud de la agregación/cardumen
Volumen	m^3	Volumen de la agregación/cardumen
Oblicuidad		Mide la simetría de la distribución de un conjunto de datos Sv (dB en m^2). Una distribución sesgada se inclina hacia la izquierda o hacia la derecha.
Compacidad de imagen		Mide la relación entre el perímetro (cuadrado) de la agregación/cardumen observado y el área del cardumen observada. Es decir, un círculo tiene una imagen compacta de 1.
Coeficiente de variación		Es la desviación estándar dividida por la media, expresada como porcentaje. Esto proporciona un método para comparar la dispersión de los datos de Sv en agregaciones con valores de Sv medios muy diferentes.

análisis de agrupamiento jerárquico (*clustering*). Todos estos análisis se realizaron usando el paquete FactoMineR en R.

Para describir los clústeres se hicieron gráficos boxplot y biplot por año de cada variable, realizado con los paquetes de ggplot2 y factoextra en R. Además, a fin de determinar la ubicación espacio-temporal de estos clústeres se hizo un ploteo georreferenciado de los clústeres por cada crucero. Para determinar las diferencias de clúster por años, se realizó un ANOVA de cada variable *vs* año, filtrando cada clúster.

Relación del krill con los parámetros oceanográficos

Para visualizar y analizar las condiciones oceanográficas superficiales de cada campaña se graficaron en una carta con la finalidad de describir patrones de las variables oceanográficas. Para cada clúster obtenido se procedió a realizar un análisis GAM utilizando los valores NASC de krill asociados con los parámetros oceanográficos superficiales obtenidos.

RESULTADOS

Caracterización de las agregaciones de krill

Se utilizó un total de 22.221 agregaciones de krill y se analizó el PCA para las tres campañas. Fueron retenidos los tres primeros componentes, explicando 81% de la varianza acumulada. La primera dimensión se asoció positivamente con el volumen, la longitud y la compacidad de imagen, también tuvo un efecto positivo en el segundo componente (Figura 2). El valor de la altura, la oblicuidad, el coeficiente de variación y NASC tuvieron efecto positivo en la dimensión 1 pero

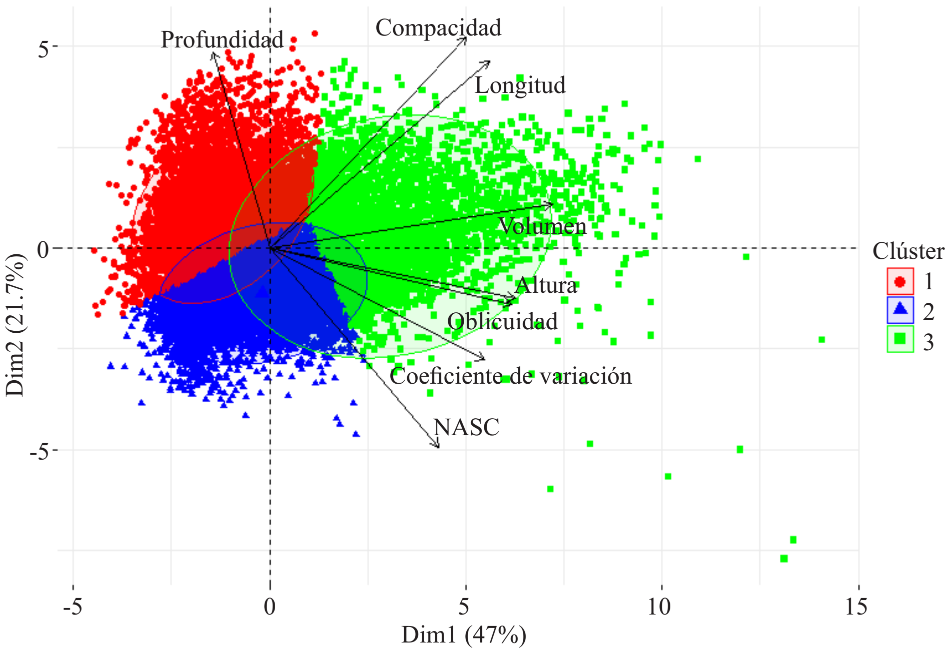

Figura 2. Biplot-PCA regiones de krill por clústeres.
Figure 2. Biplot-PCA krill's regions by clusters.

negativo en la dimensión 2. La variable de profundidad del enjambre indica migraciones verticales con efecto positivo en el segundo componente, pero negativo en el primero. Se observó que la profundidad media del enjambre presentó una relación inversa con variable de retrodispersión acústica (NASC), es decir, a mayor profundidad menores valores de retrodispersión acústica (Figura 2).

La dimensión 1 estuvo asociada a las variables morfométricas como la altura, longitud y volumen, mientras que la dimensión 2 se asoció al NASC, la profundidad y la compacidad, siendo las agregaciones de krill más pequeñas, compactas y profundas. Finalmente, la oblicuidad y el coeficiente de variación estuvieron asociados a la dimensión 3, que estarían sujetos a la simetría y dispersión de datos de cada agregación.

Luego del análisis PCA, sobre la base de las cargas de las variables en los tres primeros componentes principales, se procedió a realizar un análisis de agrupamiento de las agregaciones de krill (Figura 3). Ejecutando el método de *clustering* jerárquico, se logró distinguir tres grupos con diferentes características (Tabla 3). De los 22.221 clústeres obtenidos, 9.437 (42%) pertenecen al primer clúster, 8.618 (39%) al segundo y 4.166 (19%) al tercero, siendo el primero el de mayor número de agregaciones de krill:

- Clúster I. Este grupo se caracterizó por tener las agregaciones de krill con la menor altura media, menor a 2 m con alcance hasta los 17 m y un largo de 148 m. Estas agregaciones fueron registradas desde la superficie hasta los 250 m de profundidad, con un promedio de 80 m. Los valores de retrodispersión acústica (NASC) fueron relativamente bajos (7 m^2 mn^{-2}). La oblicuidad (falta de simetría) fue baja (en promedio 2). Los valores de compacidad de imagen se encontraron en promedio en 491; mientras que el coeficiente de variación se mostró bajo (en promedio 97). El volumen promedio se mantuvo 255 m^3. Este grupo de mayor pro-

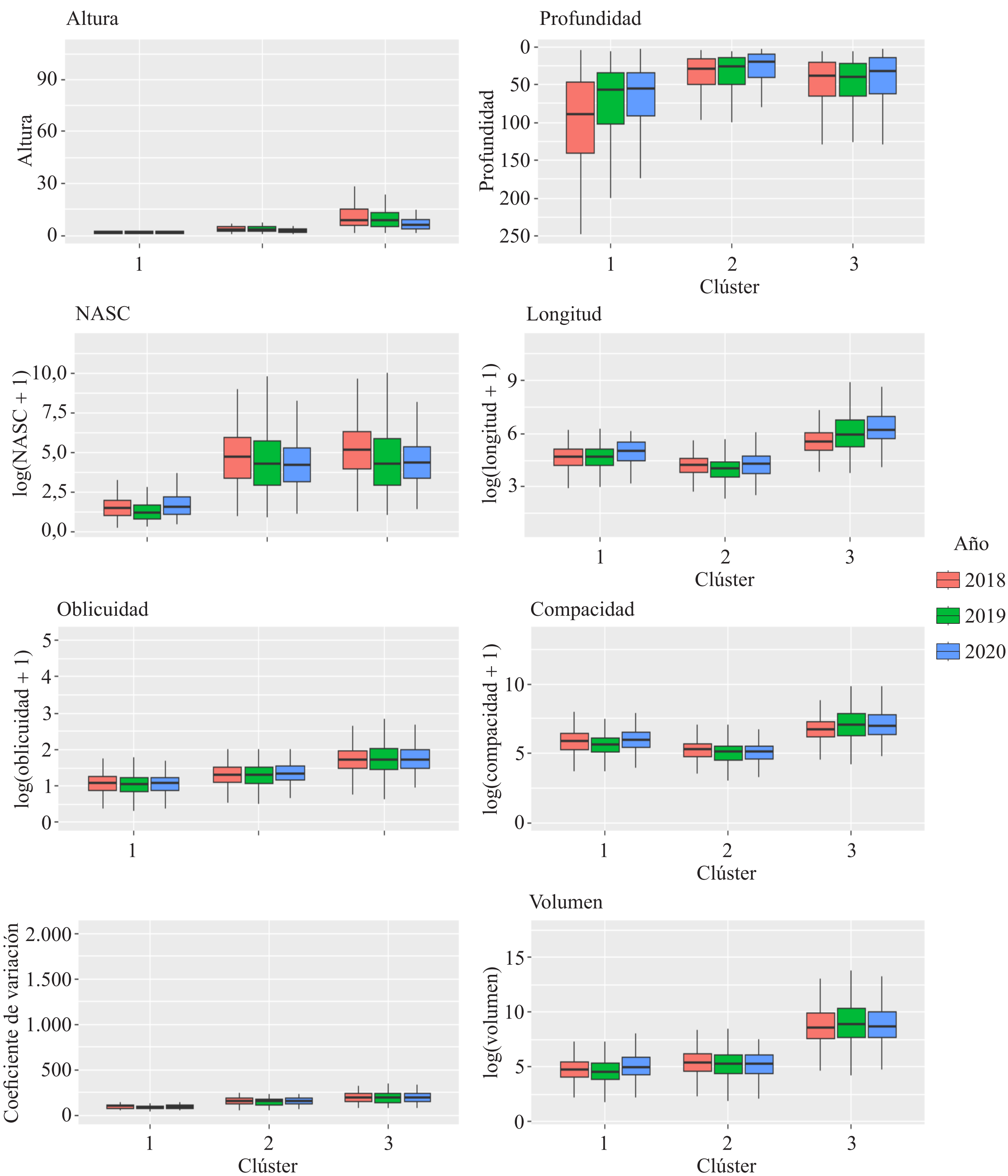

Figura 3. Boxplot de los clústeres por características de las agregaciones de krill por descriptor acústico.
Figure 3. Boxplot of clusters by characteristics of krill aggregations per acoustic descriptor.

Tabla 3. Promedio y desviación estándar (Prom + sd) de cada descriptor acústico para cada clúster.
Table 3. Average and standard deviation (Prom + sd) of each acoustic descriptor for each cluster.

	Clúster I	Clúster II	Clúster III
Descriptor acústico	Prom + sd	Prom + sd	Prom + sd
NASC (m^2 mn^2)	7 ± 20	356 ± 1.391	637 ± 4.536
Profundidad (m)	81 ± 57	34 ± 28	49 ± 42
Altura (m)	2 ± 1	4 ± 2	11 ± 10
Longitud (m)	148 ± 128	75 ± 51	849 ± 2.020
Volumen (m^3)	255 ± 511	427 ± 697	207.412 ± 1.457.917
Oblicuidad	2 ± 0,94	3 ± 1,25	6 ± 5,64
Compacidad	491 ± 900	202 ± 233	2.436 ± 12.449
Coeficiente de variación	97 ± 32	158 ± 53	213 ± 110

fundidad y de menor energía acústica retrodispersada estuvo asociado principalmente a la zona de los alrededores de la Isla Elefante entre los Antar XXV (2018) y XXVI (2019).

- Clúster II. Este grupo se caracterizó por tener las agregaciones de krill con una altura media de 4 m en promedio, ubicadas desde la superficie hasta los 208 m y con una profundidad media de 34 m. Los valores de energía acústica retrodispersada NASC promediaron los 356 m^2 mn^{-2} y la longitud de la agregación fue de 75 m. La oblicuidad fue ligeramente mayor al clúster 1, con un valor de 3. Los valores de compacidad de la imagen fueron de 202, el coeficiente de variación es idéntico al clúster I, con valor de 202 en promedio. El volumen de los cardúmenes se mantuvo con un promedio de 427 m^3.
- Clúster III. Las alturas de las agregaciones de krill de este grupo fueron mayores hasta los 107 m con un promedio de 11 m. En el caso de la profundidad, se encontraron hasta los 247 m y el valor promedio fue de 49 m. La energía acústica retrodispersada promedio fue de 637 m^2 mn^{-2} mientras que la longitud promedio fue mayor que los dos primeros clústeres, 849 m. Asimismo, la oblicuidad también fue mayor, con un promedio de 6. El coeficiente de variación tuvo un promedio de 213. Los volúmenes de este clúster fueron de mayor magnitud, con un promedio de 207.412 m^3.

Las agregaciones de krill del clúster I se caracterizaron principalmente por estar más profundas, tener menos altura y volumen, además de tener menor energía acústica retrodispersada. El clúster II se caracterizó por tener menor profundidad y longitud, además de tener las agregaciones de menor compacidad. El clúster III fue de profundidad intermedia, pero con mayor valor del resto de variables (Figura 3).

El clúster I (color rojo) de agregaciones del krill se ubicó al norte, este y sur de la Isla Elefante en 2018 (Figura 4). Para el 2019 este grupo se encontró con mayor presencia en los alrededores de la Isla Elefante, alcanzando las zonas del noreste de las Islas Rey Jorge y norte de Joinville. Para ambos años se observaron agregaciones aisladas en el Estrecho de Bransfield. En 2020 también se localizó este clúster alrededor de la Isla Elefante, aunque con mayor intensidad en la zona sur. Además, se distribuyó de manera constante a lo largo del Estrecho de Bransfield hasta el norte de Joinville.

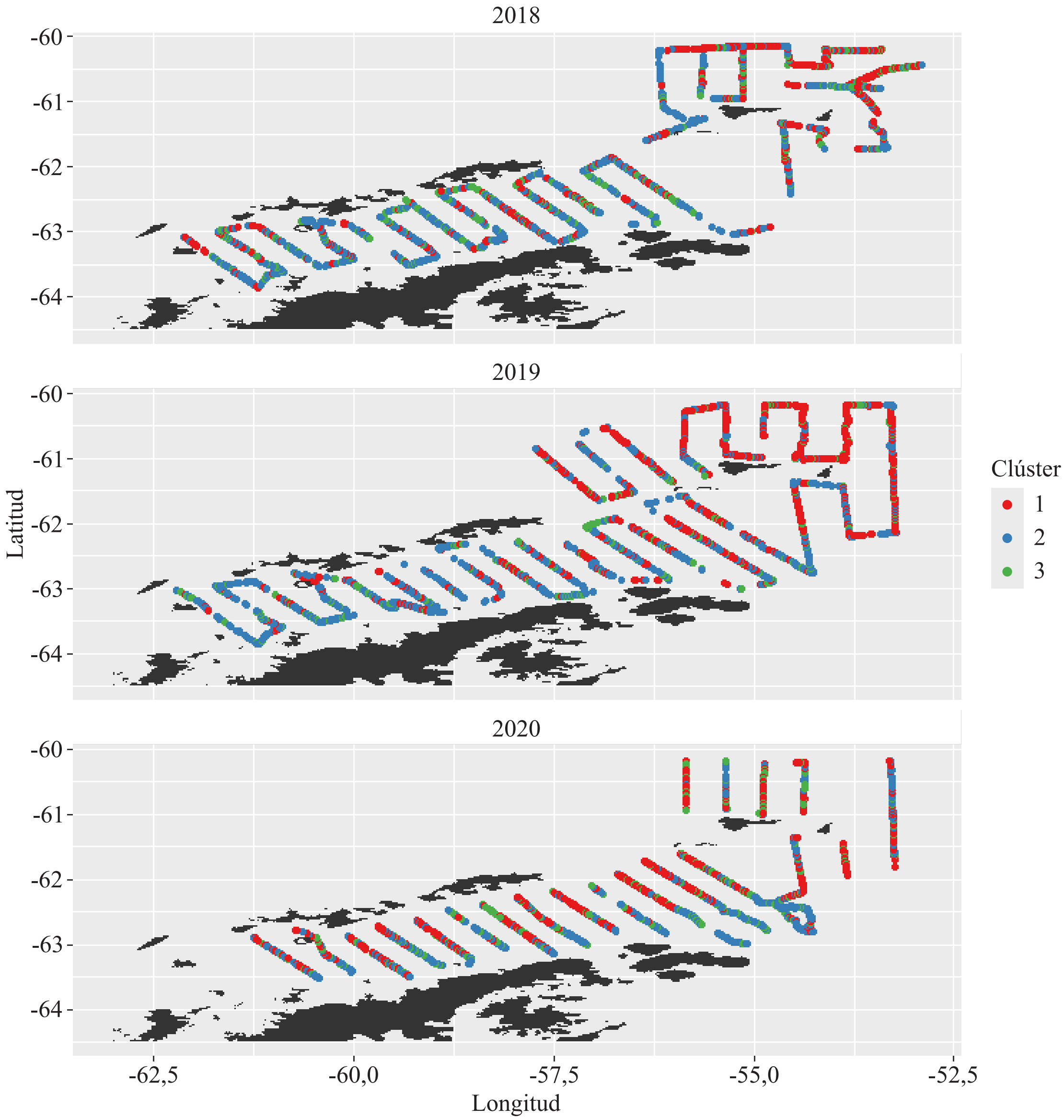

Figura 4. Clústeres de krill georreferenciados por campaña científica en la subárea FAO 48.1.
Figure 4. Krill clusters georeferenced per scientific survey in FAO subarea 48.1.

En el clúster II (color azul) las agregaciones se encontraron distribuidas en toda la zona de estudio, con mayor presencia en el Estrecho de Bransfield en 2018 y 2019. Sin embargo, se registró muy poca presencia de agregaciones de krill alrededor de la Isla Elefante y mayor en la zona norte de la Isla Trinidad. En 2020, este clúster se distribuyó con menor presencia en toda la zona de muestreo, con excepción de la zona al norte de Joinville y al este de Isla Elefante.

En 2018, el clúster III (color verde) se observó en parches aislados en tres zonas, esto es, alrededor de la Isla Elefante, en el norte del Estrecho de Bransfield y al sur de Isla Snow. En 2019, se distribuyó de manera aleatoria en toda la zona de estudio, mientras que para 2020 se distribuyó en pequeñas agregaciones en todo el recorrido del crucero.

Para observar las diferencias entre clústeres por año, se aplicó un análisis de varianza (ANOVA) teniendo en cuenta los descriptores acústicos para los distintos años. Los resultados presentaron un $p < 0{,}001$, es decir, que todas las relaciones fueron significativas, a excepción de la oblicuidad y el coeficiente de variación en el clúster I (Tabla 4). Estos resultados de ANOVA indican que sí existieron diferencias de cada clúster por años.

Parámetros oceanográficos superficiales

La temperatura superficial del mar durante la expedición Antar XXV se encontró en un rango de -1,0 a 1,5 °C, siendo el sur de islas Shetland, los alrededores de la Isla Elefante y el noreste de la Isla Joinville las más cálidas. Durante la expedición Antar XXVI, se observó un ligero calentamiento en los alrededores de la Isla Elefante, observándose temperaturas de hasta 2 °C. Durante la campaña Antar XXVII, se observó un calentamiento más elevado que llegó hasta 3 °C en la zona norte de todo el Estrecho de Bransfield y al norte de la Isla Elefante. Para las tres campañas se observó una temperatura más baja al norte de la Isla Joinville (Figura 5, izquierda).

La salinidad durante la campaña Antar XXV registró un rango de 33,5 a 34,5, siendo la zona menos salina el noreste de la Isla Elefante y la más salina el sur del Estrecho de Bransfield, con un valor de 34,5. En la siguiente campaña, la salinidad alcanzó valores cercanos a 34,0 en el Estrecho de Bransfield. Finalmente, en la campaña Antar XXVII se registró una variabilidad de la salinidad en el Estrecho de Bransfield, encontrándose núcleos de mayor salinidad (34,5) al norte de la Península Antártica y la Isla Joinville. Al sur de Islas Shetland del Sur y alrededores de la Isla Elefante, la salinidad fue de 34,0 aproximadamente (Figura 5, centro).

Con respecto al oxígeno disuelto, no se observaron patrones como en las otras dos variables. Durante la campaña Antar XXV se observaron núcleos de 6 ml l^{-1} dentro del Estrecho de Bransfield, mientras que en los alrededores de la Isla Elefante los valores de oxígeno fueron de 7 ml l^{-1} en promedio. Durante la campaña Antar XXVI, los valores de oxígeno fueron altos (~ 10 ml l^{-1}) en casi toda la zona de estudio (desde los

Tabla 4. Resumen de los análisis de varianza para cada variable analizada tomando como factor el año. Los asteriscos indican el nivel de significancia del análisis: '***' = 0,001; ' ' = 1.

*Table 4. Summary of analyzes of variance for each variable analyzed taking the year as a factor. Asterisks indicate the level of significance of the analysis. '***' = 0.001; ' ' = 1.*

Variable	Clúster I	Clúster II	Clúster III
NASC (m^2 mn^{-2})	***	***	***
Profundidad (m)	***	***	***
Altura (m)	***	***	***
Longitud (m)	***	***	***
Volumen (m^3)	***	***	***
Oblicuidad		***	***
Compacidad	***	***	***
Coeficiente de variación		***	***

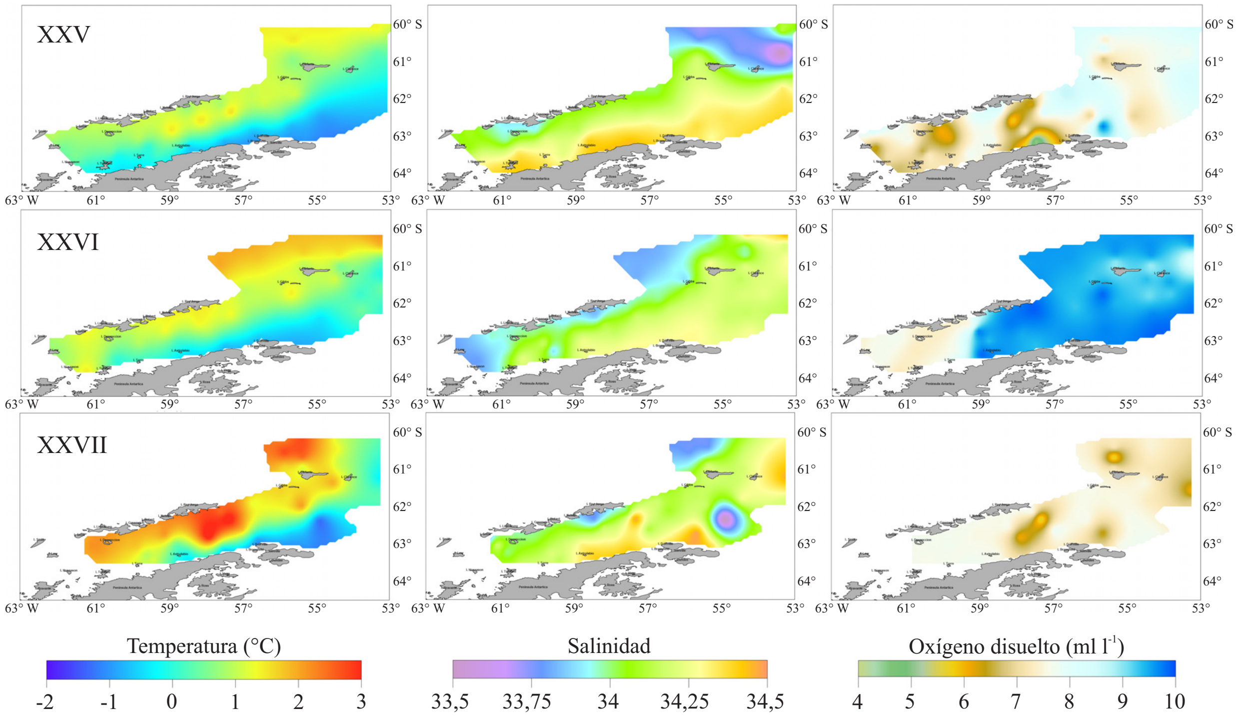

Figura 5. Temperatura, salinidad y oxígeno disuelto registrados durante los tres cruceros.
Figure 5. Temperature, salinity and dissolved oxygen recorded during the three cruises.

alrededores de la Isla Elefante llegando al sur de la Isla Nelson), para luego disminuir hasta 7 ml l^{-1}. Por último, durante la Antar XXVII, los valores de oxígeno se mantuvieron en 7,5 ml l^{-1}, con excepción de un par de núcleos con concentraciones inferiores que se ubicaron al norte de la Península Antártica y al norte de la Isla Elefante (Figura 5, derecha).

Relación del krill con las condiciones oceanográficas superficiales

Se relacionaron las agregaciones de krill con las variables oceanográficas obtenidas en cada estación. A partir de esta relación, se realizó un modelo GAM (log(NASC + 1) ~ s(OSM) + s(TSM) + s(SSM)), de familia gaussiana, para analizar los valores NASC del krill con las variables oceanográficas superficiales. Así, se observó que las tres variables resultaron significativas con $p < 0,001$ para explicar la distribución del krill. La desviación explicada fue de 13,3% y el r^2 de 0,132. La temperatura se encontró en un rango preferente de -0,5 a 1,2 °C, mientras que la salinidad estuvo entre 34,0 a 34,4 y el oxígeno disuelto entre 6,5 y 8,0 ml l^{-1} (Figura 6).

El promedio de temperatura para los tres clústeres fue de 0,8 °C, mientras que para la salinidad fue de 34,14 y, finalmente, el oxígeno estuvo en 8,16 ml l^{-1}.

DISCUSIÓN

Se han realizado diversos tipos de estudios que han permitido agrupar al krill usando análisis de PCA, ya sea según sus características biológicas (Trathan et al. 1993; Watkins et al. 1999) como morfométricas, utilizando datos hidroacústicos

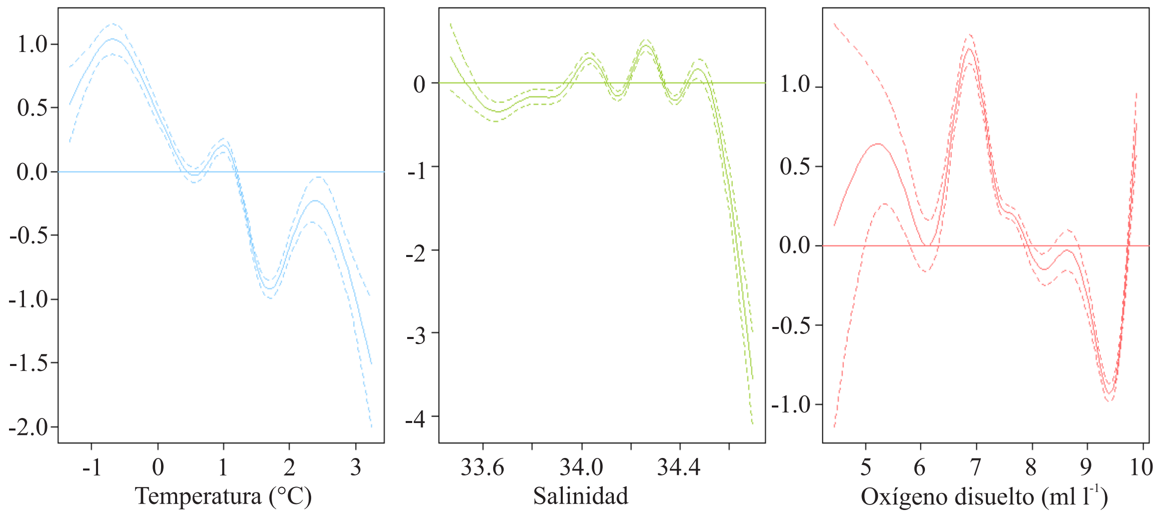

Figura 6. Gráficos GAM de las regiones de krill para cada variable oceanográfica.
Figure 6. GAM graphs of krill regions for each oceanographic variable.

(Miller et al. 1993; Tarling et al. 2009). Cuando se usan características biológicas se puede agrupar ya sea por madurez sexual, tamaños y sexualidad, entre otras. Pero cuando se utilizan metodologías hidroacústicas es posible emplear variables morfométricas de cardúmenes o agregaciones, posicionales y energéticas. Además, es factible agregar variables de hidrografía en los análisis de PCA (Krafft et al. 2010) mejorando la caracterización de los clústeres de krill. Varios autores (Kalinowski y Witek 1985; Tarling et al. 2009) describen que es posible encontrar una gran variedad de tamaños e interrelaciones de las agregaciones de krill. Sin embargo, en este trabajo se presentan tres clústeres con características acústicas, los cuales marcan diferencias significativas de variables tales como profundidad, longitud, altura, volumen y valores de energía acústica retrodispersada de las agregaciones.

Los resultados obtenidos en el presente estudio son similares a otros realizados en la zona del Estrecho de Bransfield (Kalinowski y Witek 1985; Tarling et al. 2009), donde fueron clasificados tres grupos: dos predominantes y un tercero con menor cantidad de agregaciones. Comparando el clúster I de Tarling et al. (2009) con el clúster I de este estudio, se observa que ambos comparten similitudes con respecto a su tamaño y compacidad. El clúster III de este trabajo puede ser comparado con el segundo clúster de Tarling et al. (2009). Estos clústeres son descritos como “super” agregaciones, los cuales presentan grandes dimensiones con varios kilómetros de longitud y grandes volúmenes.

Sobre la base de nuestros resultados, se observaron diferencias significativas de cada descriptor acústico con respecto al período de estudio. Este resultado difiere de que detallan Brierley y Cox (2014), quienes encontraron que los tamaños de agregaciones de krill no cambian, inclusive cuando las biomasas varían, aunque las agregaciones de krill son muy variables a microescala (Tarling et al. 2009). En este estudio se ha demostrado que los tres tipos de clúster están ubicados en las diferentes zonas de estudio, pero que presentan algunas preferencias. Existe un patrón de comportamiento para 2018 y 2019, donde se evidencia que el clúster I (color rojo) se encuentra principalmente y con mayor presencia en los alrededores de la Isla Elefante. Contrariamente, en 2020 este grupo se encontró distribuido en toda la zona de estudio; además, en este año se presentó una mayor biomasa (7,7 millones de toneladas). A pesar de que en ese año se observó un incremento en la biomasa, los patrones de comportamiento no se han visto influenciados drásticamente, pero sí cambios en su distribución espacial.

Relación de parámetros ambientales con las agregaciones de krill

La Península Antártica y alrededores han presentado temperaturas que fueron las más cálidas de las últimas tres décadas, con una anomalía térmica de + 1 °C (IMARPE 2020). Este fenómeno ha producido potencialmente un cambio en los patrones de comportamiento del krill en toda el área de estudio, además de un desplazamiento de abundancia al Estrecho de Bransfield. Esto puede deberse a que el krill es una especie estenotérmica que evita los cambios bruscos de temperatura acercándose a los frentes polares antárticos (Krafft et al. 2010). Se realizaron análisis GAM para cada clúster pero no se encontró ninguna diferencia significativa de las variables, a pesar del calentamiento que hubo en 2020, resultados similares a los encontrados por Miller et al. (1993).

Los alrededores de la Isla Elefante son una zona de altas densidades de fitoplancton (Villafañe et al. 1993) y está influenciada por los patrones de circulación de las principales masas de agua (Holm-Hansen et al. 1997) que convergen y se mezclan sobre una plataforma continental extensa (Helbling et al. 1993). Asimismo, podrían estar influenciadas por las condiciones meteorologías (Villafañe et al. 1995) que además podrían afectar la tipología de concentración del clúster I, que se encuentra más profundo y disperso, pero más pequeño en relación de altura, longitud y volumen. A diferencia de la Isla Elefante, el Estrecho de Bransfield es una zona cubierta con mejores condiciones temporales de mar. Aquí se encontraron agregaciones con mayor longitud, volumen, altura y valores elevados de energía acústica retrodispersada, que pertenecen específicamente al clúster III.

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ORIGINAL RESEARCH

The response of the natural and sewage-impacted intertidal epilithic community of the SW Atlantic to pulse (before/after summer) and chronic sewage discharges in the 1997-2014 period

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ABSTRACT. Until 2014 Mar del Plata city discharged its untreated sewage effluents to the intertidal sector. This city has a marked seasonality in the urban discharge, varying between 2.8 and 3.5 $m^3 s^{-1}$ of effluents before/after summer. The effect on the intertidal benthic community was evaluated in both spatially, in sewage-impacted and reference sites, and temporarily in both the short term, before/after summer, and in long term along nine periods between 1997-2014. The bivalve Brachidontes rodriguezii, the ecosystem engineer, reach the maximum dominance and frequency in reference areas. Spatially the presence of opportunistic and tolerant species characterized the impacted areas, while in reference sites sensitive species were prevalent. The opportunistic polychaete species *Capitella* '*capitata*' sp. and *Alitta succinea* were dominant near the sewage discharge in firsts periods. In other periods the indicator species were *Rhynchospio glutaea* or *Boccardia* spp. From 2008 the invader *Boccardia proboscidea* characterized the sewage-impacted sites building massive reefs. The crustaceans *Jassa falcata* and *Caprella* sp. were very abundant at intermediate distances from the sewage discharge, while *Monocorophium insidiosum* was very abundant in sewage-impacted areas. The tolerant and opportunistic species are favored after the summer due to the extra organic matter input. All community parameters showed lower values after the summer, and also a trend to diminish along the studied period.

Key words: Epilithic community, macrobenthos, mussel bed, long term study, chronic and pulse disturbance, tendency, SW Atlantic.

La respuesta de la comunidad epilítica intermareal natural e impactada por las aguas residuales del Atlántico SO a pulsos (antes/después del verano) y descargas crónicas de aguas residuales en el período 1997-2014

RESUMEN. Hasta 2014 la ciudad de Mar del Plata descargaba sus efluentes cloacales sin tratamiento al sector intermareal. Esta ciudad tiene una marcada estacionalidad en sus descargas, variando el caudal entre 2,8 a 3,5 $m^3 s^{-1}$ antes/después del verano. El efecto sobre la comunidad bentónica intermareal fue evaluado en la escala espacial, en sitios de referencia y sitios impactados, y también temporalmente en el corto período de tiempo, antes/después del verano, y a lo largo de nueve períodos entre 1997-2014. El bivalvo *Brachidontes rodriguezii*, el ingeniero ecosistémico, alcanza su máxima dominancia y frecuencia en áreas de referencia. Espacialmente la presencia de especies oportunistas y tolerantes caracterizó los sitios impactados, mientras que en sitios de referencia las

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especies sensibles son prevalentes. En los primeros períodos el poliqueto oportunista *Capitella* "*capitata*" sp. fue dominante cerca de la descarga cloacal, y también *A. succinea*. En períodos posteriores las especies indicadoras fueron *Rhynchospio glutaea* o *Boccardia* spp. Desde 2008 el poliqueto invasor *Boccardia proboscidea* caracterizó los sitios impactados por la descarga por masivos arrecifes. Los crustáceos *Jassa falcata* y *Caprella* sp. fueron muy abundantes a distancias intermedias de la descarga, mientras que *Monocorophium insidiosum* fue muy abundante en el área impactada por la descarga cloacal. Las especies tolerantes u oportunistas se vieron favorecidas después del verano debido al aporte extra de materia orgánica. Todos los parámetros comunitarios mostraron valores menores después del verano, y también se observa una tendencia a disminuir a lo largo de los períodos estudiados.

Palabras clave: Comunidad epilítica, macrobentos, banco de bivalvos, estudio de largo plazo, disturbio crónico y de pulso, tendencia, Atlántico SO.

INTRODUCTION

Marine scientists have realized that the sea is not an inexhaustible sink. The coastal areas first and now huge ocean areas show that marine pollution is global. Increasingly more or larger dead zones deoxygenated seas and oceans due to the discharge of untreated wastewater, which affects marine ecosystems in an area of 245,000 km^2, with implications for fisheries, livelihoods and food chains (WWAP 2017). Coastal ecosystems are highly vulnerable to multiple environmental human stressors e.g. urban and agricultural runoff of pollutants and nutrients, habitat alteration, aquaculture, fishing, acidification, etc. (Fabry et al. 2008; Halpern et al. 2008).

The main source of organic pollution is associated with the discharge of sewage generated by domestic effluents, being considered the oldest form of pollution (Pearson and Rosenberg 1978; Bishop et al. 2002; Medeiros and Bicego 2004; Borja et al. 2006; Martins et al. 2008; Muniz et al. 2006, 2013). Domestic waters provide organic and inorganic substances, including nutrients (even those that have primary and secondary treatment) that produce eutrophication that leads to changes in structure and functioning of marine ecosystems (Clarke and Warwick 2001; Gray et al. 2002). Eutrophication, i.e. over-feeding the aquatic environment by substances that induce rapid algae growth (Nixon et al. 2009; Ferreira et al. 2011). Nutrient loading in coastal waters may have direct or indirect effects on the environment. Some of the direct effects may be changes in chlorophyll levels, in primary production, in macro- and microalgae biomass and in the sedimentation of organic matter. Indirect effects include: changes in benthic biomass, benthic community structure, habitat quality, water transparency, increase in organically enriched sediments, changes in dissolved oxygen levels, mortality of aquatic organisms, changes in food chains, among others (Cloern 2001; Islam and Tanaka 2004; Díaz and Rosenberg 2008).

One of the main challenges in environmental impact assessment is to distinguish natural variability of natural communities of that variability induced by human activities. To assess the quality of the environment, and thus try to quantify the damage that man does to the ecosystem, several indices of environmental quality or ecological indices are calculated. Many of these indicators are based on benthic invertebrates because they have little or no mobility, they form associations that include species with a different degree of tolerance to stress, they respond to disturbances at supra-specific levels, such as genera, families and even classes, and finally because integrate the recent history of disturbance (Warwick 1993; Salas et al. 2006; Borja et al. 2008; Patricio et al. 2009; Dauvin et al. 2010; Muniz et al. 2013). The underlying idea that supports the concept of a biological indicator is that the selected organisms or groups provide, express or integrate information about their habitat. This can be shown through the condition, presence/absence, relative abundance or biomass, reproductive event, association structure (that is, composition and diversity), community function (such as trophic structure, or functional diversity) or any other combination of these characteristics (Muniz et al. 2013).

The city of Mar del Plata (38° S-57° W), in the SW Atlantic, is the largest summer resort in Argentina, receiving about 3 million people in summer time (Bouvet et al. 2005). Although the city has a functional submarine outfall since 2014, sewage water with only a pretreatment was discharged directly into the coastline, 9 km from the city center, for more than 30 years at a mean rate of 2.8 m^3 sec^{-1} and up to 3.5 m^3 sec^{-1} in summer (Scagliola et al. 2006). Fisheries, factories fishmeal, tourism, restaurants and textile industries are the main industrial activity in the city and therefore are responsible for the supplement large amounts of fat (12 t day^{-1} of industrial origin and 6 t day^{-1} of domestic origin) to urban wastewater (Scagliola et al. 2006, 2011). The abundance of fecal indicators (*Enterococci* 100 ml^{-1}) showed risk to human health along 15 km of beaches popular use (Comino et al. 2008; 2011).

The scorched mussel *Brachidontes rodriguezii* (D'Orbigny, 1842), an ecosystem engineer inhabits the intertidal hard substrates in large areas of the SW Atlantic, including places with sewage discharges. It is a species that has the peculiarity of dominating natural rocky coasts (Adami et al. 2004). Its community structure has served as an indicator of sewage-impact in Mar del Plata (Vallarino 2002; Jaubet 2013; Sánchez 2013; Llanos 2018). Although the short-term response of polychaetes to increases in sewage discharge during summer is partially known (Elías et al. 2006), its temporal variation in relation to community, both in short and long term periods, is unknown. In this context, the present work describe the spatial-temporal dynamics of the intertidal community in the period 1997-2014, analyzing both impacted sites by sewage (chronic) disturbance by the sewer discharge and reference sites (not impacted), as well as the community response to the events before/after the summer, in response to the increase of the sewage discharge (pulse disturbance).

MATERIALS AND METHODS

Study area

The coast of Mar del Plata city (Argentina 38° S-57° W) is dominated by sandy beaches, but occasionally there are quarzitic outcrops and horizontal abrasion platforms of consolidated loess formed by silica-cement sandstones (Teruggi 1959; Isla and Ferrante 1997). The tidal regime is regular and semidiurnal, with average heights of 60 cm in quadrature and up to 90 cm in high tides of syzygy, but very subjected to weather conditions. A strong littoral current (15 cm s^{-1}) run from South to North. During autumn-winter frequent storms from the S-SE constantly affect the coast (Manolidis and Alvarez 1994; Isla and Ferrante 1997). The climate is typically marine temperate with regular rains (850 mm $year^{-1}$). The area is influenced by derived on advected waters from the continental shelf (Subantarctic origin), with temperatures between 8 and 21° C and salinities between 33.3 and 33.8 (Guerrero and Piola 1997; Lucas et al. 2005). The present study was carried out on one of these horizontal platforms, which surrounds the sewage effluent of Mar del Plata city. Similar substrates with similar ecological conditions far from the influence of the sewage were used as reference (or control) sites.

Environmental quality

From the intertidal sewage discharge a gradient of environmental conditions was generated towards the south (sampling zone) by the flume dilution, which was function of the distance from the outfall. Vallarino and Elías (2006) revealed that salinity was almost constant in the area (around 32-33), but occasionally in some seasons (i.e. autumn-winter) lower values (26-30) were recorded in closest sites to sewage discharge (200-50 m). Dissolved oxygen showed mean values of 10 mg l^{-1} in reference site, but less than 7 mg l^{-1} at 50 m from the effluent. Values of pH ranged 8.0 to 8.3 in reference site, but low near the discharge (7.7 to 8.0). Turbidity was constant in the reference site, reaching mean values of 50 NTU, while increasing to up to 300 NTU in sites under the influence of sewage discharge, particularly in the summer (see Vallarino and Elías 2006).

Total Organic Carbon (TOC) of the interstitial sediment was elevated in patches surrounding the effluent (1.5 to 2.0%), and decreased with distance and reference sites (mean of 0.5% at 1.000 m from the effluent and also in the Reference site). Sediment accumulated among mussels also showed an environmental gradient, being more abundant in intermediate distances (20-50 kg m^{-2}) and lower in reference sites (between 20-30 kg m^{-2}), with a minimum in most near site to sewage site (10-20 kg m^{-2}).

From 1989-2014 the city's sewage effluent was discharged with only a pre-treatment over the intertidal sector. The construction of the submarine outfall lasted from 2008 to 2014. This included a breakwater that alters the dynamics of the sea by slowing the flux of littoral waters and the rates of sedimentation around it. The intertidal sewer stops in December 2014, when the current submarine outfall was opened, which discharges the city's wastewater through 130 nozzles located in the last section of 500 m long (between 3.9 and 4.4 km offshore).

Sampling design

Monitoring was carried out in three intertidal areas with different distances from the sewage outfall. Each area includes a set of three sampling sites with different condition of organic contamination (Figure 1). In the area called 1S, groups of samples (12 to 36 sampling units of 78 cm^2) were taken between 50 and 200 m from the point of discharge. In the area called 2S, groups of samples were taken between 1,000 and 1,200 m, both south from the point of discharge and the so-called Reference area (i.e. areas without sewage influence) and in different sites between 18,000 to 6,000 m north from the sewer discharge. Because differences between these 'References' were not statistically significant, values were averaged in one area. For more information, see Vallarino et al. (2002), Jaubet et al. (2013), and Sánchez et al. (2013).

Studies lasted for almost 15 years, with different objectives and therefore different sampling designs. However, the constancy of sampling impacted and reference sites and before/after summer in 9 periods was maintained. The original database includes more periods and different seasonality (monthly, quarterly), but for the present study the nine periods that have a before/after summer were considered.

In each site, sampling units were taken in the intertidal benthic community from different and independent rocks. Each sample was fixed with 10% formalin. In laboratory the material was washed and sieved through a 1 mm mesh size and the retained biological material was identified to the lesser taxonomic level, quantified, and preserved in 70% ethanol solution.

Environmental variables

Three 10 g samples of sediments were taken in each site for determination of Total Organic Carbon (TOC) by the method of Walkley and Black (1965) and expressed as percentage.

Data analysis

Factors analyzed were Sites (1S, 2S, Reference), Event (before/after summer), and Periods (9).

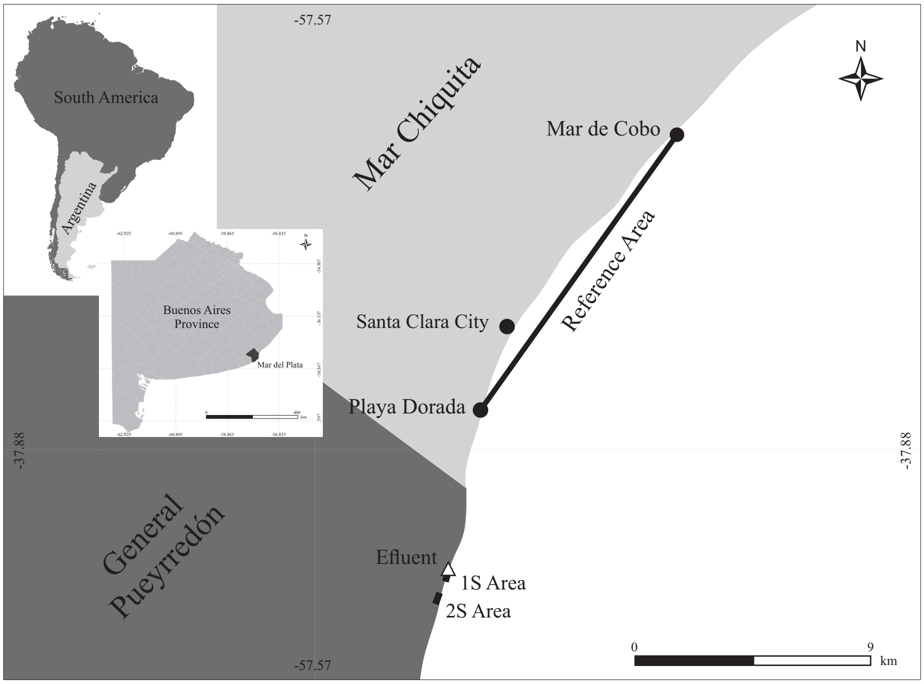

Figure 1. Sampling site. Area 1S is an average of sites located between 50-200 m south to the sewage effluent and Area 2S is an average of sites located between 1,000-1,200 m to the south of the point of discharge. The Reference Area corresponds to several reference sites (black circles). The white triangle is where the sewage discharges intertidally.

From the similarity matrix with square root transformation, a Permanova analysis was performed considering Sites, Event (before/after summer), and Periods (every before/after summer) as fixed factors. The Permanova analysis is a multivariate 'semiparametric' test which estimates parameters to adjust the distance matrix to a lineal model (Anderson 2001). Values of *p* are the result of permutations without normality suppositions, with characteristics of a free distribution test. Due to the existence of significant interactions in the Permanova analyses interpretation of results were conducted graphically.

The analysis of data included ordination by n-MDS and Cluster using a similarity matrix using the Bray-Curtis index with square root transformation to diminish the weight of dominant taxa. In the n-MDS ordination the three sites were discriminated by using different symbols. A cluster diagram was superimposed showing the groups corresponding to the factor Event (before/after summer with different color). In the same graph, the spearman correlation between sites and species was added. Species were selected from a Similarity Percentage (SIMPER) analyses, and results for every group (Sites, Event) are presented in an Appendix. This graph was made for each of the 9 periods (with before/after sampling). For the trend analysis an oversimplification was made in a n-MDS by averaging the impacted sites (1S, 2S) in one, with averaged reference sites, resulting in 18 points (= samples) for impacted sites (9 before, 9 after) and 18 reference sites. This oversimplification was, however, of little use in clarifying the spatial and temporal behavior of the community. To improve the visualization a new graph was made showing the course of the impacted site and the reference site separately.

From the same matrix the following community parameters were calculated: Richness (S), Abundance (N), Diversity (H') and Evenness (J'). These data were used to run a bi-factorial ANOVA (with Sites-Periods, and Sites-Events as fixed factors). Dominance was expressed as the abundance of the species over the total abundance, while Frequency was defined as percent of sample with the species over the total of samples.

RESULTS

Percentage of Total Organic Carbon showed significant differences among sites and periods (Table 1). Frequently the sewage-impacted sites showed the greatest values with a slight tendency to diminish. In the Period 2008-2009 a great peak of organic matter was evident in all sites, but in particular in sewage-impacted ones. Although values decrease, they remained higher compared to previous periods (Figure 2).

Table 1. ANOVA of Total Organic Carbon (%) by sites and periods. Data extended from November 1997 to March 2014.

Effect	SC	df	MS	F	P
Site	9.7	2	4.84	25.9	0.000*
Period	403.9	10	40.39	216.4	0.000*
Site*Period	51.0	20	2.55	13.7	0.000*

Biological data

Total abundance, considering the 547 sampling units analyzed, was 459,437 individual, from 95 taxa of macroinvertebrates. Only a few species were constant and abundant (Appendix, Table A1). *Brachidontes rodriguezii*, the ecosystem engineer, reached maximum dominance (57%) and frequency (84.6%). Among polychaetes, *Syllis prolixa* Ehlers, 1901 was the most abundant

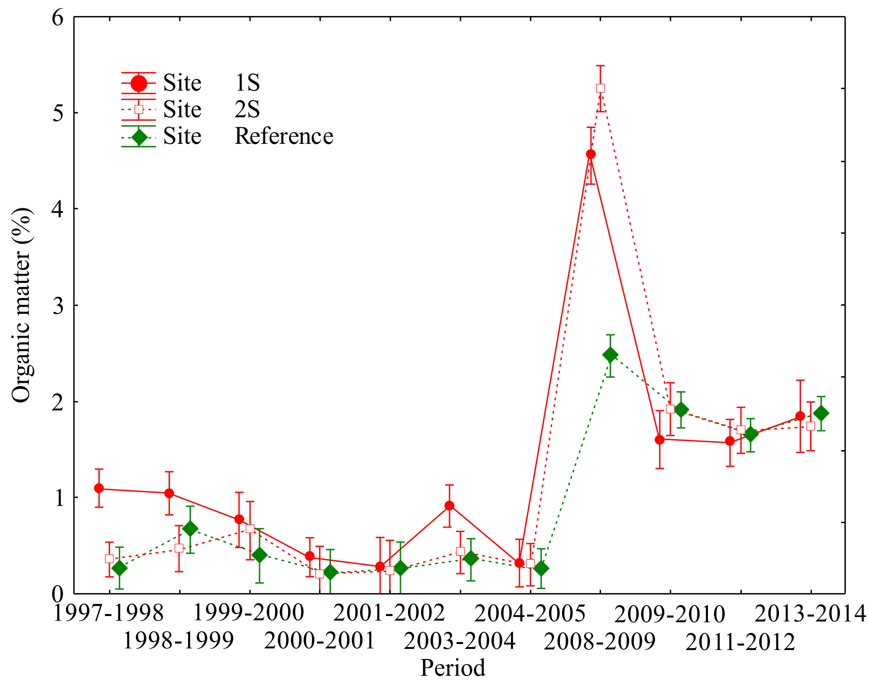

Figure 2. Organic matter from the three sampling Sites, from November 1977 to March 2014. Site 1S is the nearest to sewage (50 m) and 2S is the farest (1,000 m). Reference sites were between 9 to 18 km north. Data modified from Llanos (2018).

species (4.5%), while in frequency *S. gracilis* Grube, 1840 and *S. prolixa* were both very frequent (76 and 70.9%, respectively). *Boccardia* spp. reached 19 and 15% in dominance and frequency, respectively, while *Boccardia proboscidea* Hartman, 1940, the invader species, was the second in dominance (19.2%) but with a low frequency (15.4%).

Multivariate analysis

A permutational analysis was carried out (999 permutations), considering fixed factors: Sites (1S, 2S, and Reference), Periods (9) and Event (before/after Summer nested in Periods) (Table 2). Due to the existence of significant interactions among all factors (Sites, Periods and Event), the analyses cannot be interpreted but can be analyzed graphically by n-MDS for each period.

Ordination period-event

Period 1997-1998

The n-MDS (Figure 3 A) showed the Reference sites separated from those sewage-impacted. Some sampling units of site 2S were grouped near the Reference sites, mostly before summer. The cluster (represented by the 59% similarity line in the n-MDS) grouped sampling units from before the summer at the top of the graph, while those from after the summer were at the bottom. Sampling units corresponding to the reference sites were grouped by their affinity for each other and kept at the top, unchanged by the summer. The Simper analysis of the percentage of similarity by Sites (Appendix, Table A2) showed *B. rodriguezii* been dominant and important in the Reference area, however decreased in 1S and 2S reaching the third place in these groups. The crustaceans *Jassa falcata* (Montagu, 1808) and *M. insidiosum* (Crawford, 1937) were more important in groups 1S and 2S, as well as the polychaete *C.* '*capitata*' sp. On the other hand, the Event (before/after summer) revealed higher abundances before summer, nevertheless *C.* '*capitata*' sp. reached their maximum values after summer (Appendix, Table A3).

Period 1998-1999

The n-MDS (Figure 3 B) showed most Reference sites grouped, with some 2S sampling units, and most impacted sites (1S) and intermediate sampling units (2S) in the opposite side. Group before/after were grouped in the top and the botton of the graph, respectively. The Simper analysis by sites (Appendix, Table A4) showed a great mean abundance of *B. rodriguezii* respect the precedent period and always remained dominant in the reference site. The polychaetes *Boccardia* spp., *Capitella* '*capitata*' sp. and *S. prolixa* Grube, 1840 were dominant, the first two in sewage-impacted sites, while *Syllis* was in reference site.

Considering Events most species showed decreasing values after summer, except the most contributing species, *B. rodriguezii* and *Syllis prolixa* (Appendix, Table A5).

Period 1999-2000

The n-MDS (Figure 3 C) showed sites more or less separated according to sewage-impact,

Table 2. Results of Permanova. Si: Sites, Pe: Period, Ev: Event (nested in Period). In bold significant values.

Source	fd	SS	MS	Pseudo F	P (perm)
Si	2	1.26E + 05	63186	77.208	0.001
Pe	8	2.61E + 05	32604	39.839	0.001
Ev(Pe)	9	96942	10771	13.162	0.001
SixPe	16	1.95E + 05	12186	14.89	0.001
SixEv(Pe)	18	68098	3783.2	4.6228	0.001
Res	491	4.02E + 05	818.39		
Total	544	1.19E + 06			

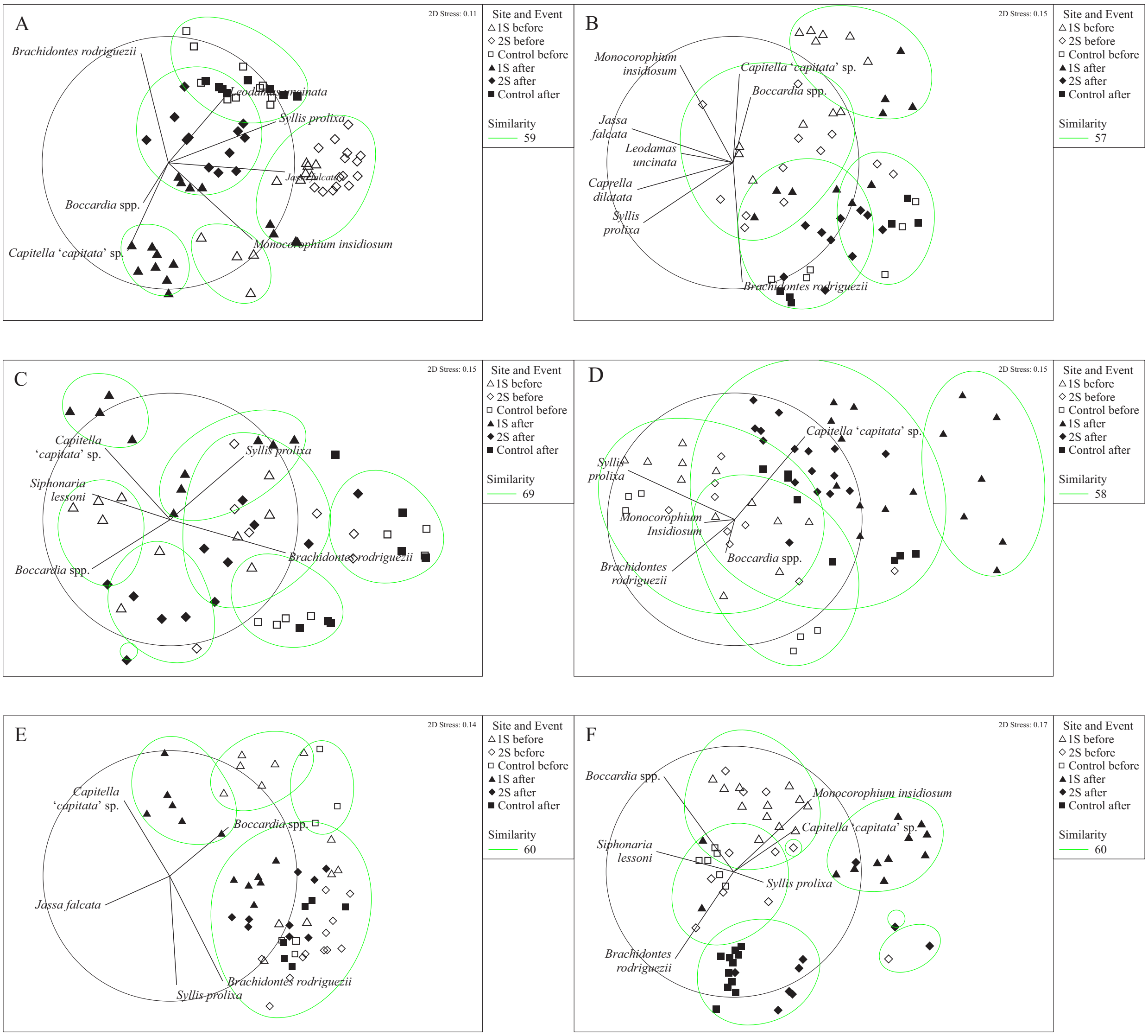

Figure 3. n-MDS showing sampling units for different periods, separated by sites (sewage-impacted, 1S and 2S, and reference site of before (white symbols) and after summer (black symbols). Green line also represents the similarity aggrupation given by the cluster analysis as before/after summer response. Black circle and vectors correspond to the spearman correlation between species (given by SIMPER) and sampling units. A) n-MDS showing sampling units of period 1997-1998 separated by sites. Green line represents the 59% similarity aggrupation. B) n-MDS of sampling units by sites in the Period 1998-1999. Green line represents the 57% similarity aggrupation. C) n-MDS in the Period 1999-2000 showing reference site and sewage-impacted sites. Green line represents the 69% similarity aggrupation. D) n-MDS showing sampling units in the period 2000-2001. Green line represents the 58% similarity aggrupation. E) n-MDS showing sampling units in the period 2001-2002. Green line represents the 60% similarity aggrupation. F) n-MDS showing sampling units in the period 2002-2003. Green line represents the 60% similarity aggrupation. G) The n-MDS by sites in the Period 2005-2006. Green line represents the 60% similarity aggrupation. H) n-MDS by sites in the period 2008-2009. Green line represents the 60% similarity aggrupation. I) n-MDS by sites in the period 2013-2014 showed two groups of sampling units; one almost avoided of intertidal life in 1S (except to a few *Boccardia proboscidea*), and the rest of sampling units. Green line represents the 60% similarity aggrupation.

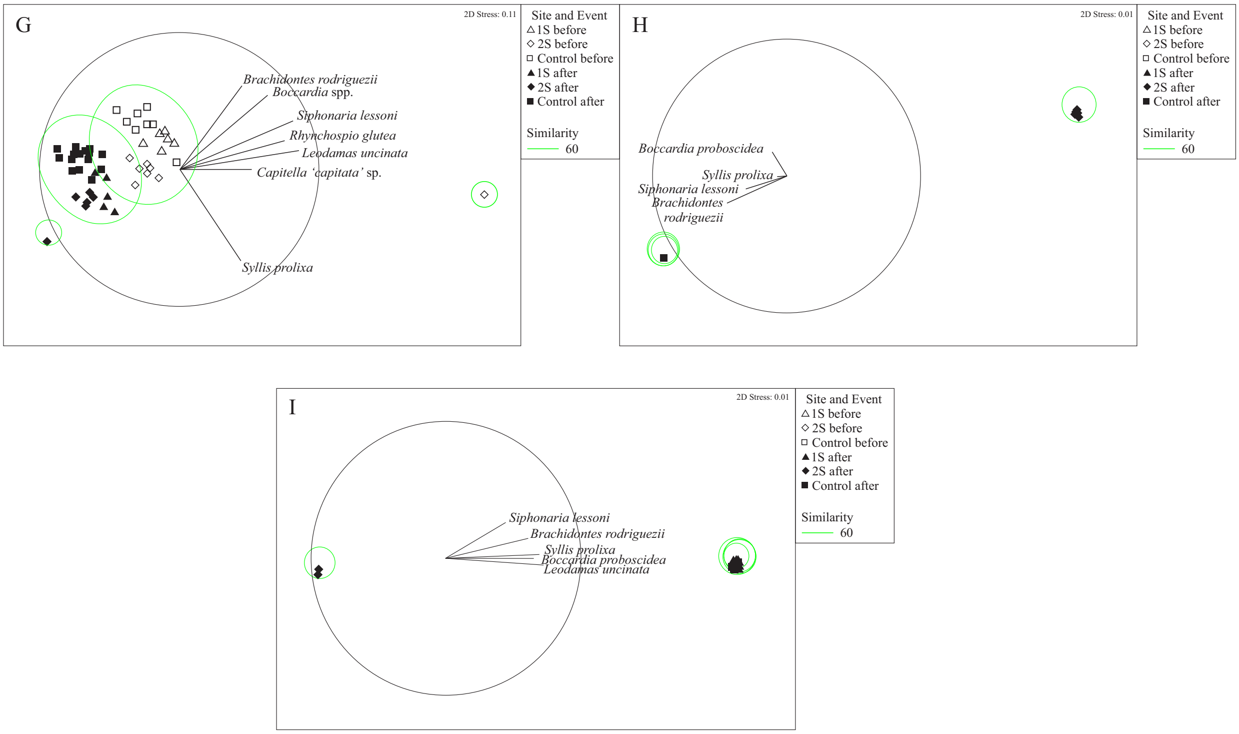

Figure 3. Continued.

except for 1S and 2S mixed, and the reference site units that are apart. The groups at the bottom of the graph represented those before summer, while the upper groups were after summer. The Simper analysis by Sites (Appendix, Table A6) showed *Brachidontes* as the main species contributing to differences among groups, with *Boccardia*, *Capitella* and *Siphonaria lessoni* Blainville, 1827 were dominant in sewage-impacted groups, while *S. prolixa* was dominant in the Reference site. In relation to factor Events most species showed decreasing values (Appendix, Table A7), except *C.* '*capitata*' sp. and *S. gracilis.*

Period 2000-2001

The n-MDS (Figure 3 D) showed a mixed pattern of sampling units, without a clear pattern about the potential gradient of sewage impact. The pattern before/after is clearly observed in the graph; sampling units before are in the left while sampling units after are in the right, related to high observed abundance of *C.* '*capitata*' sp. The Simper analysis by Sites (Appendix, Table A8) showed the polychaetes *S. prolixa* and *C.* '*capitata*' sp. as the most contributing species in sewage-impacted sites, whereas *B. rodriguezii* was in the Reference site. Respect Events most species showed decreasing values (Appendix, Table A9), but *C.* '*capitata*' sp. and *S. gracilis*.

Period 2001-2002

The n-MDS did not show a clear pattern (Figure 3 E). The pattern before/after was also unclear, although the sampling units of 1S were separated in two groups, corresponding to before and after summer, and associate to them were the polychaetes *C.* '*capitata*' sp. and *Boccardia* spp. The Simper by sites (Appendix, Table A10) revealed *B. rodriguezii* and *Caprella dilatata*

Krøyer, 1843 as dominant species in the Reference site while impacted sites are dominated by polychaetes like *Boccardia* and *Capitella*. In relation to Events most species showed decreasing values (Appendix, Table A11), except Nematode indet. and *Capitella* '*capitata*' sp.

Period 2002-2003

The n-MDS showed well-grouped sampling units, with impacted Sites in one side and Reference sites in the other (Figure 3 F). Upper two clusters represent the before summer, and the others the after summer aggrupation. Indicator species *M. insidiosum* and *C.* '*capitata*' sp. were associated with 1S site, the closest to sewage discharge. The Simper analysis (Appendix, Table A12) showed *Boccardia* spp. as the most important species in all sites, been particularly in sewage-impacted ones. *B. rodriguezii* was the second important species in the Reference site. About Events (Appendix, Table A13) several species increase their average abundances after the summer like *Mytilus platensis* d'Orbigny, 1846, *C.* '*capitata*' sp. and *M. insidiosum*, whereas other decreases.

Period 2005-2006

The n-MDS (Figure 3 G) showed a crowded pack of sampling units, more or less separated into Reference and impacted sites, except sampling units 376 and 398 (2S). Sampling unit 376 was characterized by the absence of *B. rodriguezii* and a peak of almost 4,000 individual of *Rhynchospio glutaea* (Ehlers, 1897), while 398 had low values of all species. The group at the left represent the aggrupation before summer and the other the after summer samples units. The Simper analysis by sites in the Period 2005-2006 (Appendix, Table A14) showed higher values of *Boccardia* sp. and *Rhynchospio glutaea* in 1S and 2S, respectively. In addition, *Brachidontes* was the dominant species in Reference areas. All species showed decreasing values related to Event (Appendix, Table A15).

Period 2008-2009

The n-MDS showed a pattern of sampling units ruled by the demographic explosion of the invader polychaete *B. proboscidea* (Figure 3 H) resulting that dominated species were grouped to the right, while other sampling units were in the left. The Simper by sites (Appendix, Table A16) showed the dominance of *B. proboscidea* over *B. rodriguezii* in impacted areas. About Events the species showed also the impact of the invasion of *B. proboscidea* (Appendix, Table A17).

Period 2013-2014

The n-MDS (Figure 3 I) showed two groups of sampling units. One characterized by absence of macrofaunal life (left) and the other with the rest of sampling units. The Simper by sites (Appendix, Table A18) showed site 1S avoided of the intertidal community, except a few endolithic *B. proboscidea*. This species has been described as a endolithic form (boring into the sedimentary rock), however in Argentine also develops a new types of habitat, as epilithic form, constructing tubes over the substrate. On the other hand, in the site 2S the invader polychaete is dominant over *B. rodriguezii*. In relation to Event most species showed decreasing values (Appendix, Table A19) but *Siphonaria lessoni* and *B. rodriguezii*.

Long-term trend

The n-MDS (Figure 4 A and B) produced by averaging abundances to made a single impacted or reference point (= sample) shows good stress (0.1), meaning good representation of two dimensional ordination of samples. However three Impacted samples in the upper part of the graph, far from the others due to high dissimilarity, corresponds to the high abundances of the polychaete *B. proboscidea* population (November 2008, February 2009, and November 2009). Large gap responds to the short-term effect (before/after summer, an again back to spring). The large effect of this invasive polychaete distorts the similarity

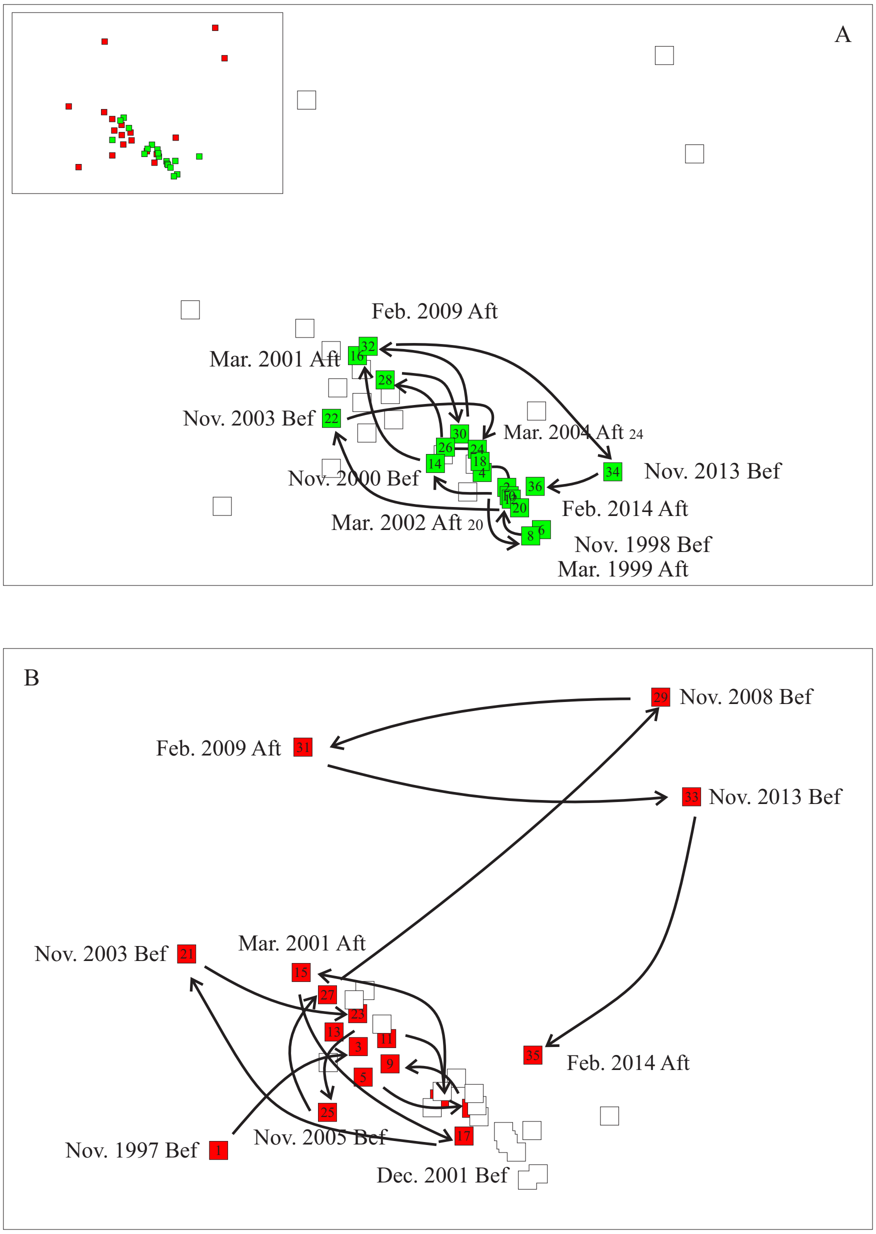

Figure 4. A) Reference site (green squares) with the general n-MDS inserted in the upper corner. B) Impacted sites (red squares). The arrows showed the drift of samples from November 1997 to March 2014. The stress is 0.1.

of the Reference and Impacted site samples. This is the reflection and consequence of the significant interactions of the Permanova that produces a confusing sampling path.

A basal group is mainly made up of samples from Reference samples (Figure 4 A), and another group of samples located slightly above the previous group mainly made up of samples from Impacted sites (Figure 4 B), both with some inserted samples from the other location.

The basal group of Reference samples (Figure 4 A) is characterized by a great abundance of *B. rodriguezii* (1.100 average abundance) and *S. prolixa* (80), while in the above group *B. rodriguezii* mean abundance is low (385) as well as *S. prolixa* (14), but *Boccardia* spp. (28).

The basal group of Impacted samples (Figure 4 B) differs from the upper group of Impacted samples in the mean abundance of *Brachidontes*, twice in basal group than upper one (767 to 332), but more close to Reference basal samples (1.100). Indicator species are more abundant in Impacted samples, like *Boccardia* spp. (27-30), *R. glutaea* (27-0), *M. insidiosum* (14-6) and *C.* '*capitata*' sp. (18-9).

Same samples were separated from these groups. For example, the first impacted sample corresponded to November 1997 (down left). It shows dominance of several crustaceans, like indicators *Jassa falcata* (302), *M. insidiosum* (169), and *Brachidontes* (216), *S. prolixa* (92), *Caprella* (52) and *S. gracilis* (29). The sample Impacted November 2003 before summer (left middle) showed a great abundance of *Boccardia* spp. (275) and low *Brachidontes* (148), and very low *Jassa* (4), *S. prolixa* (3), *Caprella* (0.1) and some *Monocorophium* (19).

The largest gaps between samples corresponded to the short-term change due to seasonal change (spring-end of summer) in Reference samples, and due to seasonal change and increasing sewage discharge in Impacted samples. Except for the gap between samples from November 2013 (before) to February 2014 (after) which in fact corresponded to the drastic reduction in the *B. proboscidea* abundance.

Community parameters

Community parameters (Richness (S), Abundance (N), Diversity (H') and Equitativity (J') were studied in the three sites (1S and 2S in the sewage-impacted area, and Reference) along the nine periods (Figure 5). Data showed highly significant differences in Reference versus sewage-impacted sites, in Periods and also interactions (Appendix, Table A20).

Mean abundance (Figure 5 A) showed the influence of the ecosystem engineer *B. rodriguezii* in the Reference Site, except in the period 2001-2002 due to the explosive increase in density of *R. glutaea* in 2S, and in the period 2008-2009 due to the demographic explosion of the invader *B. proboscidea*. On the other hand, mean richness (Figure 5 B) showed the opposite pattern, been higher in sewage-impacted sites 1S and 2S. It was observed a trend to diminish mean Richness along the studied periods, nevertheless a great increased was observed in the last one. In this last period, a peak in richness was due to the equilibrium between *B. proboscidea* and *B. rodriguezii*. Mean Diversity (Figure 5 C) and Eveness (Figure 5 D) showed a similar pattern, been higher in sewage-impacted sites rather than in Reference site, due to dominance of the ecosystem engineer in the later, and the presence of tolerant species in the first one.

The community parameters were also analyzed to Sites and Event (Figure 6). Data showed highly significant differences in Reference versus sewage-impacted sites in Event and interactions (Appendix, Table A21). All parameters showed lower values after the summer, and also a trend to decrease along the studied periods.

DISCUSSION

This was the first study in Argentina that analyzes the long-term response of the intertidal benthic community to the discharge of sewage without treatment directly to the intertidal sector. It was also the first study that analyzes the short-term response of the intertidal epilithic community before/after the summer. Due to the high seasonality of the sewage discharge, linked to the tourism peak in the summer months, the short-term variation induced by the pulse discharge was

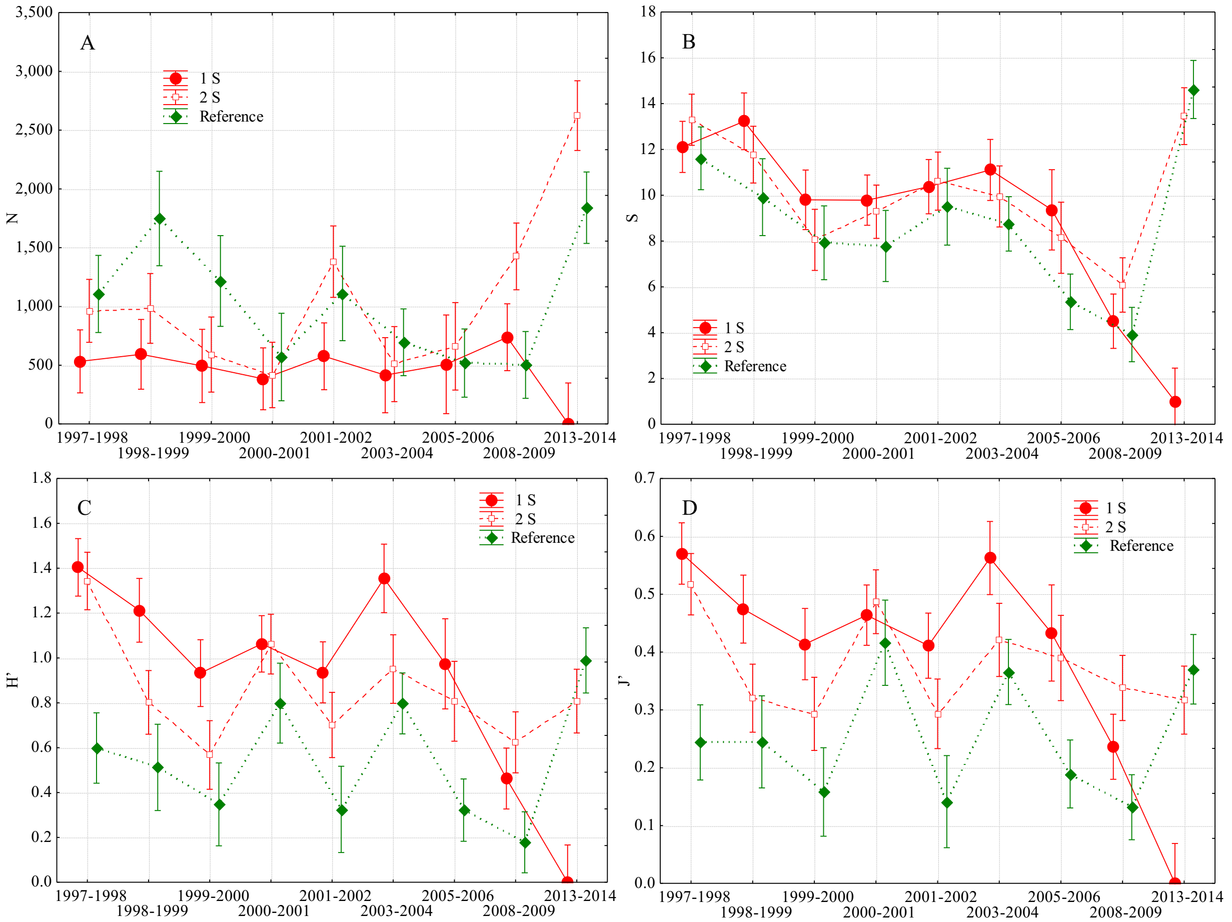

Figure 5. Mean values of Abundance (N) (A), Richness (S) (B), Diversity (H') (C), and Eveness (J') (D) in the three sites (1S, 2S in the impacted area in red lines, and Reference in green line) along the nine studied periods.

also analyzed, which in turn over-lapped with the chronic impact produced by the sewage discharge from Mar del Plata. In all cases, the study included the response of areas affected by sewers and reference areas.

There was a positive tendency for species that tolerate organic contamination to prevail in the sites affected by the sewage discharge, i.e. the polychaetes *C.* '*capitata*' sp., the classic indicator of organic enrichment, and also the now-classic *B. proboscidea* species (Pearson and Rosenberg 1978, Dean 2008).

Recently, for all Latin American and Caribbean region the indicator species belonging to the *Capitella* complex were widely mentioned and reaffirmed as an indicator polychaete, although the species identity remains to be determined in each region (Elías et al. 2021).

In some moments of the first periods *Neanthes* (= *Alitta*) *succinea* (Leuckart, 1847) was also the indicator species in agreement with classic literature (see Pearson and Rosenberg 1978; Dean 2008). In other periods the indicator species was *R. glutaea*, as well as *Boccardia* spp. (a pool of species). Firstly, the species was initially identified as *B. polybranchia* (Haswell, 1885), nevertheless through time seems to be several species. It was suspected that the invader *B. proboscidea*

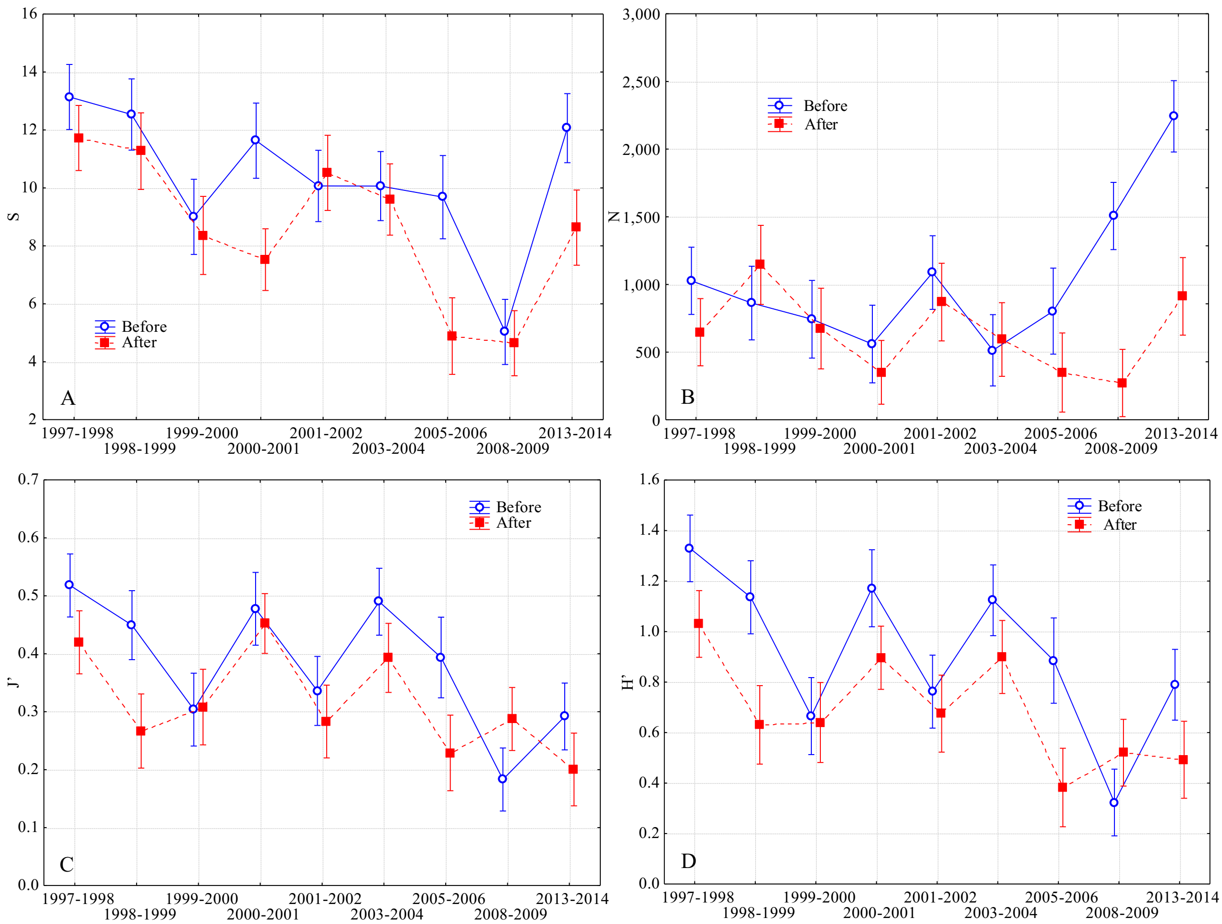

Figure 6. Mean values of Richness (S) (A), Abundance (N) (B), Eveness (J') (C), and Diversity (H') (D) in the Event before/after summer along the nine studied periods.

was present before the demographic explosion of 2008, and the present research showed that since 2006 the invader species is present as a companion species among the community.

Among the crustaceans, the tubiculous *M. insidiosum* was highly abundant in areas impacted by sewage discharge. Other crustacean indicators were also very abundant in areas located at intermediate distances from the sewage discharge (site 2S) such as *J. falcata* and *Caprella* sp. At the other extreme were the indicator species of good environmental quality, that was, sensitive or very sensitive species such as the polychaete *Leodamas tribulosus* (Ehlers, 1897), most Syllids (see below) and the flatworm *Notoplana* sp. recently detected (Cuello et al. 2017).

The Syllidae in the area initially showed a classic behavior as sensitive species (Elías et al. 2003a, 2003b). However, long-term studies (Elías et al. 2006; Sánchez et al. 2013) showed erratic behavior. In some places and on some occasions they were very abundant at intermediate distances to the sewage discharge, exhibiting a high degree of tolerance to organic contamination. This could be because these species (*S. prolixa* and *S. gracilis*) are a complex of species, similar morphologically but with a different physiologyical response. The same can be said about *N. suc-*

cinea, 'suspiciously cosmopolitan' (Sánchez et al. 2013). Probably deeper and specific taxonomic studies must show the real identity of the syllids and nereidids indicator in the SW Atlantic.

The short-term effect (before/after the summer) showed that the chronic effect produced by the sewage discharge was compounded by an aggravated effect, since all the community parameters revealed significant reductions. Also, the analysis of similarity before/after the summer showed significant reduction of the average abundances of almost all the organisms of the intertidal community but the tolerant ones, which in many cases even increased after the summer. The fact that the sewer discharge increases significantly, and that during the summer the dominant winds came from the northern sector, pushing the discharge flow to the south towards the city and the sampling sites, (Vallarino et al. 2002) had an even greater negative impact on the intertidal benthic community.

Studies in the area, including this one, made possible to detect the presence of non-native species and the explosive development of some of them, giving rise to significant changes in the structure and functioning of the intertidal community. In fact, concerning the original description of this community, more than 50 years ago (Olivier et al. 1966), structural changes were detected due to the invasion of barnacles (Vallarino and Elías 1997). Other exotic species seem not to have had significant ecological impacts on native species, although specific studies are lacking, especially of macroalgae (Becherucci et al. 2016; Palomo et al. 2016). Subsequently, the demographic explosion of the invasive polychaete *B. proboscidea* dramatically altered the structure and function of the intertidal benthic community (Jaubet et al. 2011, 2013; Garaffo et al. 2012; Jaubet 2013; Elías et al. 2015; Llanos et al. 2019).

As a first result, a series of changes were observed in the arrangement of dominant species. One of the indicator species in the sewage-impacted area were *Boccardia* spp. until year 2008. Since this year, the invader *B. proboscidea* exploded and monoculture biogenic reefs were built with extraordinary densities (Jaubet 2013; Jaubet et al. 2013). At the time no attempt to separate species was carried out, but it is suspected that the *B. proboscidea* was present with other species of the genus before the development of the reefs (2021 pers. comm. L Jaubet). Our research revealed the presence of the invader in March 2006. Most of the observed changes in the intertidal community in those periods were associated to the invasion of *B. proboscidea*, like mean values of Abundance, Richness, Diversity, and Evenness (see Elías et al. 2015). No other place in the world suffers large negative effects due to the invasion of this polychaete. In Australia and New Zealand the species has been declared 'pest' although densities are quite lower (one order of magnitude lesser respect our study area) (Hayes et al. 2005; Bradstock 2015).

The bivalve *B. rodriguezii* is the structuring organisms in the intertidal community because it provides shelter from waves and desiccation, and food for several associated species, i.e. an ecosystem engineer. On the other hand, the invasive polychaete *B. proboscidea* also build a tridimensional structure but for itself, been considered an auto-ecosystem engineer (Jaubet et al. 2013). However, *B. rodriguezii* had an unsuspected behavior about sewage-impact. Although it is the common and dominant organism of the natural community, it also shows a high level of tolerance to organic pollution. Its abundance oscillates in areas affected by the sewage discharge. In previous studies, Vallarino et al. (2002) indicated that *B. rodriguezii* was present even within 50 m from the sewage discharge in a pauperized community. However, at high and intermediate levels of organic contamination (i.e. at the sites closest to the sewage discharge, 1S and 2S) this species was competitively overcome by *B. proboscidea* (Jaubet et al. 2013; Elías et al. 2015; Llanos 2017; Llanos et al. 2018). Due to this behavior (tolerance) the calculation of ecological quality indexes was carried out with/without this mussel, been more representative the index without mussel abundance (Garaffo et al. 2017).

The organic matter underwent a jump-starting from 2005. Results from Jaubet et al. (2013) showed that from the year 2005 the value of the organic matter in the interstitial sediment of the bivalves doubled. This was probably due to the by-pass of the pre-treatment plant for sewage effluents. The maintenance of this plant (and therefore bypass) occurred twice a year, severely affecting benthic communities (Elías et al. 2009). The pre-treatment plant retained between 25 and 30 t per day of sewage sludge (Scagliola et al. 2011). The untreated wastes were released to the marine environment during bypass, aggravating the impact of the sewage on the ecosystem. It was possible that the observed increase of organic matter in the sediments could be the product of larger discharges due to the bypass.

Long-term trend

In the long term, the abundances of two species drive the changes in the epilithic intertidal community. In one hand the ecosystem engineer, *B. rodriguezii*, and in the other hand the ecosystem self-engineer, the polychaete *B. proboscidea*. While the former constructs a three-dimensional structure, which protrudes from the seafloor and allows other species to live among themselves and in the matrix they form (the definition of ecosystem engineer), the other constructs the same, but only *B. proboscidae* could live in their matrix acting as self-engineer ecosystem (Jaubet et al. 2013). In the Reference areas, the accompanying species were a few and were subject to seasonal changes, while the species of Impacted areas are mostly opportunistic or tolerant and change according to the discharge flow of the sewer of the Mar del Plata city.

In this same community, a study more limited in time but including seasonal analyzes (Elías and Vallarino 2006) showed that the great changes in similarity occur between spring-summer in impacted areas, but between winter-spring in reference areas. These changes were associated to seasonality but to sewage-induced stress in sites close to discharge. The Control site behaved in a cyclic way and in counter clock wise, but the impacted stations showed no clear pattern.

The decreasing in 'defender species' allowed the introduction of exotic species that finally become invasive species. An altered environment creates a potentially favorable environment for the establishment of introduced or non-indigenous species (Dukes and Mooney 1999). Non-indigenous species are favored in places and at times when stress is negatively affecting native flora and fauna, resulting in vacant niches available for colonization (Occhipinti-Ambrogi and Savini 2003; Piola and Johnson 2008). When several environmental quality indices were calculated, it was necessary to exclude *B. rodriguezii* from these calculations, since it induced the homogenization of sites of high index value with sites with low values (Garaffo et al. 2017).

During the 2013-2014 period, the presence of the polychaete *B. proboscidea* was observed in greater abundance at the site near the point of discharge of the sewage effluent (site 2S) but without generating the impressive reefs recorded during the years 2008-2009. However, after the demographic explosion, the population of *B. proboscidea* decreased and did not completely displace *B. rodriguezii* near the sewage discharge, giving a coexistence of both species. In this way, being the organic pollution lesser, it may on the one hand have favored the growth and development of *B. rodriguezii*. On the other hand, the smaller amount of food available for *B. proboscidea* (a polychaete that feeds by filtration or facultative by superficial deposit), would tend to diminish the reproduction rates and densities previously registered. This 'balance' could be the reason why these two species, an ecosystem engineer and the other an invasive polychaete, could coexist in this environment. Because this, the community has been described as an example of the 'bloom and bust' dynamics (Strayer et al. 2017) since the bloom of the invading *B. proboscidea* was followed by a decrease in the abundance of this polychaete (the bust) and the coexistence with the ecosystem engineer *B. rodriguezii* in areas still affected by sewage discharges (Llanos et al. 2021). Although the values of total organic matter decreased since 2008-2009, they were still almost four times higher than those recorded by Vallarino (2002) that ranged between 1 and 0.4% in site 1. This decrease in the amount of organic matter allowed *B. rodriguezii* to coexist with *B. proboscidea* because *B. rodriguezii* can tolerate average concentrations of organic matter (Vallarino et al. 2002; Vallarino and Elías 2006).

Regulation trend

What is the underlying mechanism that explains the community structure and dynamics of intertidal epilithic mussel beds? The community was described as lacking the barnacle belt, as well as lygiid isopods, littorinids snails, echinoderms and a top predatory (Olivier et al. 1966; Adami et al. 2004, 2008; Bertness et al. 2006; Hidalgo et al. 2007). Most macrophytes were seasonal, and their cycles do not significantly affect the availability of the substrate and there are also no herbivores that significantly regulate the algal cover as shown Penchaszadeh (1973). Space competition has being pointed out as the major biological structuring force in the 2-4 years period, while later successional stages were characterized by space monopolization (Nugent 1986). The regulation and stability of the intertidal community of the Mar del Plata rocky shore is also influenced by the degree and frequency of disturbance, as well as by the population dynamics of both mussels and introduced barnacles (Vallarino and Elías 1997). Therefore the top-down mechanism could not be the prevalent one. Is it a bottom-up effect?

The particulate matter from sewage discharge could be an extra supply, a bottom-up factor, but their effect is limited to the influence of the flume. Vallarino and Elías (2006) and the present work has shown great changes in sewage-impacted sites due to the short term effect before/after the summer when sewage discharge increased 60% and wind flow from the north. On the other hand, the detritus supply from subtidal macrophytes could be a structuring factor, but it was only present near the study site because there are hard substrates in the subtidal around Mar del Plata city. However, the extended distribution of *Brachidontes* beds were far away from detritus supply, because in all the distribution there were not extended subtidal macrophytes due to sand bottoms. Nevertheless, *Perumytilus* beds are distributed in southern cold-temperate regions (Patagonia), where submersed macrophytes are abundant. In here, macrophytes detritus would be present (but it was not quantified), but top-drown forces (herbivory) are weak, due to environ-mental hardness because the evaporation pressure induced by the wind and the dryness of the air (see Bertness et al. 2006; Hildalgo et al. 2007). In South Africa, Bustamante and Branch (1996) observed that the *in situ* productivity was insufficient to support the high biomass of filter-feeders on exposed shores, suggesting dependence on external subsidies, and isotope analyses showed 60-85% of the food of filter-feeders came from particulate subtidal kelp.

Bottom-up processes can have important effects on rocky intertidal community structure. Oceanographic effects could generate large ecological variability in the basal levels, increasing the input of phytoplankton, detritus, and/or larvae, and through upward-flowing food chain effects, lead to variation in top-down trophic effects. Under these conditions the invertebrates can dominate the structure and dynamics of rocky intertidal communities (Menge 2000). In this context, the continuous recruitment of *Brachidontes* (Torroglosa 2015) could be a bottom-up factor, as mentioned by Menge (2000). Other bottom-up effect was observed by Montoya et al. (2021) by showing high concentrations of Cl*a* in the period studied for the two localities near the study area (Santa Teresita and Villa Gesell), varies from 4.71 to 65.67 $\mu g\ l^{-1}$ and from 0.10 to 33.73 $\mu g\ l^{-1}$ (respectively), due to high input of nutrients in the Buenos Aires intertidal region (although with marked temporal variability).

The dominant covered of mussels mono-cultures suggests that predators did not have a strong impact in the mid intertidal, perhaps due to their small size or due to the phylogeographic history of the Patagonian region (Hidalgo et al. 2007). As a general phenomenon, physical factors appear been the dominating structuring force in these communities and were likely to be evolutionarily and ecologically responsible for weakening the effects of consumers (Bertness et al. 2006).

Ultimately the changes that take place in the intertidal community of impacted areas are changes within the same community, in response to organic contamination induced by the sewage discharge. This basically corresponds to what is stated by Pearson and Rosenberg (1978). The intertidal epilithic community dominated by the mussel *B. rodriguezii* shown a great capacity to absorb a disturbance, and back to revert to a pre-disturbance situation, to finally reach again the initial state. This mean resistance, recovery and reversibility, was defined as ecological resilience (see review by Gollner et al. 2017).

Although the sewage discharge of the Mar del Plata city no longer occurs in the coastal zone, studies of the intertidal community are still valid. Although Mar del Plata has a submarine outfall functionally since the beginning of 2014, practically all of Argentina's coastal towns discharge their sewage without treatment directly into the coastal marine environment. This situation should stopped as soon as possible, and for this, this study and the preceding ones will be of great value to evaluate the environmental impact and take eventual mitigation measures. As previously mentioned, both in Europe and in the United States, guidelines were drafted to improve the quality of recreational waters and monitor and evaluate their quality (Water Framework Directive and the Clean Water Act, respectively). The Republic of Argentina and eventually all Latin America countries should direct its attention to the environmental quality of its waters and draft its water act.

We do not predict how climate change, sea level, and acidification could affect intertidal rocky shores in the SW Atlantic. Community assemblages are expected to change in response to ocean acidification because of relative shifts in abundance between ecological winners and losers (Fabry et al. 2008). This work could be a baseline study of how the benthic epilithic community responds in both sewage-impacted and reference sites during a long term time period.

CONCLUSIONS

- The intertidal benthic community structure response to natural changes at non-impacted sites and to changes induced by organic pollution at sewage discharge sites.
- The structural changes in the community parameters and in the multivariate abundance of the benthic species showed significant changes in both the spatial and the temporal scale.
- The temporal change was reflected in both the short-term (before/after the summer) and the long-term (15 years from November 1997 to March 2014). Unexpected quickly benthic response occurs between spring and summer (three months) in relation to sewage increase and wind direction.
- Spatially the areas affected by sewage discharges showed the ecosystem engineer *B. rodriguezii* pauperized, as well as all the sensitive species, while tolerant and opportunistic

species increased, as a consequence the richness and diversity were high respect natural-low diversity areas.

- Indicator species change from period to period, tolerant/opportunistic polychaete species were *C.* '*capitata*' sp., *A. succinea*, *R. glutaea*, *Boccardia* spp., and *B. proboscidea*; while among the crustaceans were *Monocorophium insidiosum* and *J. falcata*; sensitive species were *Nemerteans*, *Syllids*, *L. tribulosus*, and recently the flatworm *Notoplana* sp.
- The abundance of organic matter in the sediments trapped by the bivalve matrix showed increasing values near the sewage discharge, and a temporal pattern of increasing values throughout the period studied, resulting in increasing environmental degradation.
- The major impact to the community was the bloom of the invader polychaete *B. proboscidea*. This community has been described as an example of the 'bloom and bust' dynamics since the bloom of the invading *B. proboscidea* was followed by a decrease in the abundance of this polychaete (the bust) and the coexistence with the ecosystem engineer *B. rodriguezii*.
- The intertidal community dominated by the mussel *B. rodriguezii* shows resistance, recovery and reversibility, i.e. resilience.

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APPENDIX

Table A1. Ranking of dominance (abundance/total abundance) and frequency (%) of all recorded species in the period 1997-2014. MOL: Mollusca, POL: Polychaeta. CRU: Crustacea, NMER: Nemertean, NEMA: Nematoda, QUIR: Quironomidae (Insecta), TUR: Turbelaria, OLIG: Oligochaeta, TAN: Tanaidacea (CRU), ANEM: Anemone, PIC: Picnogonida, SIPUN: Sipuncula, NUD: Nudibranchia (MOL), ASC: Ascidea, INS: Insecta, PRIA: Priapulida, OPHIU: Ophiura (Echinoderma).

Species	Group	Dominance	Frequency (%)
Brachidontes rodriguezii	MOL	57.08	84.6
Boccardia proboscidea	POL	19.25	15.4
Syllis prolixa	POL	4.50	70.9
Boccardia spp.	POL	3.27	47.2
Jassa falcata	CRU	2.55	33.1
Monocorophium insidiosum	CRU	2.09	32.0
Rhynchospio glutaea	POL	2.02	18.1
Siphonaria lessoni	MOL	1.38	59.4
Leodamas tribulosus	POL	1.36	43.5
Syllis gracilis	POL	1.11	76.2
Capitella ‘*capitata*’ sp.	POL	1.05	34.0
Caprella dilatata	CRU	0.81	19.9
Mytilus platensis d’Orbigny, 1842	MOL	0.72	51.2
Nemertina indet.	NMER	0.42	38.4
Nematode indet.	NEMA	0.39	23.8
Hyale grandicornis (Krøyer, 1845)	CRU	0.28	28.7
Quironomidae indet.	QUIR	0.22	16.6
Protocirrineris angelicollatio Elías y Rivero, 2009	POL	0.16	21.8
Caulleriella bremecae Elías y Rivero, 2009	POL	0.15	12.4
Idotea balthica (Pallas, 1772)	CRU	0.14	13.3
Balanus sp.	CRU	0.13	12.2
Alitta succinea	POL	0.11	25.4
Sphaeroma serratum (Fabricius, 1787)	CRU	0.09	13.3
Copepoda sp. 1	CRU	0.07	2.2
Halicarcinus planatus (Fabricius, 1775)	CRU	0.04	4.2
Lineus bonaerensis Moretto, 1971	NMER	0.04	5.5
Nereididae indet.	POL	0.04	5.3
Notoplana sp.	TUR	0.03	4.6
Spionidae indet. 1	POL	0.03	1.8
Pachycheles haigae Rodrigues da Costa, 1960	CRU	0.02	2.2
Zoeas	CRU	0.02	6.2

Table A1. Continued.

Species	Group	Dominance	Frequency (%)
Barnea lamelosa (d'Orbigny, 1841)	MOL	0.02	9.1
Copepodo harpac. indet.	CRU	0.02	5.9
Polydora sp.	POL	0.02	2.2
Lyonsia sp.	MOL	0.01	2.9
Capitellidae indet.	POL	0.01	2.9
Polychaeta indet. 2	POL	0.01	3.1
Lumbrinelidae indet.	POL	0.01	3.1
Oligochaeta sp. 4	OLIG	0.01	2.9
Oligochaeta sp. 1	OLIG	0.01	1.8
Lumbrineris tetraura (Schmarda, 1861)	POL	0.01	5.1
Polychaeta indet. 1	POL	0.001	4.2
Cyrtograpsus affinis (Dana, 1851)	CRU	0.001	2.6
Hemigrapsus crenulatus (H. Milne Edwards, 1837)	MOL	0.001	4.9
Cyrtograpsus angulatus Dana, 1851	CRU	0.001	2.9
Dodecaceria meridiana Elías y Rivero, 2009	POL	0.001	1.6
Tanaidacea	TAN	0.001	2.0
Syllis sp.	POL	0.001	3.1
Stenothoe sp.	CRU	< 0.001	1.6
Polynoidae indet.	POL	< 0.001	2.9
Erichtonius brasiliensis Dana, 1853	CRU	< 0.001	0.5
Joeropsis sp.	CRU	0.004	2.0
Syllidae indet.	POL	< 0.001	0.9
Anemona indet. 1	ANEM	< 0.001	2.2
Phyllodocidae indet.	POL	< 0.001	0.7
Phyllodoce sp.	POL	< 0.001	2.0
Isopoda Valvifera	CRU	< 0.001	0.7
Cyrtograpsus altimanus Rathbun, 1914	CRU	< 0.001	0.5
Isopoda indet.	CRU	< 0.001	0.9
Oligochaeta sp. 2	OLIG	< 0.001	0.9
Polychaeta sp. 3	POL	< 0.001	1.5
Bivalvia indet. 1	MOL	< 0.001	1.5
Pignogonida indet.	PIC	< 0.001	1.1
Sipunculida indet.	SIPUN	< 0.001	0.9
Anphipoda indet.	CRU	< 0.001	0.7
Nudibranchia indet.	NUD	< 0.001	0.7
Ascidea indet.	ASC	< 0.001	0.4
Insecta indet.	INS	< 0.001	0.7
Priapulida indet.	PRIA	< 0.001	0.5

Table A1. Continued.

Species	Group	Dominance	Frequency (%)
Pachycheles laevidactylus Ortmann, 1892	CRU	< 0.001	0.2
Isopoda Flabellifera	CRU	< 0.001	0.5
Crepidula sp.	MOL	< 0.001	0.7
Hesionidae indet.	POL	< 0.001	0.2
Polychaeta indet.	POL	< 0.001	0.5
Oligochaeta sp. 3	OLIG	< 0.001	0.5
Bivalvia indet. 2	MOL	< 0.001	0.5
Halosydnella australis (Kinberg, 1856)	POL	< 0.001	0.4
Nemertina sp. 2	NMER	< 0.001	0.4
Glycera americana Leidy, 1855	POL	< 0.001	0.4
Spionidae indet. 3	POL	< 0.001	0.2
Oeonidae indet.	POL	< 0.001	0.4
Hyperida indet.	CRU	< 0.001	0.2
Heteromastus similis Southern, 1921	POL	< 0.001	0.2
Corbula sp.	MOL	< 0.001	0.4
Anemona indet. 2	ANEM	< 0.001	0.4
Elasmopus marplatensis Alonso de Pina, 1997	CRU	< 0.001	0.5
Ostracoda indet.	CRU	< 0.001	0.2
Crysopetallidae indet.	POL	< 0.001	0.2
Terebellidae indet.	POL	< 0.001	0.2
Sabellaridae indet.	POL	< 0.001	0.2
Onuphidae indet.	POL	< 0.001	0.2
Ophiuroidea indet.	OPHIU	< 0.001	0.2
Lumbrineriopsis mucronata (Ehlers, 1908)	POL	< 0.001	0.2
Acari	INS	< 0.001	0.2

Table A2. Species that most contributed to the differences among Sites in the Period 1997-1998. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before, but the order follows the first comparison.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Jassa falcata	5.54	12.43	15.25	2.73	15.38
Monocorophium insidiosum	9.23	9.95	10.35	1.06	13.14
Brachidontes rodriguezii	14.14	17.95	9.61	30.66	19.42
Syllis prolixa	4.08	6.94	8.15	6.99	7.5
Caprella dilatata	0.3	5.31	7.09	1.35	7.06
Capitella '*capitata*' sp.	4.12	0.21	6.54	0	6.55
Nemertina indet.	0.33	3.23	5	1.38	4.35
Syllis gracilis	2.24	4.97	4.89	4.14	3.63
Leodamas tribulosus	0.55	1.95	2.61	2.86	3.4

Table A3. Species that most contributed to the differences between before/after summer in the Period 1997-1998.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Jassa falcata	12.74	2.1	15.88
Brachidontes rodriguezii	19.35	20.05	14.05
Monocorophium insidiosum	9.53	5.38	11.39
Syllis prolixa	7.95	3.82	8.32
Caprella dilatata	4.42	0.46	5.84
Capitella '*capitata*' sp.	0.71	2.53	4.37
Syllis gracilis	4.66	2.82	4.22

Table A4. Species that most contributed to the differences among Sites in the Period 1998-1999. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Brachidontes rodriguezii	393.68	774.82	57.69	1475	68.26
Boccardia spp.	36	37.23	7.85	39.08	5.99
Syllis prolixa	27.36	47.64	6.28	128	10.12
Caprella dilatata	6	36.73	4.78	8.85	3.61
Capitella '*capitata*' sp.	19.55	2.95	2.91	0	0
Syllis gracilis	26.69	3.77	1.59	26.69	2.09

Table A5. Species that most contributed to the differences between before/after summer in the Period 1998-1999.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Brachidontes rodriguezii	22.82	30.09	21
Syllis prolixa	4.95	5.78	9.46
Boccardia spp.	5.46	2.93	8.7
Leodamas tribulosus	3.1	0.34	4.97
Jassa falcata	3.09	0.56	4.84
Monocorophium insidiosum	2.97	1.13	4.69
Caprella dilatata	2.71	0.79	4.5
Capitella '*capitata*' sp.	1.88	1.65	4.4

Table A6. Species that most contributed to the differences among Sites in the Period 1999-2000. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Brachidontes rodriguezii	18.00	21.67	17.11	32.5	31.23
Boccardia spp.	4.52	4.08	15.17	1.56	8.16
Capitella '*capitata*' sp.	5.6	1.22	14.49	0	11.61
Siphonaria lessoni	4.67	2.15	10.28	1.8	3.67
Syllis prolixa	3.15	3.75	10.24	7.35	14.46
Leodamas tribulosus	0.78	1.8	5.37	0.5	1.9

Table A7. Species that most contributed to the differences between before/after summer in the Period 1999-2000.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Syllis prolixa	8.25	5.33	12.97
Brachidontes rodriguezii	18.12	14.5	11.78
Capitella '*capitata*' sp.	0.04	3.91	8.65
Rhynchospio glutaea	3.35	0	7.01
Leodamas tribulosus	2.68	0.08	5.97
Syllis gracilis	3	3.51	5.64
Jassa falcata	2.74	0.03	5.5
Boccardia spp.	1.98	1.12	5.46

Table A8. Species that most contributed to the differences among Sites in the Period 2000-2001. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Syllis prolixa	5.56	7.76	14.69	6.17	14.15
Capitella '*capitata*' sp.	4.32	1.45	11.94	0	11
Brachidontes rodriguezii	14.06	15.93	10.16	19.82	15.71
Syllis gracilis	2.35	4.54	7.75	3	6.17
Siphonaria lessoni	1.91	2.31	6.73	2.28	4.59
Boccardia spp.	1.8	1.52	6.31	0.69	4.47
Rhynchospio glutaea	1.19	1.42	5.2	1.55	4.11
Jassa falcata	1.72	0.46	4.24	1.1	4.25

Table A9. Species that most contributed to the differences between before/after summer in the Period 2000-2001.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Syllis prolixa	8.25	5.33	12.97
Brachidontes rodriguezii	18.12	14.5	11.78
Capitella '*capitata*' sp.	0.04	3.9	8.65
Rhynchospio glutaea	3.35	0	7.01
Leodamas tribulosus	2.68	0.08	5.97
Syllis gracilis	3	3.51	5.64
Jassa falcata	2.74	0.03	5.5
Boccardia spp.	1.98	1.12	5.46

Table A10. Species that most contributed to the differences among Sites in the Period 2001-2002. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Brachidontes rodriguezii	19.33	32.69	25.34	30.92	28.18
Syllis prolixa	3.41	8.81	13.21	5	10.25
Boccardia spp.	4.23	4.25	8.95	0.84	8.15
Jassa falcata	3.85	3.95	7.56	1.22	6.49
Caprella dilatata	0.81	3.35	5.09	1.8	3.48
Capitella '*capitata*' sp.	3.15	0.77	4.91	0.08	6.74
Leodamas tribulosus	0.64	2.81	4.79	0.74	2.15

Table A11. Species that most contributed to the differences between before/after summer in the Period 2001-2002.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Brachidontes rodriguezii	27.31	26.00	21.51
Syllis prolixa	7.36	3.92	12.59
Boccardia spp.	4.54	2.39	9.27
Jassa falcata	3.55	3.09	7.45
Nematode indet.	1.24	2.95	5.07
Leodamas tribulosus	2.78	0	4.97
Caprella dilatata	2.48	1.37	4.78
Capitella '*capitata*' sp.	1.45	1.82	4.65

Table A12. Species that most contributed to the differences among Sites in the Period 2002-2003. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Boccardia spp.	9.14	5.96	19.36	5.71	16.13
Brachidontes rodriguezii	13.11	16.3	12.5	22.25	18.66
Monocorophium insidiosum	4.99	0.93	9.68	0.08	9.71
Mytilus platensis	1.91	3.13	6.15	0.14	3.62
Syllis prolixa	1.22	3.28	5.62	0.45	3.03
Siphonaria lessoni	3.34	2.12	5.32	5.19	6.17
Capitella '*capitata*' sp.	2.38	0.55	5.11	0.08	4.7

Table A13. Species that most contributed to the differences between before/after summer in the Period 2002-2003.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Boccardia spp.	12.12	1.83	21.46
Brachidontes rodriguezii	13.67	19.03	14.86
Monocorophium insidiosum	1.87	3.11	6.9
Siphonaria lessoni	4.16	2.3	5.94
Leodamas tribulosus	2.98	0.36	5.62
Mytilus platensis	1.87	2.12	5.12
Syllis prolixa	0.96	2.77	4.65
Capitella '*capitata*' sp.	0.46	2.07	4.2

Table A14. Species that most contributed to the differences among Sites in the Period 2005-2006. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Boccardia spp.	7.14	1.24	16.74	3.23	16.67
Brachidontes rodriguezii	18.64	15.62	11.03	21.07	13.2
Rhynchospio glutaea	1.36	4.89	10.44	0.22	3.94
Leodamas tribulosus	2.5	4.16	10.33	0.96	7.59
Siphonaria lessoni	3.63	2.32	9.06	1.58	10.38
Capitella ‘*capitata*’ sp.	3.41	0.49	7.84	0.36	9.51
Syllis prolixa	3.08	4.99	6.33	0.8	8

Table A15. Species that most contributed to the differences between before/after summer in the Period 2005-2006.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Brachidontes rodriguezii	20.49	17.59	14.51
Boccardia spp.	6.11	1.38	13.9
Siphonaria lessoni	4.8	0.12	12.58
Leodamas tribulosus	4.91	0	12.53
Rhynchospio glutaea	4.02	0	7.82
Syllis prolixa	3.26	1.94	7.42

Table A16. Species that most contributed to the differences among Sites in the Period 2008-2009. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Boccardia proboscidea	16.75	28.59	52.18	1.5	25.11
Brachidontes rodriguezii	6.89	9.51	19.85	21.3	50.61
Syllis prolixa	2.15	1.26	4.4	0.48	3.86
Siphonaria lessoni	0.6	1.78	4.38	1.28	3.41

Table A17. Species that most contributed to the differences between before/after summer in the Period 2008-2009.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Boccardia proboscidea	24.55	6.67	44.81
Brachidontes rodriguezii	14.01	11.12	29.21
Siphonaria lessoni	1.18	1.26	3.85
Syllis prolixa	1.12	1.47	3.79

Table A18. Species that most contributed to the differences among Sites in the Period 2013-2014. The species are given in the order shown by the Simper analysis in the comparison between sewage-impacted sites, 1S and 2S with their contribution percentage. Reference site was added before.

	1S	2S		Reference	
Species	Average abundance	Average abundance	Contrib%	Average abundance	Contrib%
Boccardia proboscidea	0.1	33.12	30.56	9.26	10.06
Brachidontes rodriguezii	0	22.92	29.59	33.45	37.86
Syllis prolixa	0	5.6	6.67	3.61	3.52
Leodamas tribulosus	0	6.91	6.62	5.45	4.94
Siphonaria lessoni	0	2.84	3.89	8.08	9.86

Table A19. Species that most contributed to the differences between before/after summer in the Period 2013-2014.

	Before	After	
Species	Average abundance	Average abundance	Contrib%
Brachidontes rodriguezii	19.59	21.47	24.67
Boccardia proboscidea	22.45	7.64	22.77
Leodamas tribulosus	8.07	0.31	8.09
Siphonaria lessoni	3.56	4.37	6.38
Rhynchospio glutaea	5.86	0.11	5.43
Syllis prolixa	3.82	2.85	4.72

Table A20. Results of ANOVA between Sites and Periods. In all cases there were highly significant differences between factors and interactions.

Effect	SS	df	MS	F	p
Eveness					
Site	1.934	2	0.967	49.11	0.00*
Period	4.113	8	0.514	26.1	0.00*
Site*Period	3.825	16	0.239	12.14	0.00*
Diversity					
Site	13.23	2	6.615	57.55	0.00*
Period	27.87	8	3.484	30.31	0.00*
Site*Period	25.02	16	1.564	13.61	0.00*
Richness					
Site	157	2	78.7	8.99	0.000*
Period	2589	8	323.6	36.98	0.000*
Site*Period	2323	16	145.2	16.59	0.000*
Abundance					
Site	3.88E + 07	2	1.94E + 07	38.62	0.000*
Period	4.80E + 07	8	6.00E + 06	11.94	0.000*
Site*Period	6.84E + 07	16	4.28E + 06	8.52	0.000*

Table A21. Results of ANOVA between Event and Periods. In all cases, there were highly significant differences between factors and interactions.

Effect	SS	df	MS	F	p
Eveness					
Event	0.596	1	0.596	21.6	0.000*
Period	4.231	8	0.529	19.17	0.000*
Event*Period	0.959	8	0.12	4.34	0.000*
Diversity					
Event	6.69	1	6.692	41.13	0.000*
Period	31.67	8	3.959	24.34	0.000*
Event*Period	6.21	8	0.776	4.77	0.000*
Richness					
Event	423	1	423.2	35.94	0.000*
Period	2861	8	357.6	30.37	0.000*
Event*Period	391	8	48.8	4.15	0.000*
Abundance					
Event	2.05E + 07	1	2.05E + 07	35.56	0.000*
Period	5.34E + 07	8	6.68E + 06	11.6	0.000*
Event*Period	3.77E + 07	8	4.72E + 06	8.2	0.000*

ORIGINAL RESEARCH

An experimental study on ghost fishing in rocky coastal reefs in southern Brazil

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ABSTRACT. A ghost fishing experiment was conducted using gillnets in a rocky reef off the state of Santa Catarina, southern Brazil. Scuba divers monitored changes in the structure of the nets and catches for 92 days. One hundred and twenty-six entangled animals were observed, including target and non-target fishing species: 13 teleosts (N = 52; 43%) and four crustaceans (N = 74; 57%). The crab *Menippe nodifrons* was the most frequently entangled species (N = 36; 28%). Entanglement rates decreased over time following a logarithmic model for fishes and crustaceans, and an exponential model for both taxa combined, attributed to the degradation, and tangling of the nets and biofouling. The area of the net decreased linearly over time, collapsing after 92 days. This study provides the first experimental evaluation of the impacts of ghost fishing caused by gillnets in Brazilian rocky reefs.

Key words: Derelict gillnets, entangled, marine litter.

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Estudio experimental sobre pesca fantasma en arrecifes costeros rocosos del sur de Brasil

RESUMEN. Se realizó un experimento de pesca fantasma utilizando redes de enmalle en un arrecife rocoso frente al estado de Santa Catarina, en el sur de Brasil. Los buzos monitorearon los cambios en la estructura de las redes y las capturas durante 92 días. Se observaron ciento veintiséis animales enmallados, entre especies de pesca objetivo y no objetivo: 13 teleósteos (N = 52; 43%) y cuatro crustáceos (N = 74; 57%). El cangrejo *Menippe nodifrons* fue la especie más frecuentemente enmallada (N = 36; 28%). Las tasas de enmalle disminuyeron con el tiempo siguiendo un modelo logarítmico para peces y crustáceos, y un modelo exponencial para ambos taxones combinados, atribuidos a la degradación y enredo de las redes y bioincrustaciones. El área de la red disminuyó linealmente con el tiempo, la cual colapsó después de 92 días. Este estudio proporciona la primera evaluación experimental de los impactos de la pesca fantasma causada por redes de enmalle en los arrecifes rocosos de Brasil.

Palabras clave: Redes de enmalle abandonadas, enmallado, basura marina.

INTRODUCTION

Reports of abandoned, lost, or otherwise discarded fishing gears (ALDFG), also called derelict fishing gears, first appeared in the scientific literature in the early 1970s (Smolowitz 1978). However, it was not until the 1990s that this issue was recognized as an emerging threat (Shomura and Yoshida 1985; Goldberg 1995). Fishing, and derelict gears, have direct and indirect impacts on coastal and marine ecosystems (Macfadyen et al. 2009). The inappropriate disposal of solid waste indirectly damages aquatic populations, leading to economic losses to the fishing activity (Dayton et al. 1995). Hundreds of marine species have been affected by fishing gear entanglement and ingestion (NOAA 2014). Weather, operational fishing factors and gear conflicts are probably the most significant aspects causing the loss or discard of fishing gears at sea (Macfadyen et al. 2009), where they can entangle, trap, or kill fishes and other aquatic animals, a phenomenon called 'ghost fishing' (Kura et al. 2004).

Commercial and non-commercial species of fishes and crustaceans, birds, marine mammals, and turtles are affected by ghost fishing around the world (Matsuoka et al. 2005; Brown and Macfadyen 2007; Beneli et al. 2020), a phenomenon that has worsened with the use of synthetic, slow-degrading material, which might persist for decades in the environment. The subject has gained increasing attention in the last two decades (Gilman et al. 2012, 2016) and is currently well documented (Gilman et al. 2021). Derelict gillnets represent one of the highest risks amongst marine commercial fishing gears due to their global adverse effects (Gilman et al. 2021).

Direct measurements of fishing capacity of lost gill and trammel nets by diving observations date back to the 1990s (Kaiser et al. 1996; Erzini et al. 1997). When nets are lost, they continue to fish before becoming physically damaged or heavily colonized by incrusting biota, thus losing their catching ability. Catch rates and the evolution of lost gillnets would allow for estimating total mortality of marine life due to derelict gears (Ayaz et al. 2006; Akiyama et al. 2007; Baeta et al. 2009). A quantitative assessment of the direct impact of derelict gears on marine resources must take into account the rate of loss of such species, the effective impact lifespan of the gear, and the market value of the species caught (Gilardi et al. 2010).

A growing concern about litter in aquatic systems has also been observed in Brazil, with reports for freshwater (Iriarte and Marmontel 2013; Azevedo-Santos et al. 2022), oceanic (Santos et al. 2012; Grillo and Mello 2021), and coastal regions (Mascarenhas et al. 2008; Machado and Fillmann 2010; Vieira et al. 2011; Andrades et al. 2020; Pinheiro et al. 2021), and its subsequent impacts on estuarine fauna (Possatto et al. 2011; Dantas et al. 2012). There are also a few studies on derelict fishing gears and ghost fishing (Chaves and Robert 2009; Adelir-Alves et al. 2016), including a review of ALDFG (Link et al. 2019) and the negative impacts of ghost nets on Brazilian marine biodiversity revealed by digital media (Azevedo-Santos et al. 2021). However, till now, a quantitative assessment of the direct impact of derelict fishing gears on marine resources had not been conducted in Brazil.

This study simulated ghost fishing by derelict gillnets aiming at quantifying entanglement rates for fish and crustaceans, and at assessing net collapse over time in shallow rocky reefs off the state of Santa Catarina, in southern Brazil.

MATERIALS AND METHODS

Study site

The coast of the state of Santa Catarina is 531 km long (Figure 1), corresponding to 7% of the

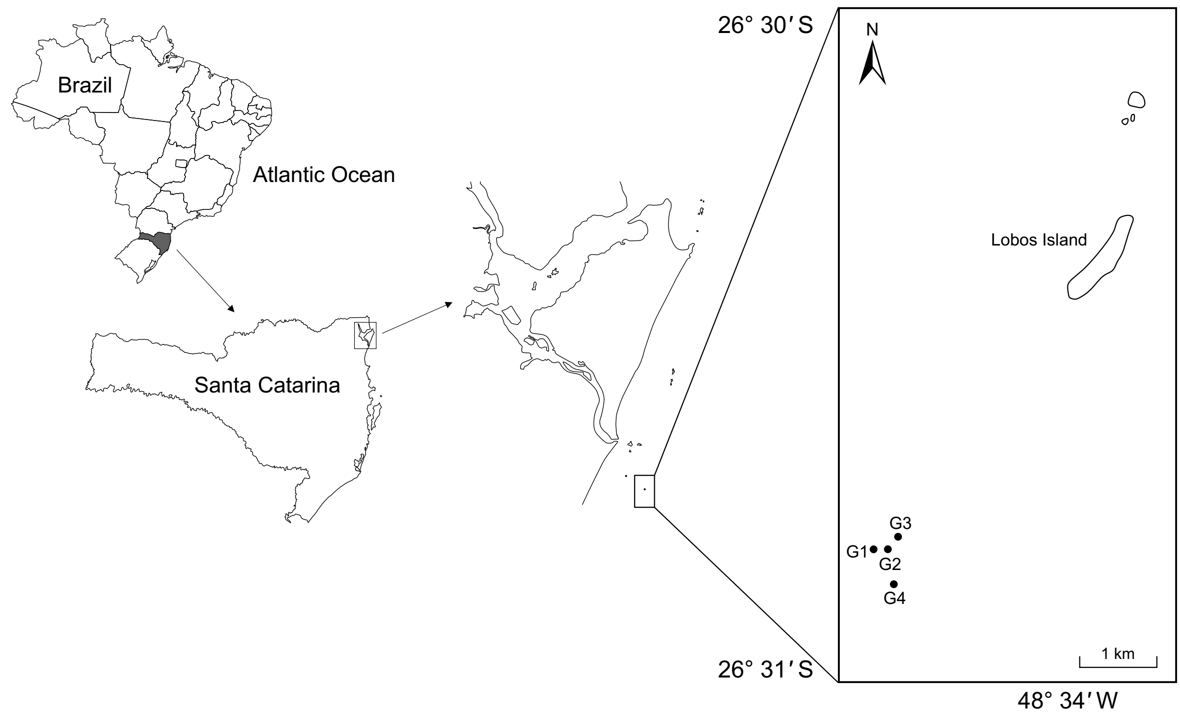

Figure 1. Location of gillnets (G1, G2, G3, G4), near Lobos Island, northern coast off the state of Santa Catarina, southern Brazil. Map: Diogo A. Moreira.

Brazilian coast, and belongs to the biogeographical province of the Southwest Atlantic or Brazilian Province (Floeter and Gasparini 2000). The region is influenced by two main atmospheric systems, the South Atlantic Subtropical Anticyclones (SASH) and the Atlantic Polar Migratory Anticyclone. These anticyclones are the generating sources of the Atlantic Tropical Mass (ATM) and the Atlantic Polar Mass (APM), respectively. The Atlantic Polar Front (APF), resulting from the contact between these two air masses, is responsible for part of the local precipitation, controlled by the presence of the mountains of Serra do Mar, Eastern Santa Catarina, and Serra Geral. Total annual rainfall is higher on the northern coast of the state (1,800 mm). The average number of cold fronts is quite similar in all seasons, with a slight decrease in the summer and a slight increase in the winter. SASH and APM anticyclones lead to an alternate prevailing wind regime between northeast (SASH) and south (cold fronts) (Carvalho et al. 1998; Pereira et al. 2009).

Experiment and data analysis

The experiment was set up in rocky reefs, 7 km (4 nm) off the northern coast of the state of Santa Catarina (Figure 1). An echosounder was used to check local depth and slope to subsequently deploy four gillnets. Nets were arranged vertically in 12 m deep areas, fixed on the rocky bottom with anchors on both sides, flagged with a surface buoy, and georeferenced (G1 26° 31′ 11.88″ S, 48° 33′ 56.16″ W; G2 26° 31′ 11.82″ S, 48° 33′ 55.20″ W; G3 26° 31′ 10.98″ S, 48° 33′ 54.48″ W; G4 26° 31′ 14.22″ S, 48° 33′ 54.78″ W). Rectangular gillnets (20 m long and 2 m high) of green polyamide monofilament (diameter = 0.7 mm; mesh size = 10 cm between opposite knots), placed around 50 m apart from each other, were used with floaters on the top line and lead sinkers on the bottom line, like those used by local fishers.

Two scientific divers conducted visual census on the nets using scuba diving (Heine 1999; Donohue et al. 2001; Pollock and Godfrey 2007). All nets were surveyed from March 29th to July 1st, 2012, with different time intervals (1, 9, 29, 55, 75, and 92 days after deployment). Each net was monitored for 30-40 min and the resulting data were recorded on a PVC clipboard using pencil; underwater photos and/or videos were taken. All entangled animals were identified to the lowest taxonomic level (Figueiredo and Menezes 1978, 1980, 2000; Fonteles-Filho 1999; Melo 1996; Menezes and Figueiredo 1980, 1985; Menezes et al. 2003), counted, and tagged with plastic clamps to prevent double counting.

To assess the entanglement, all divers followed the same procedure in each subsequent dive, recording the condition of previously observed entanglements and registering new entanglements. To evaluate the structural evolution of nets, the height between the top and bottom lines of each gillnet was measured along their length (0, 2.5, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 m) to calculate the area of the net over time. A linear model was fitted to the rate of reduction in area, and consequently in fishing capacity, due to the collapse of the net (% original area = -a ln(day + b). Sketches of each gillnet were drawn to show the reduction of the net area throughout the time. All nets were removed immediately after the final survey.

Entanglement rates (ER: number of entangled animals/day/net) were calculated by taxa (crustaceans, fishes) by counting the number of animals newly entangled between surveys and dividing by the number of days between observations (Gilardi et al. 2010). Nonlinear models (logarithmic: ER = a ln(Day) + b, and exponential: ER = a e^{bDay}) were checked for fishes, crustaceans, and total quantity (fishes and crustaceans) (Faraway 2002; Zar 2010).

The local market price of target species (USD kg^{-1}) was used as a proxy to indicate commercial importance. The recreational importance was defined based on personal observation (JAA).

RESULTS

A total of 126 animals were observed entangled in all four gillnets: four species of crustaceans (n = 74; 57%) and 13 species of fish (n = 52; 43%) (Table 1). The stone crab *Menippe nodifrons* Stimpson, 1859 was the most frequent species (n = 36; 28%). Eight of the entangled fish species and one crustacean *Panulirus laevicauda* (Latreille, 1817) have commercial importance (Table 1).

The number of entangled fishes surpassed the number of crustaceans in the first two dives, reaching 59% of total catches in Day 9 (Figure 2). After this period, the percentage of entangled fishes decreased until reaching zero, 92 days after the deployment of the nets. Crustacean entanglements increased over a longer period and decreased later.

Entanglement rates for fishes [ER = -0.269 ln(Day) + 1.1715] and crustaceans [ER = -0.164 ln(Day) + 0.826] showed higher coefficients of determination in logarithmic models (R^2 = 0.91 and 0.90, respectively) (Figure 3 A). When fishes and crustaceans were combined, the entanglement rate [ER = 1.3748 $e^{-0.032Day}$] had a higher coefficient with an exponential model (R^2 = 0.98) (Figure 3 B).

The fishing area of the nets decreased over time, due to the accumulation of detritus, biofouling, and damages such as broken meshes (Figure 4 A and 4 B). Their physical fishing capacity was reduced to 26% of the original value after 75 days of immersion (Figure 5). The rate of loss of fishing capacity was fitted to a linear model (% original area = -0.938Day + 88.112; R^2 = 0.96). Thus, it is expected that gillnets are totally collapsed within 94 days, on average, under the experimental conditions.

Table 1. List of taxa (class, family, species) caught in the ghost experiment conducted off the coast of Santa Catarina in 2012, total number of individuals caught per species (N), relative abundance (%), fishing importance (FI) in price per unit of weight (P-USD kg^{-1}), fished by recreational fishers (RF), and no commercial or recreational importance (—).

Class	Family	Species	N	%	FI
Crustacea	Xanthidae	*Menippe nodifrons* Stimpson, 1859	36	28	RF
	Majidae	*Mithrax hispidus* (Herbst, 1790)	20	15	RF
	Portunidae	*Cronius ruber* (Lamarck, 1818)	17	13	—
	Palinuridae	*Panulirus laevicauda* (Latreille, 1817)	1	1	RF/P-7.00
Teleostei	Haemulidae	*Anisotremus virginicus* (Linnaeus, 1758)	17	13	—
		Anisotremus surinamensis (Bloch, 1791)	4	3	RF/P-1.06
	Pomacentridae	*Abudefduf saxatilis* (Linnaeus, 1758)	7	6	—
	Epinephelidae	*Epinephelus marginatus* (Lowe, 1834)	2	2	RF/P-3.82
		Mycteroperca acutirostris (Valenciennes, 1828)	3	2	RF/P-2.27
	Scaridae	*Sparisoma frondosum* (Agassiz, 1831)	2	2	RF
	Monacanthidae	*Stephanolepis hispida* (Linnaeus, 1766)	1	1	RF/P-1.06
	Holocentridae	*Holocentrus adscensionis* (Osbeck, 1765)	1	1	—
	Priacanthidae	*Priacanthus arenatus* Cuvier, 1829	1	1	RF/P-1.06
	Sparidae	*Diplodus argenteus* (Valenciennes, 1830)	2	2	RF
	Carangidae	*Chloroscombrus chrysurus* (Linnaeus, 1766)	3	2	RF/P-1.06
		Caranx crysos (Mitchill, 1815)	1	1	RF/P-1.68
		Caranx latus Agassiz, 1831	1	1	RF/P-1.68
	Not identified*	—	7	6	—

*Animal in advanced state of decomposition.

DISCUSSION

This study estimated entanglement rates and the mean time of collapse of the derelict gillnets under experimental condition for the first time in Brazil. Crustaceans and fishes were the groups most affected, as has been observed around the world (Gilman et al. 2016), with the highest proportion corresponding to crustaceans (Kaiser et al. 1996; Revill and Dunlin 2003; Akiyama et al. 2007; Gilardi et al. 2010; Queirolo and Gaete 2014). In other ghost fishing experiments, the capture of mollusks was reported (Akiyama et al. 2007; Baeta et al. 2009; Queirolo and Gaete 2014), but it was not observed here. Ghost fishing is thought to be more problematic in passive fishing gears after being set and subsequently lost or abandoned, as they continue capturing animals for some time (Gilman 2015).

A temporal pattern was observed, with ghost catches initially showing a high proportion of fishes, before becoming dominated by crustaceans, as observed in other areas (Kaiser et al. 1996; Erzini et al. 1997; Akiyama et al. 2007; Brown and Macfadyen 2007; Baeta et al. 2009). The later predominance of crustaceans has been associated with their proximity to the seabed and with their scavenger habit, using dead and decomposing individuals trapped in the nets as food, resulting in cyclical catching by the fishing gear (Macfadyen et al. 2009). Thus, crustaceans are easily turned into targets for entanglement,

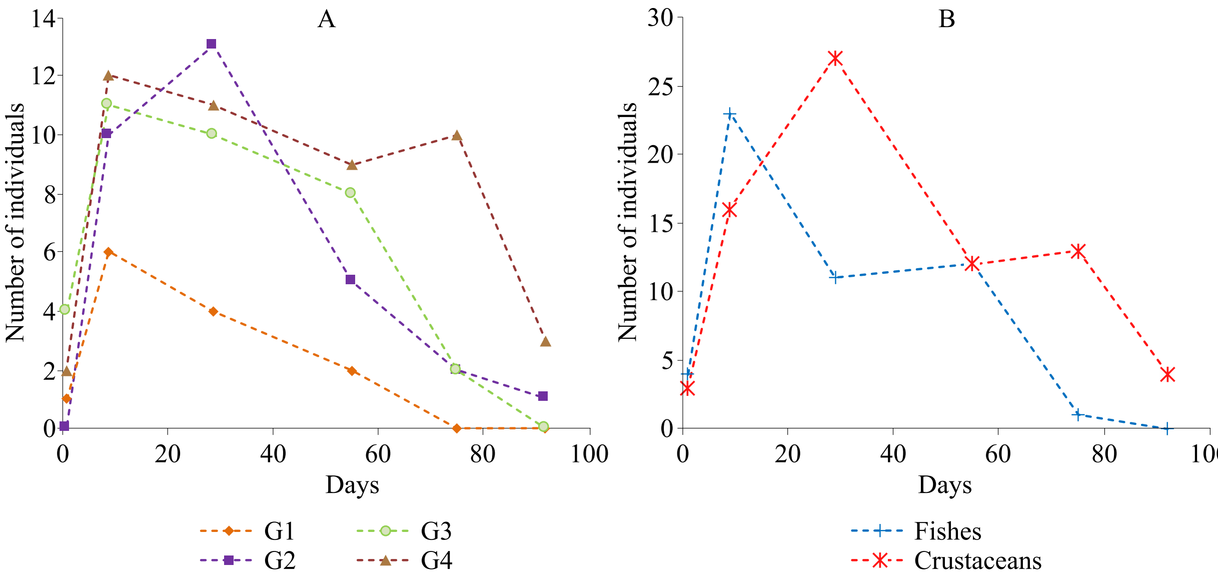

Figure 2. A) Number of individuals caught by each gillnet (G1, G2, G3, and G4). B) Total number of fishes and crustaceans caught in all four nets during the experiment.

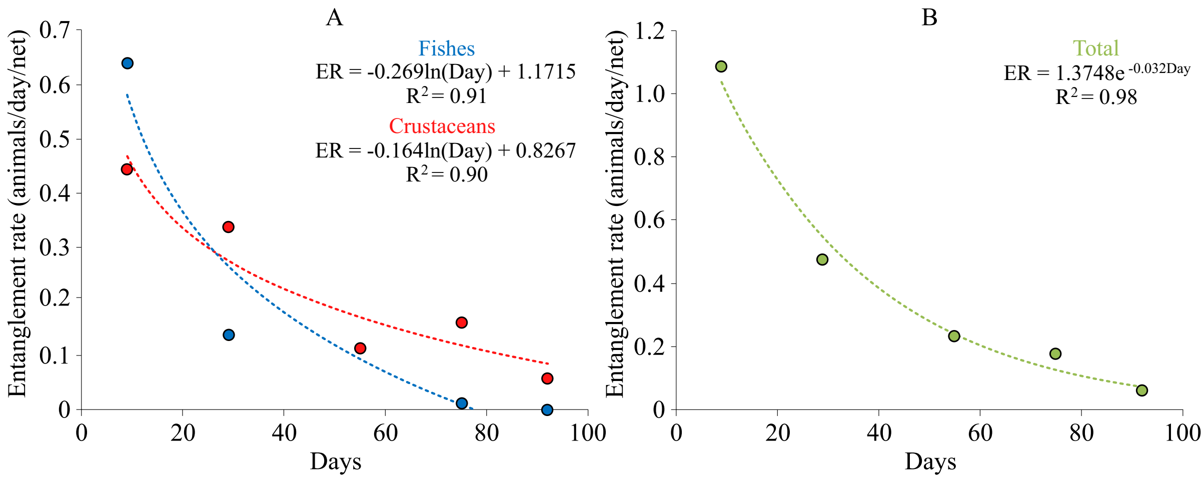

Figure 3. Entanglement rates (ER, animals/day/net) and fitted nonlinear models during the experiment conducted off the coast of Santa Catarina. A) Fishes and crustaceans (logarithmic model). B) Total (exponential model).

even after the nets present a reduction in their fishing area (Revill and Dunlin 2003; Baeta et al. 2009). Ghost fishing is often cyclical, with its duration and extent depending on the gear type, water depth, currents, and local environment, among other factors (Macfadyen et al. 2009).

Even though the analyzed nets may have been exposed to different environmental conditions, their catching efficiency followed similar patterns, with exponential models indicating rapid declines in catch rates after a few weeks (Brown and Macfadyen 2007; FAO 2009). Our study found a higher value of catch rate ($e^{-0.032t}$) compared to those reported in Izmir Bay, Turkey

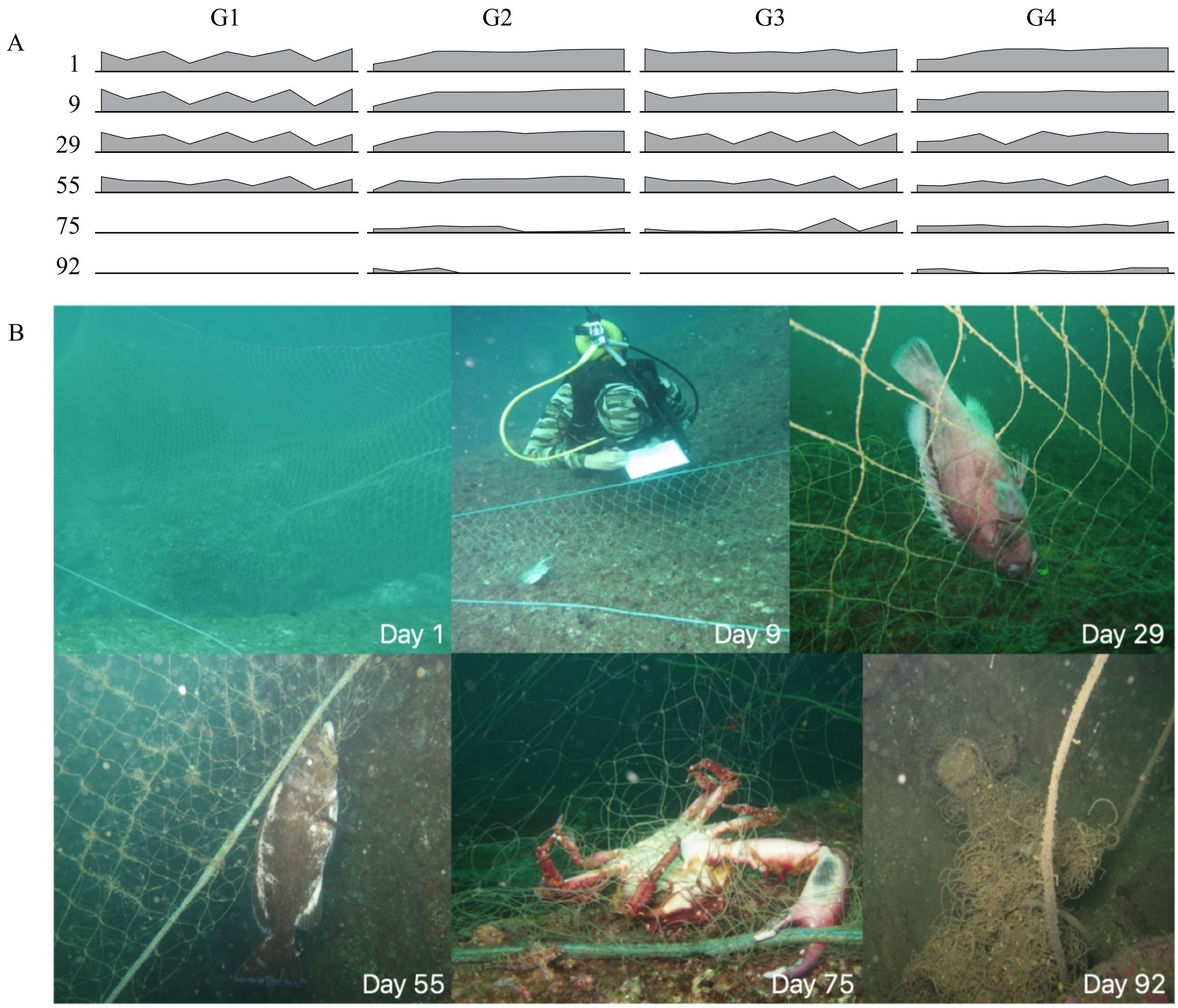

Figure 4. Fishing area of gillnets (G1, G2, G3, and G4) over a 92-day-period. A) Sketches were based on measurements of the net height (initially 2 m) and length (20 m). B) Images of the gillnet structural deterioration over the experiment period.

($e^{-0.0127t}$), in Tateyama Bay, Japan ($e^{-0.0154t}$), and in Laguna Verde, Chile ($e^{-0.0158t}$) (Ayaz et al. 2006; Akiyama et al. 2007; Queirolo and Gaete 2014, respectively), but smaller than the ones observed in Algarve, Portugal ($e^{-0.0542t}$) (Erzini et al. 1997). The catching efficiency of ghost gillnets is determined by their vertical profile, gradually declining with the exposure to storms and fouling. Biofouling, and subsequent increase in visibility, might occur rapidly in subtropical conditions, contributing to a faster decline in entanglement rates (catching efficiency) observed in our study.

A sharp decrease in the functional area of the experimental gillnets in the first weeks after deployment appears to be the pattern, followed by a gradual decline until stabilization near the bottom. Reductions to 21% of the original area after 17 weeks (Erzini et al. 1997), to 18% after 10 weeks (Revill and Dunlin 2003), and to less than 10% after 64 days of immersion (Queirolo and Gaete 2014) were reported. Thereafter, in most

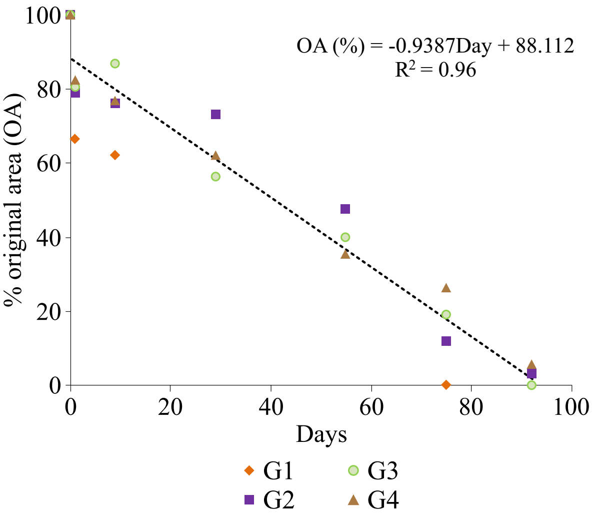

Figure 5. Reduction of fishing capacity (area) of the experimental gillnets (G1, G2, G3, and G4) over time, and linear model fitted.

studies, the rate of loss of fishing capacity decreased till reaching zero after 115 days (Queirolo and Gaete 2014), 15-20 weeks (Erzini et al. 1997), one year (Ayaz et al. 2006), or even two years of abandonment (Revill and Dunlin 2003; Tschernij and Larsson 2003). In contrast to the exponential models reported by some authors (Erzini et al. 1997; Queirolo and Gaete 2014), the fishing capacity loss rate in our experiment followed a linear model (b = -0.9387), resulting in an estimated total collapse of the nets after about 94 days. This shorter time, revealed by the linear model fitted, might have been influenced by the smaller length (and, consequently, area) of our nets, compared to those used in other experiments, making the nets more vulnerable to damages and collapse.

Degradation of the structural integrity of the net seems to be the primary cause of collapse. Some abandoned or lost gillnets may collapse immediately and have lower fishing efficiencies. Conversely, longer nets, fleets of nets, or nets snagged on rock, coral, or wrecks might be slow to collapse, or even be stretched again and continue killing for a longer time (Macfadyen et al. 2009). Length of individual nets used in the experiments around the world varied from 10 m (Akiyama et al. 2007) to 100 m (Erzini et al. 1997; Revill and Dunlin 2003). Additionally, some of the experiments used individual nets, keeping one side free or united to others by ropes, and some used fleets of 52-65 m nets, resulting in lengths of 200-250 and 378-480 m (Tschernij and Larsson 2003). The time elapsed between abandonment and total collapse of the nets varied from three months to two years and might be related to these differences in total net size, besides local environmental conditions observed. In one of the experiments with the longest collapse time reported, large fleets were employed (Tschernij and Larsson 2003); in another, the collapse time was very long when nets were deployed in wrecks, but similarly short when deployed in open areas (Revill and Dunlin 2003), as in the present study. In both cases, very few animals were caught after nine months of immersion.

In this study, all entangled crustaceans are reef-associated and have nocturnal habits (Fonteles-Filho 1999; Rieger and Girald 2001). The entangled fishes, pelagic or demersal, were also considered reef-associated, as they are included in local lists of reef fishes (Adelir-Alves and Pinheiro 2011) and depend on rocky reefs for at least part of their life cycle (Carvalho-Filho 1999). The stone crab (*M. nodifrons*), the most commonly entangled species, also inhabits reef environments (Melo 1996). Even though this species is not commonly used for human consumption in the state of Santa Catarina, probably due to its small size in relation to other commercially exploited species, it is occasionally caught by recreational fishers for their own consumption or to prepare traditional dishes for tourists (Oshiro 1999). Crabs are amongst the most abundant animals caught by ghost fishing, with *Cancer porteri* Rathbum, 1930 reaching ~ 82% in Laguna Verde, Chile (Queirolo and Gaete 2014).

Ghost fishing is undesirable from both the economic and conservation standpoints. Eight of the species entangled during our experiment are targeted by local fishers, including species of high market price, such as the dusky grouper (*E. marginatus*) and the smooth-tail spiny lobster (*P. laevicauda*). Fishes are amongst the main resources globally caught in total weight, representing 85% of the total catch but only 66% of the value, while crustaceans reach the highest market value, resulting in 22% of the total global value (FAO 2020). The dusky grouper is considered overexploited or threatened in Brazil (MMA 2016) and is categorized as vulnerable by the International Union for Conservation of Nature (Pollard et al. 2018). No marine turtle, bird, or mammal was captured during our experiment, but the risk of these species being entangled should not be disregarded, as it has been documented elsewhere (NOAA 2014). Marine mammals, reptiles, and elasmobranchs from 40 different species were recorded as entangled in, or associated with, ghost gears (Stelfox et al. 2016).

The accumulation of nets on the sea floor raises concern, as they often cover previously established benthic communities (Saldanha et al. 2003). On the other hand, fragments of gillnets colonized by sessile organisms can also act as artificial reefs, providing shelter, food, and support for fishes and invertebrates (Watters et al. 2010; Mordecai et al. 2011). Ringneck blenny *Parablennius pilicornis* (Cuvier, 1829) and yellowline arrow crab *Stenorhynchus seticornis* (Herbst, 1788) were also observed using gillnets in a similar way (JAA, personal observation). Seahorse *Hippocampus reidi* Ginsburg, 1933 has been observed using fragments of gillnets as substrate (Mai and Rosa 2009), but not in our study.

Weak monitoring and surveillance prevent the proper implementation of governance frameworks, including measures to monitor, prevent, and remediate ALDFG and ghost fishing (Gilman 2015). Effective management measures are required to augment compliance and reduce a growing worldwide threat. Experimental studies with ghost fishing are important to give support to the decision-making process on required regulations. Measures to reduce the impact of ghost fishing have been debated, including ways to prevent loss or even retrieval of derelict gears and the development of fishing gears made of biodegradable materials (Matsuoka et al. 2005; McElwee et al. 2011; Morishige and McElwee 2012). Gear retrieval programs could minimize the impact on aquatic animals. However, the most effective measure to reduce the impact of ghost fishing may be to directly inform fishers about the impact of their derelict gears and discuss mechanisms to prevent it. Thus, the FAO Code of Conduct for Responsible Fisheries (FAO 1995) could be effectively implemented.

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ORIGINAL RESEARCH

Diversity of bloom forming harmful algal species in the central Bonny estuary, Niger delta, Nigeria

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ABSTRACT. This study was carried out from December 2018 to November 2019 to examine the distribution and abundance of harmful algal species (HAS) in the central Bonny estuary. Seven sampling stations were established with ArcGIS tool. Microalgae species were sampled with 20 µm mesh plankton net. Nutrients were analyzed in the laboratory using the APHA 4500 Method, while physicochemical characteristics were determined *in situ*. Results revealed that environmental gradients were adequate to support life in that part of the estuary except for phosphate (2.90 ± 0.22-9.48 ± 1.06 mg l^{-1}). A total of 31 HASs categorized into 17 genera and three classes were determined: Bacillariophyceae (29 species), Chlorophyceae and Cyanophyceae (one species each). *Navicula amphibola* had the highest density (4.713 × 10^3 cells l^{-1}) while *Pinnularia divergens* recorded the lowest density (0.00049 × 10^3 cells l^{-1}). Total density values decreased across seasons with 9.157 × 10^3 cells l^{-1} in dry season and 8.907 × 10^3 cells l^{-1} in wet season. Checklist of species across stations showed that five species were distributed across the seven stations, while two were found only in Station 2 and 7. Diversity indices revealed Shannon's index ranged between 3.17 and 3.25 and species evenness ranged between 0.78 and 0.88, while Margalef range value (3.09-3.31) was considered moderately stable. Therefore, there is a need for proper management practices which could help to reduce the level of nutrient discharge into the central Bonny estuary.

Key words: Distribution, season, abundance, HAS, environmental gradients.

Diversidad de floraciones de especies de algas nocivas en el área central del estuario del Río Bonny, delta del Níger, Nigeria

RESUMEN. Este estudio se llevó a cabo entre diciembre de 2018 y noviembre de 2019 para examinar la distribución y abundancia de especies de algas nocivas (HAS, por sus siglas en inglés) en el área central del estuario del Río Bonny. Se establecieron siete estaciones de muestreo con la herramienta ArcGIS. Las especies de microalgas se muestrearon con una red de plancton de 20 µm de malla. Los nutrientes se analizaron en laboratorio mediante el Método APHA 4500, mientras que las características fisicoquímicas se determinaron *in situ*. Los resultados revelaron que los gradientes ambientales fueron adecuados para sustentar la vida en esa parte del estuario, excepto por el fosfato (2,90 ± 0,22-9,48 ± 1.06 mg l^{-1}). Se determinaron un total de 31 HAS categorizadas en 17 géneros y tres clases: Bacillariophyceae (29 especies), Chlorophyceae y Cyanophyceae (una especie cada una). *Navicula amphibola* tuvo la mayor densidad (4,713 × 10^3 células l^{-1}) mientras que *Pinnularia divergens* registró la menor densidad (0,00049 × 10^3 células l^{-1}). Los valores de densidad total disminuyeron a través de las estaciones con 9,157 × 10^3 células l^{-1} en la estación seca y 8,907 × 10^3 células l^{-1} en la estación húmeda. La lista de especies en las estaciones mostró que cinco especies se distribuyeron en las siete estaciones, mientras que dos se encontraron solo

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en las estaciones 2 y 7. Los índices de diversidad revelaron que el índice de Shannon osciló entre 3,17 y 3,25 y la uniformidad de las especies osciló entre 0,78 y 0,88, mientras que el valor del rango de Margalef (3,09-3,31) se consideró moderadamente estable. Por lo tanto, existe la necesidad de prácticas de gestión adecuadas que podrían ayudar a reducir el nivel de descarga de nutrientes en el área central del estuario del Bonny.

Palabras clave: Distribución, estación, abundancia, HAS, gradientes ambientales.

INTRODUCTION

The estuarine ecosystem is an ecotype affected by sea inflow and neighboring freshwater, which results in high levels of nutrients in the water body (Jha et al. 2014). According to Kress et al. (2002), estuaries are places for human settlements and activities (shipping, urban and industrial waste) making them vulnerable to changes caused by pollution, climate change and overfishing, which in turn alter the water body's productivity. Dynamics in biological populations, especially planktonic communities, represent variations in physical and chemical processes in estuaries (Marques et. al. 2007).

Harmful algal blooms (HABs) have become the preferred scientific term instead of red tides because these outbreaks have no connection to the tides and may or may not color the water red (Sverdrup et al. 2003). Additionally, some algae species may bloom and color water, which is not harmful. HABs are thus defined as high propagation of algae, ensuing transformation. Such algae have the probability of producing toxins (Boesch et al. 1997).

Approximately 300 algal species are said to cause these blooms. It is understood that almost one-fourth produce toxins (IOC 2015). A very small number remains potentially harmful, which can pollute aquatic organisms through contaminants resulting in health problems in human beings, as well as multiplying and changing habitats in ways that may be considered unfavorable to them (Brand et al. 2012). Under the right conditions, these groups of algal species form an algal bloom provided that sufficient nutrients, water column steadiness, enough light and ideal temperatures are present (Hall et al. 2013). Nutrient enrichment is the key mechanism through which fertilizer or nutrient loads of nitrates and phosphates are discharged into a waterbody, such as livestock farming waste (Larsson et al. 1985) and industrial or municipal waste (Larsson et al. 1985; Gilbert et al. 2005). Runoffs transport these nutrients through river systems and eventually to marine or freshwater systems. Many algal blooms can damage aquatic species, and adverse blooms are the result of an occasional accumulation of toxic algae which can create hypoxic conditions, producing damaging effects on aquatic ecosystems (Gilbert et al. 2005).

Several new bloom species are assumed to follow the discovery of concealed flora communities (Smayda 1998) that had been there for years in these lakes, but were not identified as detrimental until more subtle toxin-revealing techniques or an increase in the measurement and teaching of observers were employed (Anderson et al. 1994). The coastline is vulnerable to HABs, particularly enclosed embayments, due to urbanization, tourism, and industrial waste (Anderson et al. 2002; Sellner et al. 2003). Furthermore, the flow of water, relaxation, and development of cysts are factors in the creation of blooms (Sellner et al. 2003). There are several environmental factors (both physical and chemical), including nutrient availability and temperature changes, which are being described as important drivers to harmful algal species diversity (Giannuzzi et al. 2012). The aim of this study was to assess the diversity of bloom forming harmful algal species in the central Bonny estuary of the Niger delta.

MATERIALS AND METHODS

Study area

The Bonny estuary is among the numerous low-land coastlines of the Niger delta complex. It is located between 4° 25′ and 4° 50′ N latitude and 7° 0′ and 7° 15′ E longitude in Rivers State of Nigeria (Figure 1). It extends in length to about 180 km from its mouth to the upper limits of saline influence. It is mainly brackish and consists of a main river channel and creek. The Bonny estuarine channel has the highest tidal flow of all river systems, which is influenced mostly by tidal movements. The Bonny estuary extends from the mouth of the estuary (lowest reach) close to the Atlantic Ocean, where salinity is > 30 in dry season and about 28 in rainy season, to the uppermost reaches of the Iwofe area, where salinity is < 5 and < 3, respectively (Dangana 1985).

Sampling stations

Seven geo-referenced stations were set up along the estuary course using the ArcGIS tool, through a reconnaissance preliminary survey: Station 1 (Nembe waterside), Station 2 (Ebetu), Station 3 (Isaka open river), Station 4 (Isaka main town), Station 5 (Back of Ibeto cement), Station 6 (Macoba), Station 7 (NPA dockyard) (Figure 1).

Sample collection, preparation and analyses

Samplings were carried out at low tide in seven geo-referenced stations previously described. Surface water hauls, collected in triplicate at various sampling stations by filtering 100 l of surface water over a 20 µm phytoplankton net. For analysis and identification (Hansen 2002), they were kept in 15 cm Nalgene storage bottles. Fifty percent were preserved immediately using 2% formaldehyde while the other 50% (live samples) were stored in an insulated box to prevent rapid

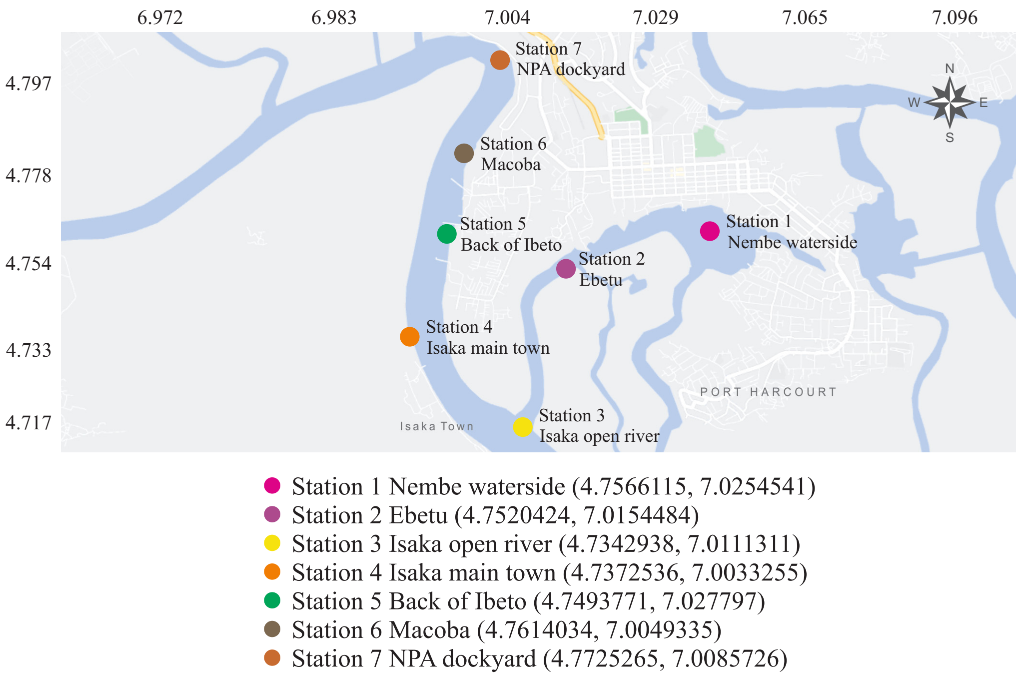

Figure 1. Study area indicating sampling stations in central Bonny estuary, Niger delta.

temperature change (IOC 2015). Temperature, salinity, Total Dissolved Solids (TDS), pH and DO were measured *in situ* with a Horiba water checker (Model Extech D0700) at each sampling location. Triplicate surface water samples for nutrients (phosphate PO_4, nitrate NO_3, nitrite NO_2, and sulphate SO_4) were collected at neap tide at a depth of 5 cm with pre-cleaned plastic container, kept in ice-chest box and taken to the laboratory for further nutrient analysis. Laboratory determination of nutrients followed the standard procedures of water and wastewater analysis of the American Public Health Association for PO_4, NO_3, NO_2 and SO_4 (APHA 2012).

Enumeration of harmful algal species

Microalgae were counted using the Lackey Drop Micro-transect Counting Method (APHA 1998). The sample was mixed well before subsampling a drip of 0.05 ml onto a glass-slide in triplicate with cover-slip. The processed volume and the number of observed microalgae were known in a given volume; their abundance was counted with a low power objective with an inverted microscope (Leica DMIL). Microphotographs of harmful algae were taken by employing a camera fixed to the microscope. Identification of algae was done by following references of Taylor (1987), Hallegraeff et al. (1995), and Tomas (1997). Density was calculated as:

$$\text{Number (No) individuals ml}^{-1} = \frac{C \times TA}{A \times S \times V}$$

where, C = number of organisms counted; TA = area of the cover slip, mm^2 ; A = area of one strip, mm^2; S = number of strips counted; and V = volume of sample under the cover slip, ml.

Data analysis

Physicochemical and nutrient parameters of samples were analyzed using one-way analysis of variance of SPSS version 20. Fixed effect ANOVAs were done in replicates. Tukey HSD was used to separate the mean differences at a 95% confidence interval ($p < 0.05$). Spatial variation of the various environmental parameters and harmful algal species across the season was done using the T-test. The diversity of HAS in the estuary was calculated with harmful algal species abundance, using the PRIMER software version 6.1.6 (Clarke and Gorley 2006).

Simpson's diversity indices

The term Simpson's diversity index is any of three (3) closely related indices (Simpsons 1949).

- Simpson's Diversity Index (D): it measures the possibility of randomly picked individuals from a sample:

$$D = \sum (n/N)^2$$

$$D = \frac{\sum n(n-1)}{N\,(N-1)}$$

- Simpson's Diversity Index 1-D: here, the index denotes possibility of entities randomly picked in a sample.
- Simpson's Reciprocal Index 1/D: it represents a community of one species and a higher value.
- Shannon-Weiner Diversity Index (H'): the level of uncertainty of forecasting a random sample is associated to a community. Community with one species (low diversity) (Shannon and Wienner 1963):

$$H' = -\sum p_i \, \text{In} P_i$$

where p_i is the proportion of individuals of the *i* species.

Species richness (S) is a total number of dissimilar species in an area. It is intensely hooked on sample size and strength (Begon et al. 1990):

- Margalef's Diversity Index:

$$Dmg = \frac{S-1}{InN}$$

- Menhinick's Diversity Index:

$$DMn = \frac{S}{\sqrt{N}}$$

RESULTS

Results from physicochemical parameters and seasonal variation of the surface water from the sampled sites in the central Bonny estuary revealed that there was a significant a difference in pH, DO and turbidity ($p < 0.05$), while temperature, salinity, BOD, conductivity and TDS showed no significant difference ($p > 0.05$) across stations. Mean values of pH, DO, salinity, BOD, conductivity, and TDS decreased across the season (dry to wet), while temperature and turbidity values increased across the season (dry to wet) (Table 1).

Nutrient composition of sampled sites from the central Bonny estuary indicated that there was a significant difference across stations ($p < 0.05$). PO_4 and NO_3 decreased across seasons (dry to wet), while NO_2 and SO_4 increased from dry to wet season. Nitrate and sulphate showed significant differences ($p < 0.01$ and $p < 0.05$, respectively) across seasons (Table 2).

Three classes of major groups were represented in the samples: Bacillariophyceae, Chlorophyceae and Cyanophyceae, with 15 genera and 31 species (Table 3). Families Pinnulariaceae and Stephanodiscaceae recorded two species each; Pleurosigmataceae and Coscinodiscaceae recorded three

Table 1. Physicochemical parameters at different stations and seasons in the central Bonny estuary. T: temperature, DO: dissolved oxygen, BOD: biological oxygen demand, COND: conductivity, TDS: total dissolved solids.

Station	pH	T (°C)	DO (mg l^{-1})	Salinity	BOD (mg l^{-1})	COND (µS cm^{-1})	Turbidity (NTU)	TDS (mg l^{-1})
1	6.85 ± 0.09^a	29.97 ± 0.71^a	4.97 ± 0.37^b	15.09 ± 1.41^a	2.36 ± 0.09^a	21.39 ± 2.39^a	7.17 ± 1.02^a	19.57 ± 9.30^a
2	7.11 ± 0.07^b	29.48 ± 0.82^a	4.64 ± 0.36^{ab}	16.20 ± 0.98^a	2.12 ± 0.19^a	20.82 ± 2.32^a	7.31 ± 0.57^a	17.44 ± 2.33^a
3	7.26 ± 0.05^{ab}	28.97 ± 0.56^a	4.46 ± 0.28^{ab}	19.40 ± 2.40^a	2.49 ± 0.18^a	25.19 ± 3.51^a	9.90 ± 1.07^b	18.34 ± 2.24^a
4	7.26 ± 0.05^{ab}	28.95 ± 0.58^a	4.39 ± 0.35^{ab}	19.47 ± 2.29^a	2.30 ± 0.21^a	25.24 ± 3.46^a	10.45 ± 1.02^b	18.33 ± 2.31^a
5	7.32 ± 0.20^c	28.96 ± 0.50^a	3.89 ± 0.16^a	18.24 ± 2.22^a	2.42 ± 0.21^a	24.21 ± 3.04^a	6.06 ± 0.65^a	17.16 ± 2.44^a
6	7.34 ± 0.05^c	29.07 ± 0.53^a	3.97 ± 0.17^a	18.88 ± 2.04^a	2.42 ± 0.13^a	24.27 ± 3.11^a	5.20 ± 0.63^a	18.67 ± 2.24^a
7	7.35 ± 0.05^c	28.96 ± 0.54^a	4.32 ± 0.20^{ab}	17.81 ± 1.59^a	2.34 ± 0.13^a	22.84 ± 2.67^a	7.20 ± 0.35^a	16.87 ± 2.21^a
Season								
Dry	7.39 ± 0.03	28.32 ± 0.39	5.10 ± 0.20	24.10 ± 1.24	3.00 ± 0.08	29.91 ± 1.10	5.42 ± 0.29	19.20 ± 1.60
Wet	7.08 ± 0.03	29.85 ± 0.25	3.97 ± 0.77	13.20 ± 0.33	1.83 ± 0.06	14.84 ± 1.0	19.26 ± 0.50	17.20 ± 0.85
t value	6.94	3.481	6.762	9.55	11.97	13.32	6.094	1.178
p value	0.62	0.00*	0.00*	0.00*	0.00*	0.22	0.13	0.00*

Superscripts of the same alphabet are not significantly different across the column ($p > 0.05$). Superscripts of different alphabets are significantly different ($p < 0.05$).

Table 2. Nutrients (phosphate PO_4, nitrate NO_3, nitrite NO_2, and sulphate SO_4) across stations and seasons in the central Bonny estuary.

Station	PO_4 (mg l^{-1})	NO_3 (mg l^{-1})	NO_2 (mg l^{-1})	SO_4 (mg l^{-1})
1	3.14 ± 0.2^{a}	0.72 ± 0.07^{a}	0.0034 ± 0.0004^{a}	$1,010.43 \pm 9.02^{c}$
2	2.90 ± 0.22^{a}	2.62 ± 0.92^{b}	0.0039 ± 0.0007^{a}	$1,026.14 \pm 6.32^{c}$
3	3.70 ± 0.25^{a}	0.53 ± 0.04^{a}	0.0046 ± 0.0007^{a}	967.90 ± 9.20^{a}
4	6.71 ± 0.53^{b}	0.66 ± 0.06^{a}	0.0048 ± 0.0003^{a}	978.81 ± 6.76^{ab}
5	9.48 ± 1.06^{c}	0.71 ± 0.06^{a}	0.0067 ± 0.0003^{b}	$1,004.10 \pm 14.03^{bc}$
6	3.73 ± 0.24^{a}	0.54 ± 0.04^{a}	0.0043 ± 0.0002^{a}	$1,029.62 \pm 10.19^{c}$
7	5.40 ± 0.67^{b}	0.49 ± 0.08^{a}	0.0043 ± 0.0006^{a}	962.381 ± 6.43^{a}
Season				
Dry	4.68 ± 0.548	1.12 ± 0.33	0.0044 ± 0.0003	974.06 ± 6.03
Wet	5.26 ± 0.32	0.73 ± 0.03	0.0047 ± 0.0002	$1,014.30 \pm 4.54$
t value	1.059	1.371	0.601	5.439
p value	0.11	0.00**	0.13	0.02*

Superscripts of the same alphabet are not significantly different ($p > 0.05$). Superscripts of different alphabets are significantly different ($p < 0.05$).

*Significant at $p < 0.05$

**Significant at $p < 0.01$

species each; Naviculaceae recorded six species; Triceratiaceae recorded five species; and Bacillariaceae recorded four species, while other families recorded one species each. Class Bacillariophyceae had 29 species, while Chlorophyceae and Cyanophyceae recorded one species each.

The Family Naviculaceae had the highest density followed by Bacillariacea, while the Family Microcoleaceae recorded the least density (Figure 2). *Navicula* spp. recorded the highest percentage (19%) followed by *Nitzschia* spp. (12%), while the lowest (1%) was recorded for *Thalassiosira eccentrica* (Figure 3). Mean cell density plotted against species in the study area indicated that *N. amphibola* recorded the highest mean abundance (4,714.38 cells l^{-1}), followed by *N. dicephala* (4,603 cells l^{-1}), while *Pinnularia divergens* (4.9 cells l^{-1}) recorded the lowest abundance (Figure 4). Abundance across seasons indicated that 17 species decreased with season (dry to wet), while 11 species increased across seasons (dry to wet). Two species (*Gyrosigma stigma* and *P. divergens*) recorded mean value only during the dry season, while only one species (*N. hybrida*) recorded mean values in the wet season (Figure 5). Total density values increased across season with 9,157 cells l^{-1} and 8,907 cells l^{-1} in dry and wet seasons, respectively. The Class Bacillariophyceae recorded the highest percentage composition of harmful algal taxa (75%), followed by the Class Chlorophyceae (15%), and the Class Cyanophyceae (10%).

Cyclotella meneghiniana, *Cymbella turgidula*, *Diploneis finnica*, *N. amphibola* and *Tretraedron tumidulum* were fairly distributed across the seven sampling stations, while two species, *P. divergens*, *N. hybrida* were the least distributed harmful algal species in stations 2 and 7, respectively (Table 4). Diversity indices showed that the highest taxa value (31) was recorded in Station 4 and the least taxa value (29) was recorded in Sta-

Table 3. Harmful algal species (HAS) composition in the Central Bonny estuary.

Class	Family	Species
	Cymbellaceae	*Cymbella turgidula* (Grunow, 1875)
	Pinnulariaceae	*Pinnularia undulata* (Sensu Cleve, 1891)
		Pinnularia divergens (W. Smith, 1853)
Bacillariophyceae	Naviculaceae	*Navicula amphibola* (Cleve, 1891)
		Navicula dicephala (Ehrenberg, 1838)
		Navicula oblonga (Kützing, 1844)
		Gyrosigma fasciola (Griffith and Henfrey, 1856)
		Gyrosigma stigma (Hassall, 1845)
		Gyrosigma acuminatum (Rabenhorst, 1853)
	Catenulaceae	*Amphora holsatica* (Hustedt, 1925)
	Tabellariaceae	*Asterionella japonica* (Cleve and Möller, 1882)
	Diploneidineae	*Diploneis finnica* (Cleve, 1891)
	Stephanodiscaceae	*Cyclotella antigua* (Smith, 1853)
		Cyclotella meneghiniana (Kützing, 1844)
	Bacillariaceae	*Nitzchia hybrida* (Cleve and Grunow, 1880)
		Nitzchia sigma (Smith, 1853)
		Nitzchia vermicularis (Hantzsch, 1860)
		Bacillaria paxillifera (Muller and Hendy, 1951)
	Surirellaceae	*Surirella robusta* (Ehrenberg, 1841)
	Pleurosigmataceae	*Pleurosigma elongatum* (Smith, 1852)
	Coscinodiscaceae	*Coscinodiscus concinnus* (Smith, 1856)
		Coscinodiscuss granni (Gough, 1905)
		Coscinodiscuss radiatus (Ehrenberg, 1840)
	Triceratiaceae	*Odontella aurita* (Agardh, 1832)
		Odontella longicruris (Hoban, 1983)
		Odontella mobiliensis (Grunow, 1884)
		Odontella sinensis (Grunow, 1884)
		Triceratium favus (Ehrenberg, 1839)
	Thalassiosiraceae	*Thalassiosira eccentrica* (Cleve, 1904)
Chlorophyceae	Clorococcaceae	*Tretraedron tumidulum* (Hansgirg, 1889)
Cyanophyceae	Microcoleaceae	*Microcystis aeruginosa* (Kützing, 1846)

tion 2, while taxa values of 30 were recorded in Stations 1, 3, 5, 6, and 7 (Table 4). The number of individuals with the highest value (9,625) was observed in Station 7, while the lowest value (8,496) was reported in Station 2. The highest value of dominance D (0.049) was reported in Station 4, followed by 0.048 in Station 2, while the lowest value (0.043) was reported in Stations 2 and 7. The Shannon index with the highest value (3.25) was reported in Station 7, followed by Stations 2 and 6 (3.23), while the lowest value (3.17) was reported in Station 3. The highest

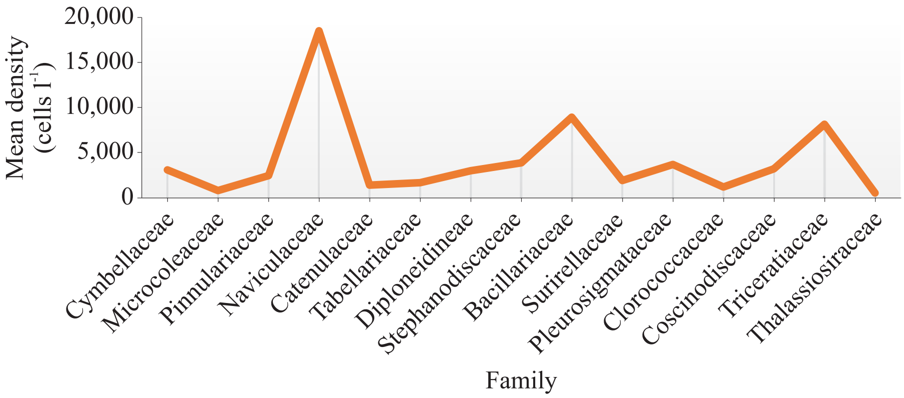

Figure 2. Mean density of harmful algal family in the central Bonny estuary.

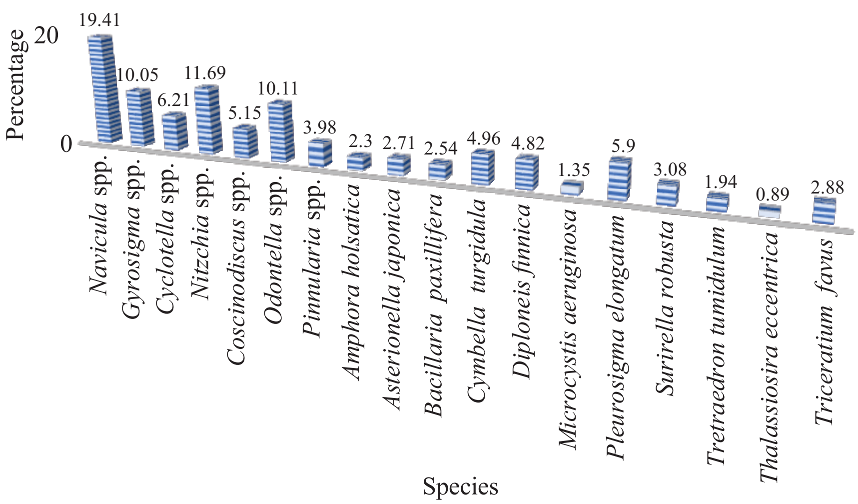

Figure 3. Percentage composition of harmful algal species (HAS) in the central Bonny estuary.

value of evenness (0.88) was registered in Station 2, followed by 0.86 in Station 7, while the least value (0.78) was reported in Station 4. The highest Margalef value (3.31) was reported in Station 4, followed by 3.19 in Station 5, while the least value of 3.09 was reported in Station 2 (Table 4).

DISCUSSION

The pH reported in the central Bonny estuary was well within the preferred pH range limits of 6.5 to 9.0 for optimal fish and aquatic life (Boyd and Lichktopller 1979) recommended by the World Health Organization (WHO 2008). Vincent-Akpu and Nwachukwu (2016) reported a pH value of 7.7 ± 0.1 in Bonny. Valsaraj et al. (1995) reported increased pH on days of extreme photosynthetic activity. The seasonal difference in pH values recorded was in line with results of earlier studies conducted by Dublin-Green (1990) in the Bonny estuary, where lower values of pH were recorded in the rainy season. However, studies by Nweke (2000), Ebere (2002), and Clarke (2005) registered higher pH in the dry season than in the wet season.

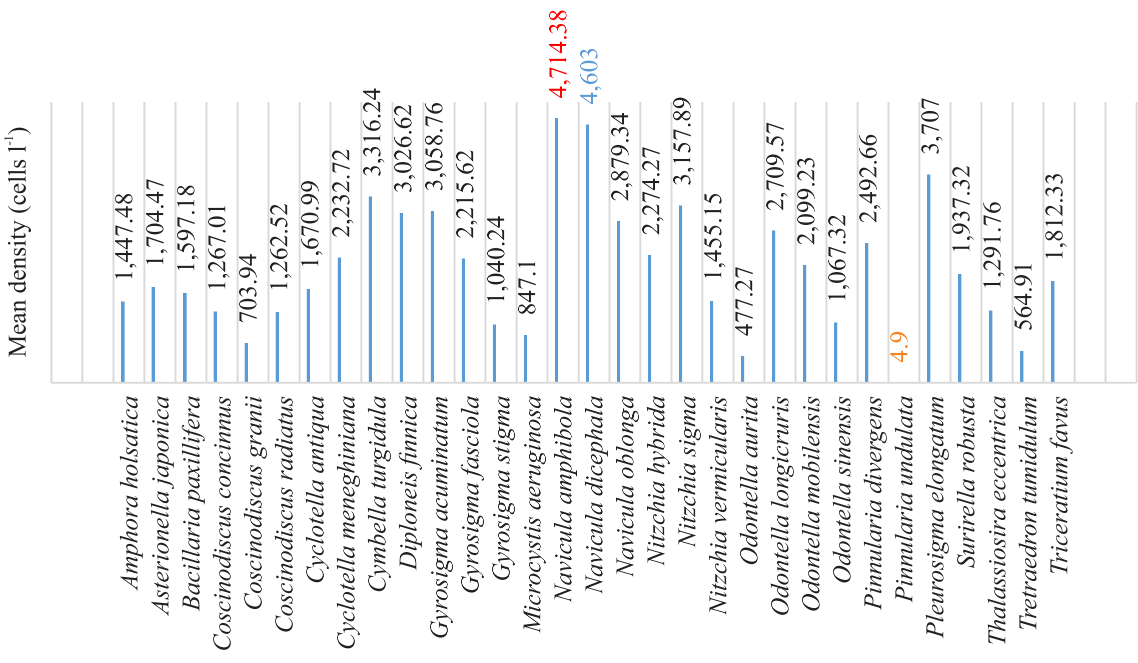

Figure 4. Abundance of harmful algal species (HAS) in the central Bonny estuary.

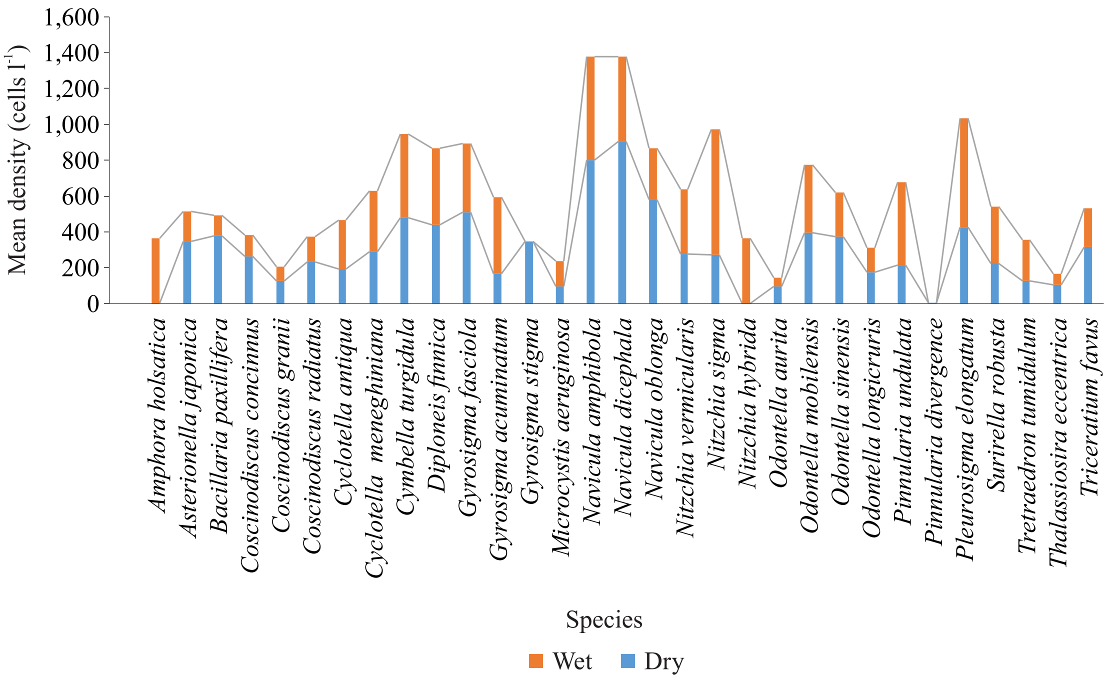

Figure 5. Mean density of harmful algal species (HAS) across seasons in central Bonny estuary.

Table 4. Checklist and diversity of harmful algal species (HAS) in the central Bonny estuary.

Species	Station						
	1	2	3	4	5	6	7
Bacillariophyceae							
Amphora holsatica	-	+	-	+	+	+	+
Asterionella japonica	+	-	+	+	-	+	-
Bacillaria paxillifera	+	+	+	+	-	-	+
Coscinodiscus concinnus	+	+	+	+	+	-	+
Coscinodiscus granii	+	+	-	-	+	-	+
Coscinodiscus radiatus	+	+	+	+	+	-	+
Cyclotella antiqua	+	+	+	+	+	+	-
Cyclotella meneghiniana	+	+	+	+	+	+	+
Cymbella turgidula	+	+	+	+	+	+	+
Diploneis finnica	+	+	+	+	+	+	+
Gyrosigma fasciola	-	+	+	+	+	-	-
Gyrosigma acuminatum	+	-	-	-	+	-	+
Gyrosigma stigma	+	+	-	-	-	-	-
Navicula amphibola	+	+	+	+	+	+	+
Navicula dicephala	+	+	+	+	-	+	-
Navicula oblonga	+	-	+	+	+	-	+
Nitzchia vermicularis	+	-	+	+	+	+	+
Nitzchia sigma	-	+	+	+	+	+	-
Nitzchia hybrida	-	-	-	-	-	-	+
Odontella aurita	+	-	-	-	-	-	+
Odontella mobilensis	+	-	+	+	-	+	+
Odontella sinensis	+	-	+	-	+	+	-
Odontella longicruris	-	+	-	-	-	+	-
Pinnularia undulata	+	-	+	+	+	+	+
Pinnularia divergens	-	+	-	-	-	-	-
Pleurosigma elongatum	+	-	+	+	+	+	+
Surirella robusta	+	+	-	-	+	+	+
Thalassiosira eccentrica	+	-	+	+	+	+	+
Triceratium favus	+	-	+	+	+	+	-
Chlorophyceae							
Tretraedron tumidulum	+	+	+	+	+	+	+
Cyanophyceae							
Microcystis aeruginosa	-	-	+	+	+	-	-

Table 4. Continued.

	Station						
	1	2	3	4	5	6	7
Taxa_S	30	29	30	31	30	30	30
Individuals	9,282	8,496	8,832	8,628	8,903	9,237	9,625
Dominance_D	0.046	0.043	0.048	0.049	0.045	0.044	0.043
Shannon_H	3.21	3.23	3.17	3.19	3.22	3.23	3.25
Evenness_e^H/S	0.83	0.88	0.79	0.78	0.83	0.84	0.86
Margalef	3.17	3.09	3.19	3.31	3.19	3.18	3.16

Temperatures across stations and seasons were normal with reference to their location in the Niger delta. Ansa (2005) stated between 25.9 °C and 32.4 °C. Uedema-Naa et al. (2011) reported a range between 28.94 °C and 29.72 °C. Vincent-Akpu and Nwachukwu (2016) measured temperatures of 28.0 ± 0.5 °C in Bonny. Onwugbuta-Enyi et al. (2008) reported DO values ranged from 4.6 to 11.8 mg l^{-1}. These findings are in contrast with the results of this study, which may be due to seasons. Davies et al. (2008) also stated reduced DO in the wet season as compared to the dry season and attributed it to a decrease in photosynthetic events of algae, which agrees with the result of this study. The reason for the reduced mean DO values was attributed to the turbidity of the water due to influxes from run-offs and degeneration of waste in the water. Water with DO above 6 mg l^{-1} will sustain fish and desirable forms of aquatic biota, whereas water with 2 mg l^{-1} DO will support mainly decomposers.

The present salinity and conductivity records showed a similar trend within the acceptable range for coastal waters. Chindah and Nduaguibe (2003) obtained salinity values from 11.5 ± 1.8 to 20.3 ± 3.0 in the lower Bonny. Clarke (2005) registered higher salinities in the dry season than in the wet season, which is in line with the results of this study. Dibia (2006) described conductivity values increasing during the dry season due to absorption of ions. Values of BOD recorded in the study are within the tolerable range for aquatic environments (WHO 2008). Vincent-Akpu and Nwachukwu (2016) reported a lower value of 2.80 mg l^{-1} in Nembe and 2.50 mg l^{-1} in Bonny estuary. Also, the observation of Braide et al. (2004) on water quality in the Eastern Niger delta showed that the BOD load in this study did not pose a hazard to the aquatic environment. Boyd (1981) reported that turbidities in natural waters rarely go beyond 20,000 mg l^{-1} and even muddy waters frequently have less than 2,000 mg l^{-1}. Also, the observed turbidity level in this study corroborates the range of 2 NTU to 47 NTU stated by Asonye et al. (2007). Turbidity from plankton is not harmful to fish when it is at a mild level. Fish harvesting is made easier as they are less suspicious (Swann 2006). Roelke et al. (2007) reported that stability of light energy is expected to regulate algae ecosystem structure. Vincent-Akpu and Nwachukwu (2016) reported TDS values of 13.1 mg l^{-1} in Nembe and 14.9 mg l^{-1} in Bonny estuary. The higher total dissolved organic solid concentration observed in this study may be ascribed to high surface runoff, overland flow, as well as higher release of organic wastes into the river.

Higher phosphate values were recorded in the wet season than in the dry season, which is in contrast with the findings of Chinda and Braide (2001), who reported higher phosphate in the dry season. This may be ascribed to the higher biomass of phytoplankton and epiphyton in the wet season. Natural inputs from decay of organic matter might be a contributor to the high phosphate levels in this estuary. Davies et al. (2009) and Davies (2013) recorded a higher nitrate value in the dry season than in the wet season, which is in line with the finding of this study and might be ascribed to high anthropogenic inputs. Nitrate does not pose a health threat, but it is readily reduced to nitrite by the enzyme nitrate reductase, which is widely distributed and abundant in both plants and microorganisms (Glidewell 1990).

Abowei et al. (2012) reported algae families including Baccillariophyceae, Dinophyceae, Chlorophyceae, and Cyanophyceae along the shor eline of Koluoma Creek in Bayelsa. Baccillariophyceae were the dominant and constituted 60% of the phytoplankton biomass. Babu et al. (2013) recorded 101 phytoplankton species on India's East-west coast, in which 76 species corresponded to Bacillariophyceae, 17 to Dinophyceae, 5 to Cyanophyceae, 2 to Chlorophyceae), and 1 to Chrysophyceae.

According to Elliot (2010) the distribution pattern of phytoplankton of Black Volta waters in Ghana showed that all species, except two species of *Euglena* sp. and *Phacu spyrum* (Euglenophyceae), were fairly distributed in the four hydrological seasons. He further stated that the impoundment of Black Volta might, however, be the main factor responsible for the discontinuous seasonal distribution of *Euglena* sp. and *P. spyrum* observed in the study. The result was similar to other research indicating that Bacillariophyceae were the dominant genera in water samples (Badsi et al. 2012). Abubakar (2009) stated that in tropical regions, dry and rainy seasons showed distinct fluctuations with an abundance of algae. Swann (2006) reported that algae was among the reasons for turbidity, as high turbidity during the rainy season was probably attributed to runoff. Iqbal et al. (1990) reported that monthly variability in algal population resulted in major seasonal disparity in the physicochemical parameters in Hub Lake. The higher abundance during the wet season was due to nutrients and the water level at the time. This finding is in contradiction to the results of this study. Total density values decreased across seasons, with 9.157×10^3 cells l^{-1} and 8.907×10^3 cells l^{-1} recorded in the dry and the wet season, respectively. Seasonal differences in algal abundance in the dry season have also been reported by Erondu and Chinda (1991) and Ogamba et al. (2004) in the Niger delta. Indabawa and Abdullahi (2004) also recorded higher algal cells in the dry season than in the rainy season.

Diversity is dependent on key ecological practices such as competition, predation and succession, therefore, changes in these processes can alter the species diversity index through modifications in evenness (Stirling and Wilsey 2001). According to the classification of the Shannon-Wiener index, if the diversity index is lower than 1, then biota communities would be regarded as unstable, whereas a diversity index of 1-3 would be considered moderately stable, and a value higher than three would signify a stable or prime condition (Mokoginta 2016). Shannon-Wiener indices above three recorded in most of sampled stations confirmed that these stations were moderately stable and not under pollution stress, suggesting that central Bonny estuary is relatively vulnerable to environmental changes. Ofonmbuk and Lawrence (2015) reported low Margalelf's diversity values from 2.871 to 3.513 in the Qua Iboe estuary. This was similar to 2.93 reported by Ogbuagu and Ayoade (2012), which is in agreement with the findings of this study. This indicated that the harmful algal community was stable among seasons in the study area. Minimal variations in the density of harmful algal species as reflected by Shannon-Wiener, Pielous evenness, and Margalef species richness, may be ascribed to uniform physical and chemical conditions (Ogamba et al. 2004). The high diversity of values could be attributed to the influence of bunkering activities, which are highly likely in the estuary, as also reported by Adesalu and Nwankwo (2008).

CONCLUSIONS

This study provides clear information regarding the level of diversity and environmental gradients in relation to species abundance and distribution in the central Bonny estuary. Observed cell densities serve as an early warning for future bloom occurrences of potential impacts if the density increases significantly beyond a determined threshold. In addition to effective education, dissemination, and communication of the available information, there is a need for an adequate monitoring program warning on the formation of harmful algal blooms which requires easy regulation of the aquatic resource in the central Bonny estuary.

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Declaration of interest

The authors declare that there is no conflict of interest.

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ORIGINAL RESEARCH

Microplastics found in the World Heritage Site Cocos Island National Park, Costa Rica

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ABSTRACT. Microplastics (MPs) defined as 'small' pieces of plastic < 5 mm have been found in almost every marine habitat around the world, and studies have shown that we can find them in the ocean surface, the water column, the seafloor, the shoreline, in biota and in the atmosphere-ocean interface. This study aimed to assess both marine and freshwater environments of Cocos Island, Costa Rica, in the Pacific Ocean, by sampling sediments and biota to determine the presence and abundance of this pollutant. Sediment samples were superficial and weighed one kilogram each. For the sampling of freshwater fish and shrimps, nonselective capture with small nets was made in rivers with access by land, while fishing rods were used for the marine fish sampling, and cage and scuba diving for lobsters. Plastics were found in all types of samples: 93% of marine sediments, 32% of freshwater sediments, 20% of freshwater fish, 15% of freshwater shrimps, 27% of marine fish, and 51% of marine lobsters. Like many reports around the world, it was expected to find MPs at marine samples, and it was concluded that ocean currents, tourism activities, and discarded fishing gear from illegal fishing activities could be the sources of marine pollutants. In contrast, the amount of MPs found in freshwater environments was not expected. Their possible sources are unclear at this moment.

Key words: Marine ecosystem, freshwater ecosystem, sediments, oceanic island, fish, lobsters, shrimps.

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Microplásticos encontrados en el Parque Nacional Isla de Cocos, Patrimonio de la Humanidad, Costa Rica

RESUMEN. Los microplásticos (MP), definidos como "pequeñas" piezas de plástico < 5 mm, se han encontrado en casi todos los hábitats marinos del mundo, y los estudios han demostrado que podemos encontrarlos en la superficie del océano, en la columna de agua, en el fondo marino, en la costa, en la biota y en la interfaz atmósfera-océano. Este estudio tuvo como objetivo evaluar los ambientes marinos y de agua dulce de la Isla de Cocos, Costa Rica, en el Océano Pacífico, mediante el muestreo de sedimentos y biota para determinar la presencia y abundancia de este contaminante. Las muestras de sedimento fueron superficiales y pesaron un kilogramo cada una. Para el muestreo de peces de agua dulce y camarones, se realizó una captura no selectiva con redes pequeñas en ríos con acceso por tierra. Para el muestreo de peces marinos se utilizaron cañas de pescar, y para langostas se utilizaron jaula y buceo con escafandra autónoma. Se encontraron plásticos en todo tipo de muestras: 93% de sedimentos marinos, 32% de sedimentos de agua dulce, 20% de peces de agua dulce, 15% de camarones de agua dulce, 27% de peces marinos y 51% de langostas marinas. Al igual que muchos informes en todo el mundo, se esperaba encontrar MP en las muestras marinas, y se concluyó que las corrientes oceánicas, las actividades turísticas y los aparejos de pesca desechados por actividades de pesca ilegal podrían ser fuentes de contaminantes marinos. Por el contrario, no se esperaba la cantidad de MP encontrada en ambientes de agua dulce. Sus posibles fuentes no están claras hasta el momento.

Palabras clave: Ecosistema marino, ecosistema de agua dulce, sedimentos, isla oceánica, peces, langostas, camarones.

INTRODUCTION

Anthropogenic litter on the marine environment has significantly increased over the recent decades. Initially described in the marine environment in the 1960s, marine litter is nowadays commonly observed across all oceans (Bergmann et al. 2015). Plastic, the main component of litter, has become ubiquitous and sometimes represents up to 95% of the waste that accumulates on the shorelines, the sea surface and the seafloor. Together with its breakdown products, mesoplastics (5-25 mm) and microplastics (< 5 mm) (GESAMP 2019) have become more abundant in the marine environment than any other pollutant (Bergmann et al. 2015).

The term ‘plastic’ is used in many fields and has different definitions. For the purpose of this study, it is defined as a sub-category of the larger class of materials called polymers, including thermoplastics and some thermoset materials such as polyurethane foams, epoxy resins, and some coating films that are generally counted within the category of ‘plastics’ in marine debris (GESAMP 2015). Traditionally, the term microplastic (MP) has been widely adopted as a generic form for ‘small’ pieces of plastic (< 5 mm) (Andrady 2011; Gewert et al. 2017; Miller et al. 2017; Herrera et al. 2018; Froese and Pauly 2021).

There are primary and secondary sources of MPs. The difference relies on whether particles are originally manufactured to be < 5 mm (primary) or if they are a result from the breakdown of larger pieces of plastic (secondary) (GESAMP 2015; UNEP and GRID-Arendal 2016). Some primary MPs include production pellets/powders and engineered plastic microbeads used in cosmetic formulations, cleaning products, and industrial abrasives. On the other hand, secondary MPs are degraded and then fragmented, such as textile fibers, tire dust, and water bottles, among others (UNEP and GRID-Arendal 2016).

Visual characterization is the most used method for the identification of MPs (using size, type, shape, and color as criteria). The minimum resolution is allocating into bin sizes of 100 µm (Hanke et al. 2013). Type categories are usually defined as fragments, pellets, filaments, films, foamed plastics, granules and Styrofoam. Colors are diverse and have been reported as transparent, crystalline, white, clear-white cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, and yellow (Hanke et al. 2013).

Studies on MP pollution in marine environments have received significantly greater attention compared to those of freshwater and terrestrial environments. Sampling efforts have been done in the beaches, water column, ocean surface, subtidal sediments and biota (Zobkov et al. 2018; Gola et al. 2021; Ugwu et al. 2021). However, in recent years, studies have expanded to freshwater and terrestrial ecosystems (mainly surface water and sediments) (Wong et al. 2020; Baho et al. 2021).

Global effects of MP pollution have even been documented in remote regions, such as the snow from mountains (Free et al. 2014; Napper et al. 2020), the Arctic (Bergmann et al. 2019) and arctic polar waters (Bergmann and Klages 2012; Lusher et al. 2015), and deep-sea sediments (Van Cauwenberghe et al. 2013), to mention a few. In high-altitude remote areas, the presence of MPs is mostly due to atmospheric transportation (by wind, storm, or rain); therefore, MPs can easily reach different isolated ecosystems (Allen et al. 2019) and spread into terrestrial systems (Rilling 2012). Also, remote coastal areas, where local pressures are low or even absent, are expected to be less affected by environmental pollution (Zhao et al. 2015; Çomakli et al. 2020). Additionally, there is increasing evidence of MP pollution in remote coral reef systems (Imhof et al. 2017; Ding et al. 2019; Tan et al. 2020). These remote systems are considered healthy ecosystems now facing the potential threats of the emerging MP contaminants as well. Despite this, little is known about key issues such as the spatial distribution of MPs within these remote uninhabited areas or the possible sources and input pathways of MPs into these regions (Tan et al. 2020).

The research of MPs in Costa Rica is just beginning, and the information is scarce. Currently, Costa Rica has only two studies of MPs in marine organisms. The first report of MPs was in a sample of 30 sardines from the Family Clupeidae, with *Opisthonema libertate* (filter feeders' fish) from the Pacific coast. Researchers detected MPs in all individuals with an average of 36.7 pieces per fish, 79.5% were microfibers and 20.5% were other types of plastic particles (Bermúdez-Guzmán et al. 2020). In another study a year later from the Pacific coast, 27 individuals from seven different species of fish from higher trophic levels were sampled. Eighty-nine percent of the fish had MPs, with an average of 3.75 MPs per fish and 93% of these particles were microfibers. Also, they sampled 29 benthonic carnivorous crabs (*Callinectes arcuatus*), 76% of which had MPs with 2.64 MPs per crab, and 93% microfibers (Astorga-Pérez et al. 2022).

Moreover, Cocos Island National Park is the only oceanic island of Costa Rica, located 535 km from the Costa Rican Pacific coast, far away from any populous city. The highest elevation point is Cerro Iglesias with an altitude of 575.5 m above sea level. The island is covered by tropical rainforest and its average annual precipitation varies between 4,500 to 6,000 mm (Alfaro 2008). Herrera (1985) suggested that the high precipitation is because the island is strongly influenced by the north-south movement of the Inter-Tropical Convergence Zone. The island is drained by three main watersheds: the Genio River, which flows north and empties into Wafer Bay; the Iglesias River, which flows from north to south and empties into Iglesias Bay; and the Lièvre River watershed, which flows from east to west and empties into Chatham Bay. In addition, the hydrographic network of this island is formed by permanent rivers and streams, which differentiates it from other oceanic islands located in the Eastern Tropical Pacific, such as those from the Galapagos archipelago, which are more arid (Bergoeing 2012; Gutiérrez-Fonseca et al. 2013).

Furthermore, Cocos Island is regarded as one of the few effective Marine Protected Areas around the world and it has become famous because of its large aggregations of pelagic species (Naranjo-Elizondo and Cortés 2018). Apart from a few park rangers and some facilities provided for regular visitors such as researchers and volunteers, the island is almost inhabited (Díaz-Bolaños et al. 2012). Because of this, plastic residues in the National Park could be generated from daily human activities (e.g. cooking, cleaning, among others), the majority of which happen at the Wafer base. Besides this local production of plastic residues, the confiscation of illegal fishing gear around the island and marine debris carried by marine currents to the coasts of Cocos Island National Park could also be important sources of plastic pollution (SINAC 2017).

Because Cocos Island is a remote island with low anthropogenic influence, the aim of this research was to assess the presence and abundance of MPs and estimate differences between terrestrial and aquatic ecosystems regarding this pollutant. This study examined sediments and biota from both ecosystems.

MATERIALS AND METHODS

Sampling site

Cocos Island is part of the Cocos Marine Conservation Area, a site managed by the Costa Rican National System of Conservation Areas (Sistema Nacional de Áreas de Conservación, SINAC). This island with a surface area of 24 km^2 possesses an extensive diversity of ecosystems at both land and marine levels, where the cloud forest and coral reefs predominate (Díaz-Bolaños et al. 2012; Alvarado et al. 2016). It has abundant fresh water with numerous streams that flow along the coast surrounding the two main rivers of the island (Genio River and Iglesias River). The legal category of this protected area only allows diving activities, while hunting, fishing and any other activity threatening the different ecosystems' health are banned (González-Andrés et al. 2020).

Two field expeditions were conducted to obtain samples. During the first expedition (June 18th to July 7th 2019 under Permission 2019-I-ACMC-08) most of the samples of sediments, freshwater fish, freshwater shrimps and marine fish were collected (Figure 1). Later, in the second expedition (October 3th to October 15th 2020 under Permission 2020-I-ACMC-08) the majority of the marine lobster samples were collected.

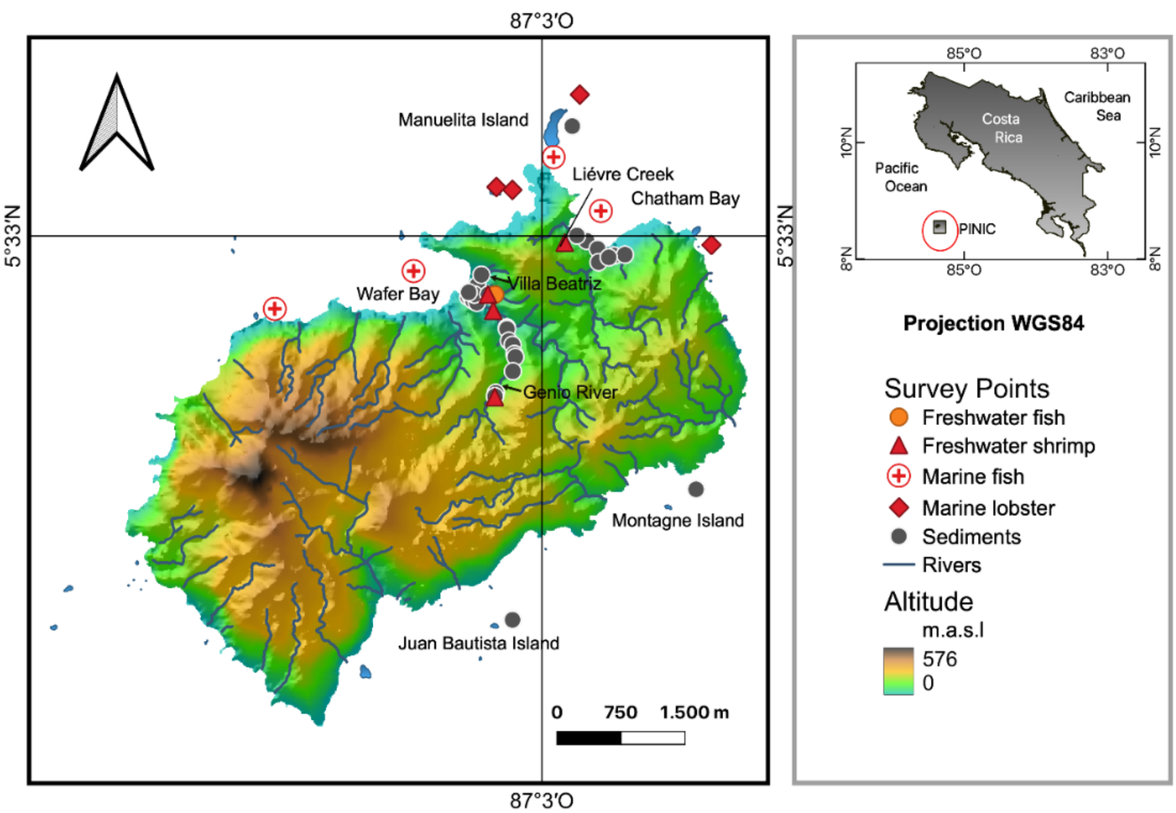

Figure 1. Collection sites and sample types from Cocos Island (m.a.s.l = meters above the sea level).

Sample collection

Marine and freshwater sediments

All sediment samples were superficial and weighed one kilogram each. They were sampled by taking approximately the top 5 cm of the sediment in a 50 50 cm square with a clean stainless steel hand trowel and stored in metal containers previously washed with distilled water. Metal containers were stored in freezers at 0 °C prior to processing (Herrera et al. 2018).

Freshwater organisms

All freshwater fish species were indistinctly caught with small nets in the rivers, which were accessed by land. Fishing nets were also used at two more sites, Liévre creek and Genio River, for freshwater shrimps sampling.

Marine organisms

Fishing rods were used for the marine fish sampling at the north side of the island due to unfavorable environmental conditions on the south side of the island. Captured freshwater and marine fish were placed in coolers with ice for transportation to the processing point in the island. All instruments were sterilized with alcohol and quantitatively washed with distilled water. Tables were covered with bags and cotton garments to avoid cross-contamination. Collected organisms were weighed (g) and measured (total body length in cm) (Table 2). Subsequently, lobsters and fish were dissected to remove the entire gastrointestinal tract (GIT) following the procedures described by Boerger et al. (2010) and Lusher et al. (2013). Extracted GITs, composed by stomach, intestine, liver, pancreas, and pyloric cecum were weighed separately and preserved in glass containers with 70% alcohol. In the case of freshwater shrimps, the digestive system could not be extracted due to their small size, therefore, the exoskeleton was carefully removed and the whole animal was preserved in glass containers with 70% alcohol.

Sample processing

In order to prevent and/or reduce potential contamination from external sources, such as airborne fibers, the laboratory workspace was frequently cleaned, and work was performed in a laminar airflow cabinet, particularly for preparing solutions, sieving and filtrating, when possible. In addition, glassware was washed thoroughly, oven-dried and covered with aluminum foil when not in use.

Sediments

For sediment samples, a density separation and filtration method using aqueous solutions was performed. The aim of this process was to utilize density differences to separate different types of polymers from organic and inorganic natural particles such as the sediment, sand or silt particles (Kershaw et al. 2019). One kilogram of every sediment sample was mixed with a NaCl solution (1.2 g cm^{-3}) and stirred for at least 2 h for sand samples, and 24 h for silty sediment samples (Hidalgo-Ruz et al. 2012; Qiu et al. 2016; Martin et al. 2017; Enders et al. 2020). After agitation, the sample was allowed to settle (covered with aluminum foil) for 24 h, allowing denser constituents to sink and less dense particles to float or to remain in suspension. After 24 h, the supernatant was filtered with a Büchner funnel and passed through a 10 μm retention glass fiber filter paper. In most cases, a triplicate was required while filtering since the presence of silt made the process difficult. Filter papers were removed in a laminar cabin and stored in sealed Petri dishes prior to examination under a stereo microscope.

Organisms

Freshwater shrimps and GITs of lobsters, marine and freshwater fish, were chemically digested to extract MPs. Extraction was carried out according to the method described by Cole et al. (2014), Kühn et al. (2017), and Bessa et al. (2019). A solution of 10% KOH to digest the organic matter was added. The volume of the liquid did not exceed 50% of the total volume of the Erlenmeyer (250 or 500 ml). To obtain a dissolved solution, Erlenmeyers were covered with aluminum and placed in an oscillating incubator at 60 °C at 300 rpm for 24 h. Subsequently, the digested content from the chemical process was sieved through a 60 µ stainless steel sieve and transferred to a clean Petri dish. The excess of water was evaporated in an oven at 45 °C for 30 h. Glass Petri dishes were covered with aluminum foil with small holes to allow water to evaporate and prevent possible airborne plastic contamination (Enders et al. 2020).

Identification and validation of microplastic

All particles were identified, measured, and photographed using a stereo microscope OPTIKA SZ-ST2 with image analysis system AMSCOPE MU1000 Camera with AMPSCOPE software. Plastic particles < 5 mm were classified as MPs; if their size was > 5 mm they were excluded from the analysis (Andrady 2011). They were also classified by type as fibers (elongated), fragments (irregular pieces), pellets or films (thin and transparent) and categorized by their color (Hidalgo-Ruz et al. 2012; Qiu et al. 2016; Martin et al. 2017; Enders et al. 2020). Knots (fragments of fishing nets between 5-25 mm) were photographed and counted into the frequency of occurrence but not considered in the calculation of the average MPs/lobster, since they are not considered MPs.

Quality control of experiments

Glassware, plastics and dissection tools were rinsed three times with distilled water to reduce possible contamination (Li et al. 2015; Lusher et al. 2015). Tap water, saline water and sodium hydroxide were filtered with a 1 mm glass fiber filter before use, and samples were covered with aluminum foil to prevent any kind of pollution. To prevent contamination by airborne MPs, sample handling was performed in a laminar flow cabinet (Zhang et al. 2017; Mason et al. 2018; Oßmann et al. 2018; Wang et al. 2018). Negative controls (Jabeen et al. 2017) were carried out during sodium hydroxide treatments, observation, identification and validation of MPs, resulting in a total of 22 controls. All particles identified in these controls were fibers, and any similar particles found at sediments and tissues samples were excluded from the analysis.

Statistical analysis

The number of MPs data in different organisms and ecosystems were not normally distributed according to the Shapiro normality test at 95% of confidence. Therefore, a Mann-Whitney Test for two independent samples were performed to determine differences of MPs abundance between marine and freshwater sediments, marine fish and lobsters, freshwater fish and shrimps, marine and freshwater fish, and marine lobsters and freshwater shrimps. Statistical analyses were performed using R Statistical Software (R Core Team 2020).

RESULTS

All types of samples resulted positive for the presence of MPs: 93% of marine sediments, 32% of freshwater sediments, 27% of marine fish, 20% of freshwater fish, 51% of marine lobsters, and 15% of freshwater shrimps. In addition, two types of MPs were observed: fibers and fragments (see Supplementary Material for the most representative images of MPs found). In marine lobsters, pieces bigger than > 5 mm were found, photographed, classified as fibers and knots but excluded from statistical analysis (Appendix, Figure A1).

Contamination from the laboratory was detected from 22 contamination controls. An average

2.3 ± 2.0 plastic/control was determined. Particles found in negative controls were fibers with sizes > 5 mm. These particles could be derived from the air pollution in the laboratory, although many sources of contamination were avoided. Fibers that were consistent in shape and color in the controls were not considered in any sample.

Sediment samples

Marine sediment samples were collected from sandy beaches (14 samples) and shallow water (4 samples) at different locations. In addition, 14 freshwater sediment samples were collected from Genio River, Villa Beatriz creek, and Liévre creek (Table 1). All sediment samples were taken at depths less than 10 m. A frequency of occurrence of 93% was obtained in marine ecosystems with an average of 3.35 ± 4.30 MPs per sample, and 32% in freshwater ecosystems with an average of 1.00 > 1.47 MPs per sample (Figure 2). Mann-Whitney Test showed a significantly higher quantity of MPs in marine ecosystems (p = 0.025) when comparing it to the freshwater ecosystems.

Organism samples

For marine fishes, a total of 31 Jordan's snappers (*Lutjanus jordani*) from the Family Lutjanidae were caught. For freshwater fishes a total of 30 organisms from four families (Gobiidae,

Table 1. Locations and sediment textures of sampling sites located in marine and freshwater sediments of Cocos Island.

Location site	Sediment texture	Number of samples
Wafer Bay	Sandy beach	10
Liévre creek Bay	Sandy beach	4
Montagne Island	Shallow water	1
Manuelita Island	Shallow water	1
Juan Bautista Island	Shallow water	1
Liévre creek Bay	Shallow water	1
Genio River	Muddy sediments	10
Villa Beatriz creek	Muddy sediments	3
Liévre creek	Muddy sediments	1

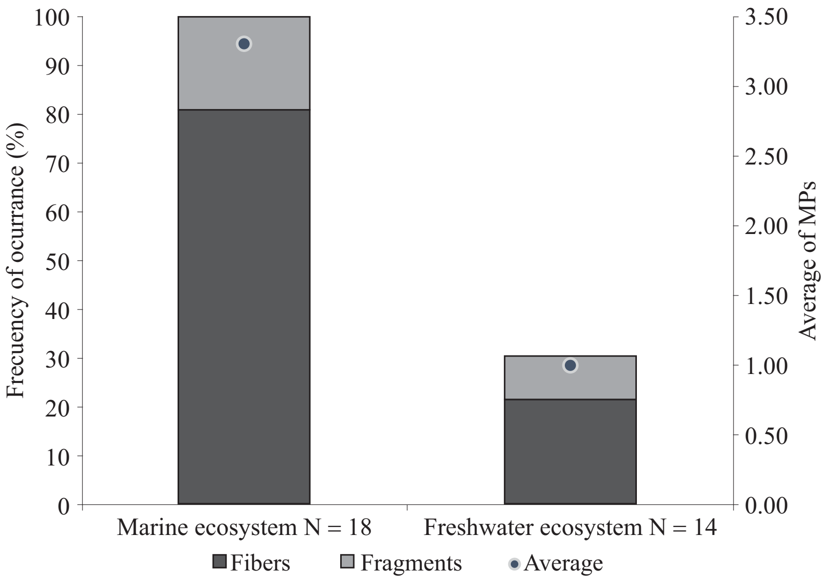

Figure 2. Comparison of microplastics (MPs) in sediment samples between marine and freshwater ecosystems from Cocos Island, Costa Rica.

Mugilidae, Eleotridae and Gobiesocidae) were captured. Species from each family were respectively: eleven organisms *Sicydium cocoensis*, nine organisms *Dajaus monticola*, six organisms *Eleotris picta* and four organisms *Gobiesox fulvus.* During the first expedition, 14 green spiny lobsters of the species *Panulirus gracilis* (Family Palinuridae) were captured with traps. Because of high predation by whitetip reef sharks in the traps, in the second expedition the lobsters were captured directly through scuba diving, resulting in 39 lobsters from the same Family Palinuridae: 11 *Panulirus gracilis* and 28 *Panulirus penicillatus.* Also, a total of 32 shrimps of the Genus *Macrobrachium* sp. were captured. Table 2 describes the characteristics of the captured organisms.

A frequency of MPs occurrence of 27% was obtained in marine fishes, with an average of 1.37 ± 0.51 MPs per organism (Figure 3). A total of 11 MPs were found, 82% of them were fibers, while only 18% were fragments (Table 3). Ninety percent of the MPs had a mean size < 3 mm and the main color was black, followed by red and blue. In the case of freshwater fishes, a frequency of occurrence of 20% was obtained with an average of 1.16 ± 0.40 MPs per organism. A total of 7 MPs were found, 57% of them were fibers, while 43% were fragments. All sizes of the MPs were < 3 mm and the main color was blue, followed by black and red.

In marine lobsters, a frequency of occurrence of 51% was obtained with an average of 1.42 ± 0.75 MPs per organism. A total of 18 MPs were found, 56% of them were fibers, while 44% were fragments. Eighty-three percent of the MPs found in marine lobsters had mean size < 3 mm, but they were the only organism with three pieces bigger than > 5 mm (11.25 mm, 10.22 mm, and 9.85 mm), identified as knots but excluded from the analysis, since they are not considered MPs

Table 2. Morphometric characteristics of the organisms collected in the Cocos Island National Park to determine the presence of microplastics (MPs) in their tissues.

Fish species	N	Characteristics	
		Average of body weight (g)	Average of total length (cm)
Marine fishes			
Lutjanus jordani	30	567 ± 109	35 ± 2 (31.6-81.5)
Freshwater fishes			
Dajaus monticola	9	5.9 ± 3	13 ± 6 (7.5-23.5)
Eleotris picta	6	205 ± 168	25 ± 6 (19.5-34.1)
Gobiesox fulvus	4	14 ± 3	11 ± 4 (8.4-17)
Sicydium cocoensis	11	13 ± 9	9 ± 2 (5.3-11.5)
Marine lobsters			
Panulirus gracilis	23	302 ± 87	10 ± 1 (7.8-12)
Panulirus penicillatus	11	650 ± 340	11 ± 3 (7.3-19)
Freshwater shrimps			
Macrobrachium sp.	39	4 ± 6	5 ± 2 (3.5-13.3)

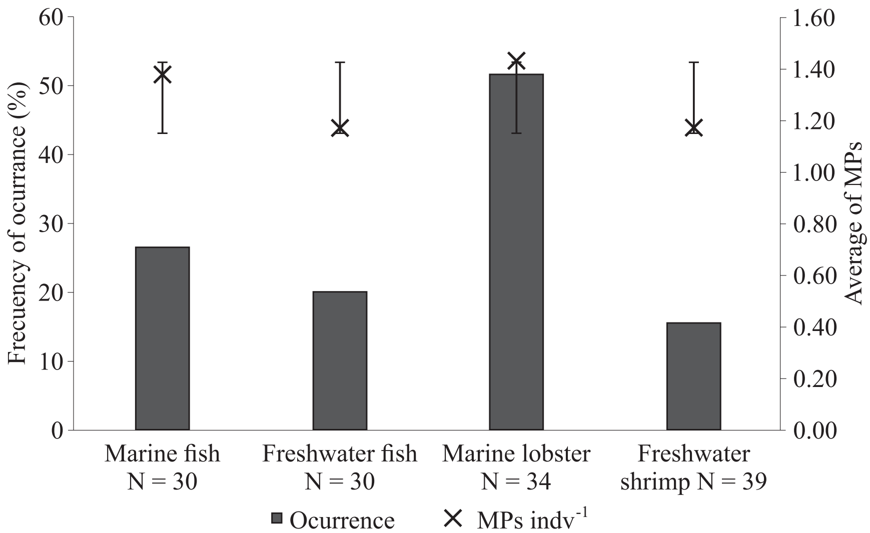

Figure 3. Microplastics (MPs) found in marine and freshwater organisms at Cocos Island National Park, Costa Rica.

Table 3. Types, sizes, and colors of microplastics found in the biota of Cocos Island National Park, Costa Rica.

Characteristic	Classification	Marine fish (%)	Freshwater fish (%)	Marine lobster (%)	Freshwater shrimp (%)
Form*	Fibers	82	57	56	100
	Fragments	18	43	44	0
Size	< 1mm	45	57	83	67
	1-3 mm	45	43	17	33
	3-5 mm	9	0	0	0
Color	Red	36	14	28	33
	Black	45	29	22	17
	Blue	18	43	22	33
	Others	0	14	28	17*

*Even though other categories of forms were considered in this research, such as fiber, fragment, films and pellet, only two types of MPs were found (fibers and fragments). Predominant colors were blue, black and red. Category 'other' included green, transparent and white.

(Appendix, Figure A1). The main color of MPs in marine lobsters was red. In freshwater shrimps, the frequency of occurrence was 15% with an average of 1.16 ± 0.40 MPs per organism. A total of 6 MPs were found, 100% of them were fibers. Mean size of the MPs were all < 3 mm and the main color was blue and red, followed by black.

Microplastics were observed in all organisms. The number of MPs between marine and freshwater fish was not statistically significant (Mann-Whitney Test, p = 0.4895). The abundance of plastics by items/individual was significantly higher in marine lobsters than in freshwater shrimps (Mann-Whitney Test, p = 0.0056).

DISCUSSION

Cocos Island National Park is sparsely populated and far away from the continent and big cities; despite that, MPs were found at both freshwater and marine ecosystems. Furthermore, higher frequency and quantity of MPs were found in marine sediments compared to freshwater sediments. In the marine environment, the presence of MPs has been demonstrated in a wide diversity of regions and concentrations because of their persistence and long-range transportation by wind, rainfall and/or currents. Particularly, beaches and subtidal sediments appear to function as sinks for MPs (Duis and Coors 2016; Katare et al. 2021; Nammalwar 2021).

Studies of the surface circulation in the South Pacific Ocean have shown an accumulation of debris in the eastern-center region of the South Pacific Gyre (Martinez et al. 2009). Also, large debris concentrations were found just north of the North Pacific Transition Zone within the North Pacific Subtropical Convergence Zone (Pichel et al. 2007). Cocos Island is located between these two giant gyres (North Pacific and South Pacific), which could be important sources of MPs. In fact, the California Current, which can carry materials from the North Pacific Gyre, is the possible source of MPs of the north coast of Costa Rica (Johnson et al. 2018).

A lower abundance of MPs was expected in samples from terrestrial ecosystems, but an occurrence of 32% was determined in sediments, 20% in freshwater fish, and 15% in shrimps. According to Lwanga et al. (2016), the majority of MPs particles entering the freshwater are primarily from i) secondary plastics generated by the breakdown of larger plastic items (single-use packing, tires, fibers from synthetics fabrics and road paint particles); and ii) effluent discharges from wastewater and sewage. Additionally, it was determined that population density and quality of waste management can be established as the key anthropogenic factors affecting the presence and abundance of MPs in the freshwater environment (Free et al. 2014; Lwanga et al. 2016). Nevertheless, due to the low density of people that inhabit the island (SINAC 2017) and the pristine origin of the island's rivers, the sources of plastic contamination in freshwater ecosystems remain unclear.

One possible source are MPs from atmospheric transportation by wind and/or storms. The tropical area where the island is located receives large volumes of annual rainfall, and its humidity comes from the Pacific Ocean (Alfaro 2008). Recently, it has been demonstrated that MPs can travel within the air as 'urban dust' (Dehghani et al. 2017; Dris et al. 2017), which usually originates from road dust from tires, paint particles, or fibers from synthetic textiles (GESAMP 2015; Dris et al. 2017; Horton et al. 2017). Also, studies on atmospheric fallout in Paris, France (Dris et al. 2016) and Dongguan, China (Liqi et al. 2017) suggest an atmospheric MPs conveyance and subsequent deposition. Remote and pristine areas are also affected. Research in a remote area of the Pyrenees mountains provided evidence of direct atmospheric fallout of MPs deposition (Allen et al. 2019). Additionally, the detection of MPs in glacier surface snow collected from an isolated area from human impact on the Tibetan Plateau, indicated that MPs can be transported over long distances (Zhang et al. 2021).

The majority (63%) of the MPs found in organisms were classified as microfibers, this result is consistent with those of Celik (2021), Makhdoumi et al. (2021) and Pan et al. (2021). According to the literature, microfibers are considered as the major marine pollutant throughout the world. Some authors estimate a million tons of coastal synthetic fabric waste entering the ocean each year, affecting different marine ecosystems. They also point out that there is an urgent need for development of cost-effective and efficient remediation technologies, legislative action towards the source and public awareness (Mishra et al. 2019; Singh et al. 2020).

The present study analyzed two groups of organisms in marine and freshwater ecosystems: fish and crustaceans, whose different feeding habits, behaviors and habitats, play important roles in the ingestion of debris. Indeed, an increase in the abundance of plastics will also increase the bioavailability of this pollutant to other organisms (Boada et al. 2015; Jabeen et al. 2017). The marine fish analyzed (*Lutjanus jordani*) are usually found over hard bottoms in the inshore reef areas and are carnivorous, feeding mainly on invertebrates and smaller fish (Fischer et al. 1995; Bussing and López 2005). The freshwater fish were caught in shallow and turbulent rivers, and the different genus of the species found are reported to feed on zooplankton, algae, and small benthic invertebrates (Bussing 1998). Freshwater fish caught are considered 'benthonic fish' and the MPs found in these organisms could be related to heavy plastics in the benthic zone, unlike marine fish that consume plastics floating in the water column. Marine lobsters and freshwater shrimps are both benthic macroinvertebrates associated to rocky bottoms, hence they are more exposed to the debris deposited on the sea bottom or stream bed (Naranjo 2011; Figueroa and Mero 2013; García-Guerrero et al. 2013).

It could be predicted that marine lobsters will have a higher exposure to debris than freshwater shrimps. Naranjo-Elizondo and Cortés (2018) found anthropogenic debris at Cocos Island, between 200 and 350 m depth, from which 60% of the items were plastics from local boats and fishing gears. Fishing gears comprised lost lines and most of fishing debris observed in contact with fish or crabs (Naranjo-Elizondo and Cortés 2018). These authors' concern about the possible plastic ingestion by different organisms eventually confirmed it with the present study. Nylon fishing knots found inside marine lobsters' digestive system were > 5 mm (11.25 mm, 10.22 mm, and 9.85 mm), and were in the process of conversion to MPs. Only a higher number of items per individuals was determined in marine lobsters versus freshwater shrimps. This coincides with the result of a higher number of MPs in marine sediments versus freshwater sediments. This last could be influenced by the feeding mode of each organism and the abundance of MPs in the habitat in which they are found.

Statistical analyzes did not detect differences in the type of plastic particles ingested by marine and freshwater organisms. Therefore, investigation regarding types and abundance of debris in both ecosystems should continue. However, strategies that involve behavioral change, removing/cleaning-up and mitigation measures to reduce the inputs of plastics from land or sea bottom sources are being taken to tackle this complex problem (Ogunola et al. 2018).

CONCLUSIONS

It was conclusive that both marine and freshwater ecosystems are being affected by MP particles. It is important to continue investigating the sources and impacts of MPs in both ecosystems to find solutions that can be effectively implemented. Although the sources of MPs in the freshwater ecosystem are so far unclear, transportation of MPs from seabirds or by wind are possible hypothesis that should be investigated in future assessments.

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APPENDIX

Pieces between 5-25 mm were found in three of the marine lobster's digestive systems. Figure A1 shows the particles identified as fishing knots made from nylon fibers. Besides the knots, a considerable amount of nylon fibers was also extracted and counted. Lobsters had 81, 14, and 37 nylon fibers resulting from the fragmentation of the knots, respectively.

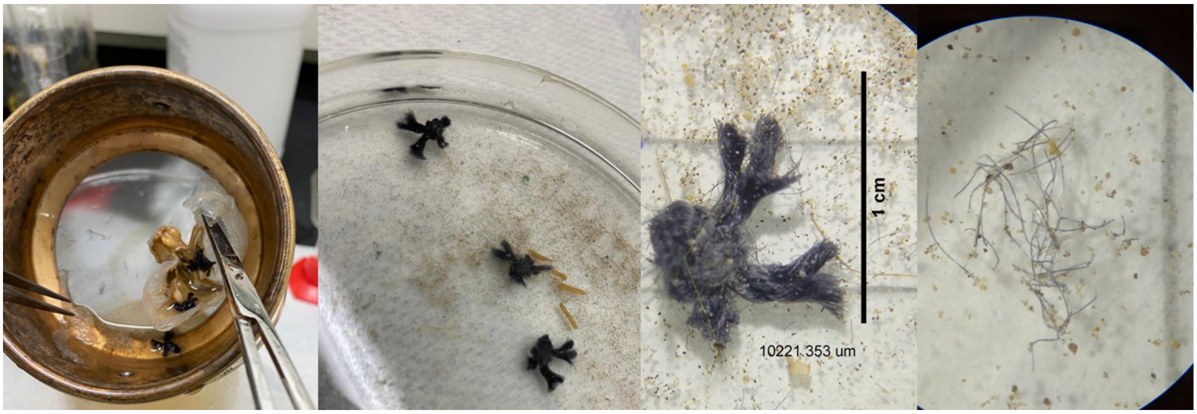

Figure A1. Mesoplastic extracted from a marine lobster digestive system.

REVIEW

Recent advances on research of the native prawn *Macrobrachium americanum* (Decapoda: Palaemonidae) with aquaculture and conservation purposes

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ABSTRACT. There has recently been great concern for proper conservation and use of living natural resources where good management practices on aquaculture are mandatory. The economic and ecological importance of prawns of the Genus *Macrobrachium* cause an impact at a global level involving economic, academic and social aspects. *Macrobrachium americanum* appears as one of the genus species with high nutritional value and an economic demand in the national and international markets, as well as a vital income for fisherman and producers of this species. For researchers, it is a challenge to find solutions to culture and propose conservation measures for *M. americanum* with emphasis on development, nutrition and reproduction. Although there are scientific studies supporting the economic importance of this species, our knowledge about its cultivation, reproduction and conservation is limited. This paper summarizes the latest studies made in cooperation with *M. americanum* in research lead by the Centro de Investigaciones Biológicas del Noroeste, Mexico. After several years of continuous research, it is considered that those efforts have produced useful information for the sustainable exploitation, conservation and basic management practices of this species.

Key words: Freshwater ecosystem, growth rate, native prawn species, conservation, sustainable fishing.

Investigaciones recientes sobre el langostino nativo *Macrobrachium americanum* (Decapoda: Palaemonidae) con fines de acuicultura y conservación

RESUMEN. En los últimos tiempos ha existido una gran preocupación por la conservación y uso adecuados de los recursos naturales vivos, donde las buenas prácticas de manejo en la acuicultura son importantes. La importancia económica y pesquera de los langostinos del Género *Macrobrachium* causa un impacto a nivel global que incluye aspectos económicos, académicos y sociales. *Macrobrachium americanum* se presenta como una de las especies del género con alto valor nutritivo y demanda económica en el mercado nacional e internacional y constituye un importante ingreso para los pescadores y productores de esta especie. Para los investigadores es un desafío encontrar soluciones para la conservación del *M. americanum* con énfasis en el desarrollo, la nutrición y la reproducción. Aunque existen estudios científicos que avalan la importancia económica de esta especie, nuestro conocimiento sobre su cultivo, reproducción y conservación es limitado. Este artículo resume algunos estudios en colaboración sobre *M. americanum*, liderados por el Centro de Investigaciones Biológicas del Noroeste, México. Después de varios años de investigación continua, se considera que esos esfuerzos han producido información útil para la explotación sostenible, conservación y las posibles medidas de manejo para esta especie.

Palabras clave: Ecosistema de agua dulce, tasa de crecimiento, especie nativa de langostino, conservación, pesca sostenible.

INTRODUCTION

Freshwater prawns

The goal of aquaculture activity is the production of useful techniques for the rearing, cultivation and commercialization of aquatic animals and plants (Vega-Villasante and Chong 2006). Prawns or freshwater shrimps, which are the most diverse crustaceans within the Palaemonidae family, are of major importance in aquaculture. They have a wide geographic distribution including many species living in estuarine and freshwater ecosystems (Hernández-Sandoval 2008). Within this family, the Genus *Macrobrachium* Bate, 1868, has been of great interest due to the number of species it comprises (more than 230), its geographical distribution and its economic value in many countries (Vega-Villasante et al. 2011b; Méndez-Martínez et al. 2018b). Traditionally, several species of this genus have been cultured or extracted from rivers for human consumption owing to its good flavor and high protein content. Since they are omnivorous with carnivorous trends, there are many possibilities of developing diets with a variety of nutrients. According to Vega-Villasante et al. (2011a, 2011b), the Genus *Macrobrachium* is distributed in the tropical and subtropical regions around the world in areas where precipitation fluctuates between 400-1,350 mm a year. They may be found at sea level up to 800-1,500 m altitude, delimited by the isotherm of 18 °C, with minimum and maximum annual temperature of 16 °C and 32 °C, respectively (Arroyo-Rentería and Magaña-Ríos 2001). Freshwater prawns can successfully live in rivers, estuaries, swamps and most kind of coastal water bodies (Valencia and Campos 2007). This ability and flexibility to live in a variety of habitats, makes prawns of this genus a good option to explore its potential for aquaculture.

In Mexico, several native species and one exotic (*Macrobrachium rosenbergii*) have been reported (Arredondo-Figueroa and Ponce-Palafox 2011). A total of 16 species have been registered in both sea sides of Mexico: in the Atlantic, bordering the Gulf of Mexico and the Caribbean Sea; and in the Pacific, from Baja California to Chiapas (Hernández et al. 2015).

In recent years, many freshwater prawn populations have decreased or disappeared due mainly to overfishing and water pollution. This phenomenon is particularly serious in river basins. García-Guerrero et al. (2013) described some actions required for the conservation of the species that, if accomplished, will benefit the communities settled on the banks of the rivers by providing them with protein-rich food. Unmanaged fisheries for this and other species should be stopped to allow their reproduction and conservation.

Macrobrachium americanum (Figure 1) is a prawn reaching a large size in the wild and is distributed in the Pacific coast from northern Mexico to Peru (Wicksten and Hendrickx 2003; Hernández et al. 2007; Méndez-Martínez et al. 2017) (Figure 2). There are some studies on the growth, nutrition, biochemistry, distribution and ecology of this species (Hernández et al. 2007;

Figure 1. *Macrobrachium americanum* adult male (left) and adult females (right) collected in Oasis San Pedro de la Presa, Baja California Sur, Mexico (17° 03′ 36.14″ N, 100° 01′ 35.03″ W). Picture from Soberanes-Yepiz.

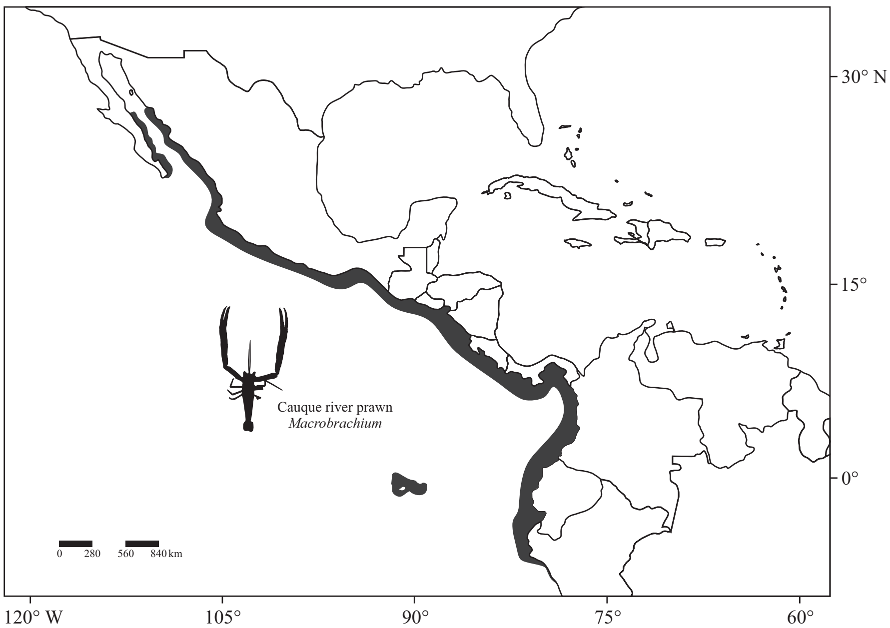

Figure 2. Geographic distribution of cauque river prawn *Macrobrachium americanum*.

García-Guerrero et al. 2011; Méndez-Martínez et al. 2018a; Soberanes-Yepiz et al. 2018; Soberanes-Yepiz 2020). However, it should be noted that there is little knowledge on culture practices or conservation goals. To address this issue, this study aims to present the latest advances on reproduction and nutrition of this prawn carried out by researchers of the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR).

Economic facts of *Macrobrachium americanum*

At present, several research groups from CIBNOR La Paz, B.C. S. CUCOSTA Guadalajara and CIIDIR Oaxaca in Mexico, have performed several proposals to determine the biological or ecological features of *M. americanum*. This is a species of high economic value based on their taste, high protein content and appearance, which is expensive for cuisine (García-Guerrero et al. 2013; Méndez-Martínez et al. 2018a). Average prices per kilogram range between USD 2.61 to USD 6.55 in a local market depending on demand, size and quality of the product. Globally speaking, this market is supplied mainly by *M. rosenbergii*, an Asian native species commonly cultivated in countries such as India, China and Thailand, and exported to Europe, Asia and North America (García-Guerrero et al. 2013). In Latin America, *M. americanum* is one of the largest prawns of the genus with a maximum of 290 mm in total length (Méndez-Martínez et al. 2017). It almost reaches the size of *M. rosenbergii*, which is perhaps the largest species of this genus, with up to 320 mm in total length (Ibarra and Werthmann 2020). Therefore, the cultivation of *M. americanum* has become very attractive for aquaculture.

Recent research focused on *Macrobrachium americanum*

Studies carried out with sub-adults of *M. americanum* by López-Uriostegui et al (2020) revealed that larger specimens were collected at sites with temperatures ranging from 26 °C to 29 °C and salinity from 3 to 10.5. Meanwhile survival decreased with temperatures above 30 °C and salinities greater than 10. An optimum level for survival was set at temperatures of 23-29 °C and a salinity from 0 to 5. In another research, Sainz-Hernández et al. (2016) observed that 29 °C seems to be the best temperature for egg rearing, since at this temperature eggs increased their volume by 50%, hatching rate was 100%, and larvae survived for a longer time. They also observed that at 26 °C eggs increased 25% in volume, 40% of the eggs hatched, and larvae died in early stages. Larvae of eggs incubated at 33 °C died one day after hatching.

Based on the study of physical and chemical parameters from two sampling sites (Coahuayan river with salinity of 1.03, and the artificial irrigation channel Zanja Prienta with salinity of 0.2 in Michoacán), which are natural habitats of *M. americanum* in Mexico, it is suggested that this prawn is a better option for culture on water with high alkalinity and total hardness in comparison with *M. rosenbergii* (García-Guerrero et al. 2013). The latter authors also reported adults *M. americanum* at temperatures as low as 21 °C, however, post-larvae were present only at temperatures above 25 °C. In general, *M. americanum* prefers to live under aquatic vegetation, rocks, crevices and holes or dug in the mud leaving their shelters to feed at night (González-Vera et al. 2018).

On the other hand, García-Guerrero and Hendrickx (2009) described the embryonic development of the species and found that the incubation period was 18 days at 24 °C. Likewise, García-Guerrero (2009, 2010) studied the proximal composition of eggs incubated at different temperatures and detected that the main source of energy were lipids and that proteins were the most abundant component. Regarding reproduction techniques, Aquiñaga-Cruz et al. (2012) showed that, even though eyestalk ablation of female *M. amer-*

icanum did not trigger reproduction, such procedure was recommended because the growth rate increased twice and the aggressiveness between animals was reduced, increasing survival.

Another research stated that *M. americanum* has effective dispersal capabilities due to its life history, including a pelagic larval stage and behavioral characteristics, such as positive phototaxis as a juvenile and the ability to disperse in terrestrial environments (García-Guerrero et al. 2013). On the other hand, McNamara et al. (1983) observed little tolerance of first larval stages of *M. americanum* to salinity on survival, respiratory rate and molting. García-Guerrero and Apun-Molina (2008) evaluated the effect of density and the use of shelters on the survival and growth of juveniles and concluded that those kept at low density and with shelter had better growth. Additionally, García-Guerrero et al (2011) studied the oxygen consumption of specimens and corroborated that both the temperature was the determining factor, and that the mass-specific oxygen consumption rate of small prawns was higher than that of large prawns for all of the temperatures studied.

In relation to nutritional studies, Pérez-Rodríguez et al. (2018) analyzed the effect of five diets with different amounts of crude protein (27, 33, 38, 43 and 48%) on growth and survival of wild *M. americanum* prawns. The option with 33% protein level resulted in the best treatment for the experimental design they followed.

Another important issue considered was eating habits. Despite its daytime feeding preferences, Lopez-Uriostegui et al. (2017) noticed that *M. americanum* can be fed also at night or during the dark period. Therefore, it is recommended to offer food at both scenarios for this prawn, while for species such as *M. tenellum*, feeding must be given only during the light period.

Santamaría-Miranda et al. (2018) studied the polyculture of *M. americanum* prawn and tilapia (*Oreochromis niloticus*) with different densities, finding that their polyculture at room temperature in freshwater with a density of 14: 5 (tilapia: prawn) was like monoculture with beneficial effects for both species.

Advances in reproduction and nutrition

Pérez-Rodríguez (2017) evaluated the effects of three diets (Camaronina 35 Purina®, 100% pelletized commercial food, and a 50:50% of food: fresh food with sardine and squid meat) on spermatophore production and sperm quality. Prawns (15-130 g weight interval) were collected in the oasis of San Pedro de la Presa, Baja California Sur, Mexico. Specimens were fed daily for 244 days under controlled laboratory conditions and sampled every 24 days. Results showed no significant differences caused by diet between sperm quality variables and reproductive exhaustion (decrease in spermatophore weight, percentage of sperm produced and increase in the average number of dead sperm). Additionally, a comparison with specimens recently captured from the wild in July 2017 revealed that all sperm quality variables were significantly higher in wild specimens than in specimens kept in controlled laboratory conditions. Since no significant differences in sperm quality were observed between the three diets and considering that the pellet is less expensive and easier to use than pelletized food, the former is more recommended for feeding reproductive *M. americanum* males. However, morphological or anatomical aspects of this reproductive structure still need to be clarified.

In addition, Yamasaki-Granados et al. (2012) evaluated the larval survival for different combinations of stocking density and feeding from larvae cultivated in green water. From these combinations, larvae fed with *Artemia* nauplii at a density of 50 larvae l^{-1} had the highest survival. Currently, supplies of juveniles are limited because hatchery and laboratory-reared larvae are difficult to raise.

Méndez-Martínez (2017) performed three related experiments with larvae and juveniles of

M. americanum obtained under laboratory conditions from the spawning of wild females. Experiments consisted of the evaluation of the effect of *Artemia* nauplii enriched with microalgae on the growth and survival of *M. americanum* larvae. Three different diet combinations were used. Those larvae fed with *Artemia* metanauplii I enriched with *Chaetoceros calcitrans* diet achieved the highest survival and growth. In Experiment II, the effect of four concentrations of crude protein (30.7, 37.2, 41.8 and 46.8%) in the diet on the productive response, proximal composition, and body amino acid of juvenile *M. americanum* was determined. Survival was 100% in all treatments. Dietary protein content had a significant effect on the proximal composition and the amino acid profile. Under experimental conditions, prawns fed with 37.2% crude protein diet reached a significantly highest final weight. In experiment III, the effect of different levels of protein (35 and 4%) and lipids (6, 10 and 14%) in the diet with factorial arrangement (3 × 2) was evaluated for six protein/energy relationships (17, 18, 19 20, 21, and 22 mg kJ^{-1} g^{-1}). The effect on productive and nutritional variables such as hepatopancreas cytology, biochemical and hematological composition was evaluated. Results showed that the diet containing 35% protein and 10% lipids with a P/E ratio of 18 mg kJ^{-1} g^{-1} seemed to be optimal for juveniles. It has been demonstrated that a proper amount of dietary protein is helpful to minimize cannibalism (Mendez-Martinez 2017). Since *M. americanum* has carnivorous trends, it can be assumed that this will also work with this species.

On the other hand, Raso-Ramírez (2019) carried out a population genetic study of this prawns with specimens from Baja California Sur (Oasis San Pedro de la Presa, La Paz, BCS) and from the Coyuca river, Guerrero (Coyuca de Benítez, Gro.). The main purpose was to evaluate the variation and genetic structure in two sites with different environmental conditions using primers for three mitochondrial genes (16S rDNA, COI mtDNA, and control region). Results indicated that genetic diversity was within the interval normally observed for other decapod crustacean species and other *Macrobrachium* species, showing high values (Hd = 0.99; π = 0.011). The genetic diversity observed in some sites of the two places (BCS and Gro.), indicated them as potential places for the conservation tasks, and both seemed to be proper locations for future genetic management programs as well as for the development of cultivation biotechnologies. In this study, values of genetic differentiation (φst) were low and significant (φst = 0.006-0.2). Results of ANOVA did not indicate the existence of two or more genetically different populations.

In another study carried out by Soberanes-Yepiz (2020), adult specimens of *M. americanum* were sampled in the Coyuca river, Guerrero, México (August to September 2015) and the Oasis of San Pedro de La Presa, BCS (June 2017 to October 2018). The author reported a positive allometric growth for males ($b > 3$) and a negative allometric growth for females ($b < 3$), finding that female weight was a better predictor of total fertility of *M. americanum* than length. The resulting equation describing the relationship between weight and fecundity of females was: Fertility (as number of eggs) = 1,863.6 Wt^{-23482}. Regarding the experimental diet in terms of oxidative stress prevention, it was suggested that a suitable diet for juveniles should have a protein/lipid ration of 35/10. According to the gonadal stage, four stages were identified: previtellogenesis, early vitellogenesis, late vitellogenesis, and spawning. Reproduction occurred mostly during the rainy season (from July to October), and ovigerous females appeared mainly from September to October.

Therefore, *M. americanum* females are considered multiple spawners. This suggests that the main period of capture of *M. americanum* prawns occurs during the rainy season coinciding with the pick of reproduction. Currently, there is no regulation at all for their capture, which has caused a significant decline in their populations in both places (García-Guerrero et al. 2013, 2015). Because of this, the total annual catch is reducing every year, which is no longer a good business for local fishermen. Consequently, they look for a variety of other activities, different from fishing. It is considered that a permanent monitoring of this living resource is required, as well as continuing the research on reproductive and feeding behavior aspects of this prawn both in the wild and in laboratory to propose and execute measures that promote its conservation through sustainable management.

CONCLUDING REMARKS

There are several studies, most of them carried out with the Genus *Macrobrachium* at the Centro de Investigaciones Biológicas del Noreste S.C., based on culture improvement, larval production juvenile feeding, reproduction techniques, assays on growth and survival in ponds or tanks. The study of native aquatic species like *M. americanum* is of great importance because they are key elements in the recirculation of energy in fragile and degraded ecosystems that are of primary social and economic significance. Although there are several studies on this species, it is required to investigate on the relationship between density, stocking size and the growth under mono and polyculture conditions. Most research efforts focused on domestication by selecting best features or attributes that makes the species valuable, like low aggressiveness and large size.

Studies on genetics are required to select specimens with higher growth rate, since only prawns of 50 to 60 g were obtained in farming systems of rustic ponds. *M. americanum* seems to be a species with good potential for commercial culture, given its large size and high market price. This last should encourage researchers and justify financial support to study this species. Finally, we found that, after several years of continuous research by researchers from different countries (Mexico, Costa Rica, Brazil, Ecuador, USA), those efforts have produced useful information for the sustainable exploitation and prevention of disappearance of this species.

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NOTE

Size structure of Areolate grouper (*Epinephelus areolatus*) from the Saudi coast of the Arabian Gulf

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ABSTRACT. Areolate grouper, *Epinephelus areolatus*, is one of the reef-associated fish species which is highly sought-after in the seafood trade. Consequently, a high market demand resulted to overexploitation and population decline of the species in the wild. This paper aimed to determine the size structure of *E. areolatus* from the Arabian Gulf. A total of 355 specimens of *E. areolatus* collected over the 12-month sampling period revealed high proportions of females observed throughout the year and in size class. Males (29.3 cm, 358.44 g) were bigger and heavier than females (28.8 cm, 326.66 g). The 'b' values ranging from 2.86 to 2.88 indicated negative allometric growth. The relationship between length and weight showed significant positive correlations with $p < 0.0000$ and r^2 values ranging between 96.05-97.12%.

Key words: Arabian Gulf, areolate grouper, length-weight relationship.

Estructura de talla del mero areolado (*Epinephelus areolatus*) de la costa saudí del Golfo Arábigo

RESUMEN. El mero areolado, *Epinephelus areolatus*, es una de las especies de peces asociadas a los arrecifes que es muy buscada en el comercio de productos del mar. Consecuentemente, una alta demanda de mercado resultó en la sobreexplotación y la disminución de la población de la especie en la naturaleza. Este trabajo tuvo como objetivo determinar la estructura de tamaño de *E. areolatus* del Golfo Arábigo. Un total de 355 muestras de *E. areolatus* recolectadas durante 12 meses de muestreo revelaron altas proporciones de hembras durante todo el año y en la clase de talla. Se observa que los machos (29,3 cm, 358,44 g) fueron más grandes y pesados que las hembras (28,8 cm, 326,66 g). Los valores de "b" que oscilan entre 2,86 y 2,88 indicaron un crecimiento alométrico negativo. La relación entre longitud y peso mostró correlaciones positivas significativas con $p < 0,0000$ y valores de r^2 que oscilaron entre 96,05-97,12%.

Palabras clave: Golfo Arábigo, mero areolado, relación largo-peso.

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Areolate groupers, *Epinephelus areolatus* (Forsskål, 1775), are reef-associated fishes found mainly on depths ranging from 6-200 m (Froese and Pauly 2013). They are usually inhabitants of seagrass beds or fine sediment bottoms near rocky reefs, dead coral, or alcyonarians, in shallow continental shelf waters (Tupper and Sheriff 2008; Froese and Pauly 2013; Sanaye 2014). Despite the wide distribution of Family Epinephilidae, this species is found only in the Indo-Pacific region (Russell and Houston, 1989; Heemstra and Randall 1993; Ottolenghi et al. 2004; Sanaye 2014) (Figure 1). Areolate groupers are important fisheries and aquaculture species. Together with other grouper species, they are considered most highly sought-after food fishes in the seafood trade (Kuo 1995; Sadovy et al. 2003). However, the high demand of grouper in the market generated a negative ecological impact. It is the most

intensively exploited group in the live fish trade, implying that this group is heavily overfished (Morris et al. 2000). Studies reveal that trade often follows a pattern of sequential overexploitation where the most sought species is targeted and fished out first before the less valuable species (Johannes and Riepen 1995; Sluka 1997). Barrowman and Myers (1996) and Reinert et al. (2005) indicated that the removal of considerable number of sexually mature fish in the stock would compromise the overall stock reproductive output, especially the species forming concentrated and brief spawning aggregations (Sadovy and Domeier 2005), such as this group. Population decline was already observed in some areas and is attributed to overfishing, habitat degradation and pollution, destructive fishing techniques, high export demand, etc. (Johannes 1997; Sadovy 2000). Together with the compounding effect brought about by natural and anthropogenic causes, stocks cannot sustain their population. As a result, natural stocks are depleted or even worse, some species are threatened to become extinct. At present, some species of groupers are already considered 'threatened organisms' according to IUCN categories and criteria.

In the region, fisheries are considered as the most important natural resource next to oil (Carpenter et al. 1997). In Saudi Arabia, production figures reveal an increasing trend from 1996 to 2005 (Ministry of Agriculture Saudi Arabia, 2007). Only in 2005, a total of 74,779 t of seafood products were produced from traditional fishing, industrial fishing, fresh and marine aquaculture. Nearly three-fourths (70.5%) of total catch contributed from traditional fishing. Industrial fishing and aquaculture account only for 10.2% and 19.2%, respectively. There are two main grouper species commonly sold in the market, namely *E. coiodes* and *E. areolatus*. Their prices range from USD 15-25 and USD 8-11 kg^{-1}, respectively.

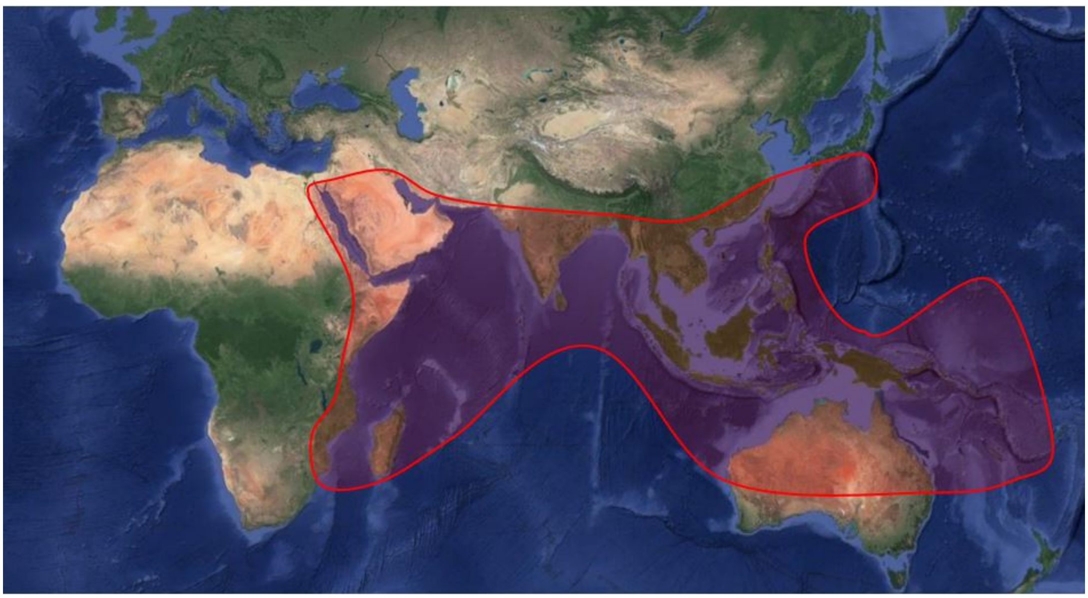

Figure 1. Geographical distribution of *Epinephelus areolatus*.

The purpose of this study was to determine the size structure of areolate grouper, *E. areolatus* from the Arabian Gulf. It particularly focuses on the length-weight relationship which can be a useful tool in management and conservation of the species in area.

Specimens of *E. areolatus* were collected on a monthly basis from Jubail Fish Market and Auction Center, a major fish landing site on the eastern province of Saudi Arabia, western part of the Arabian Gulf. A total of 355 samples were collected over the 12-month sampling period from February 2014 to January 2015. The relationship between total length (TL) and whole-body weight (BW) of *E. areolatus* was analyzed by measuring length and weight of the specimens. The statistical relationship between these parameters of fishes was determined by using the parabolic equation formulated by Froese (2006) as follows:

$$W = aL^b$$

where, W = weight of fish (g), L = length of fish (mm), a = constant and b = an exponential expressing relationship between length-weight. Such relationship when converted into the logarithmic form gives a straight-line relationship graphically. The same equation mentioned above is written in logarithmic form as:

$$\text{Ln } W = \text{Ln } a + b{*}\text{Ln } L$$

where, b and the coefficient of determination r^2 were estimated at 95% confidence limit. Statistical analyses were computed using EXEL STAT for Windows (XLSTAT 2007).

The length of the gathered samples ranged between 17.1-47.1 cm, with the mean length of 28.95 cm. The weight ranged between 58.10-1,343.43 g, with the mean of 334.80 cm. It was further observed that males (29.3 cm, 358.44 g) were bigger and heavier than females (28.80 cm, 326.66 g). Maximum length and weight reported in other areas were as follows: 50.5 cm and 1.94 kg in Egypt (Abd-Allah et al. 2015), 29.5 cm in the Philippines (Gumanao et al. 2016), 49.5 cm and 1.5 kg in India (Nair et al. 2021), and 30.2 cm and 0.38 kg in Indonesia (Fadli et al. 2022).

The ‘b’ value was 2.88 for males while for females it was 2.86, and for combined sexes it was 2.87. Results indicated that the weight of *E. areolatus* increased with the increasing length (Figure 2). Analysis of regression shows that there was a significant relationship between the two variables with $p < 0.0000$ and r^2 values ranging between 96.05-97.12%. Typically, growth in fish is explained by von Bertallanfy curve (Hopkins 1992; Pauly 1994; Jobling 2002) represented by an asymptotic sigmoid curve in many species (Ricker 1979). In reef fishes like *E. areolatus*, fast growth is exhibit during pelagic and juvenile stages, however, it slows down during transitions into adulthood to apportion more energy for breeding (Jobling 1994; Hutchings 2003; Claro and Garcia-Arteaga 2014). In the present study, computed ‘b’ values were 2.87 (combined sexes), 2.88 (males) and 2.86 (females). This is an indication that the fish shows a negative allometric growth implying that the parts of the fish grow slower compared to its body as a whole. This growth performance index is similar to the studies conducted from the Gulf of Suez (2.83), north coast of Aceh, Indonesia (2.86-3.31), southwest coast of India (2.95-3.2) and Davao Gulf, Philippines (3.03) (Abd-Allah et al. 2015; Gumanao et al. 2016; Nair et al. 2021; Fadli et al. 2022).

The relationship between length and weight showed a positive correlation, suggesting that the weight of *E. areolatus* increased with the increasing length. According to Jennings and Polunin (1997), this relationship is a morphometric measurement of how a species allocates mass allometrically. The association between the two variables can be useful to estimate standing crop biomass (Abd-Allah et al. 2015), assess species fitness overtime (Bolger and Connolly 1989) and provides information on production capabilities (Jobling 2002).

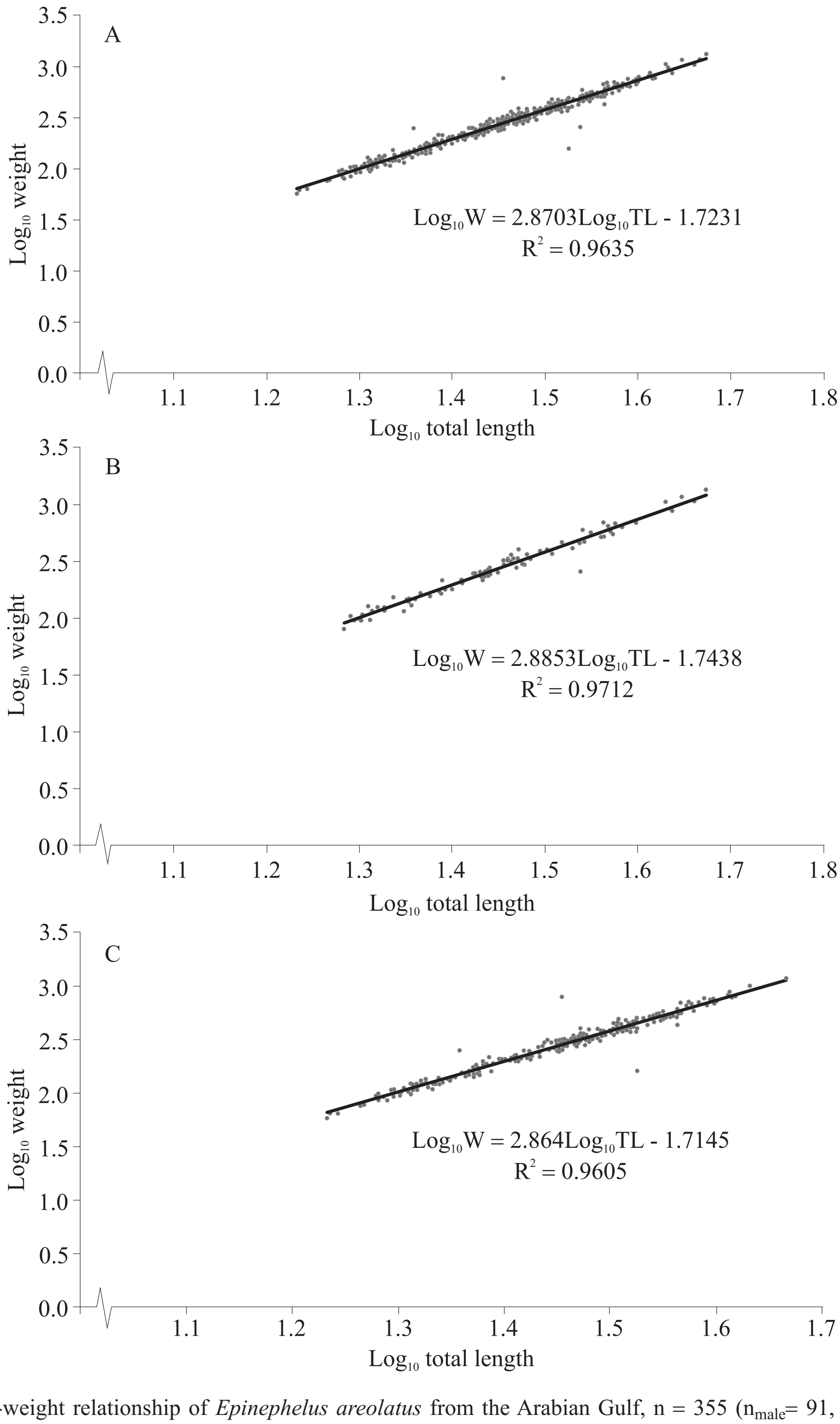

Figure 2. Length-weight relationship of *Epinephelus areolatus* from the Arabian Gulf, n = 355 (n_{male}= 91, n_{female} = 264). A) Combined sexes. B) Male. C) Female.

Similar to the reproductive capability of a species, growth is largely associated with the prevailing local environmental circumstances (Roff 2000). Many studies have uncovered that the pronounced growth variability within and between populations is due to the combination of both genetic and environmental factors such as temperature, photoperiod, pH, salinity, and food availability (Werner and Gilliam 1984; Manooch 1987; Conover 1990; Sadovy et al. 1992; Sale 1998; Lombardi-Carlson et al. 2008; Munday et al. 2008; Claro and Garcia-Arteaga 2014).

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NOTE

New inland records of the bull shark *Carcharhinus leucas* from Sumatra, Indonesia

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ABSTRACT. Six new records of the bull shark *Carcharhinus leucas* from five different river basins of Sumatra, Indonesia, were reported as a result of captures by artisanal and recreational fishers, including records from rivers of northern and western Sumatra for the first time. These findings may highlight the importance of Sumatran river basins for the reproduction of this threatened species in Indonesian waters. Inland records of *C. leucas* in Southeast Asia and in particular Indonesia are scarce but important for nature conservation purposes and sustainable future fishery management.

Key words: Biogeography, Carcharhinidae, elasmobranchs, freshwaters, conservation, data-poor area.

Nuevos registros continentales del tiburón toro *Carcharhinus leucas* de Sumatra, Indonesia

RESUMEN. Se reportaron por primera vez seis nuevos registros del tiburón toro *Carcharhinus leucas* en cinco cuencas fluviales diferentes de Sumatra, Indonesia, como resultado de las capturas de pescadores artesanales y deportivos, incluidos registros de ríos del norte y oeste de Sumatra. Estos hallazgos resaltan la importancia de las cuencas de los ríos de Sumatra para la reproducción de esta especie amenazada en aguas de Indonesia. Los registros continentales de *C. leucas* en el Sudeste Asiático y, en particular, en Indonesia, son escasos pero importantes para la conservación de la naturaleza y la gestión pesquera sostenible en el futuro.

Palabras clave: Biogeografía, Carcharhinidae, elasmobranquios, agua dulce, conservación, área con datos escasos.

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The Indonesian Archipelago can be considered as a data-poor area, as there is currently no systematic study on the status of its shark populations (Jaiteh et al. 2017). Although the Malay Archipelago has been identified as a hotspot of elasmobranch biodiversity, containing about 30% of the more than one thousand shark and ray species in the world (Last and Stevens 2009), this region remains poorly investigated and a major blind spot for conservation. This lack of knowledge can be understood as a lack of concerted research effort on both marine and non-marine elasmobranchs in Indonesia, due to financial limited research support and logistical difficulties in conducting research in remote, hard-to-access, and politically unstable areas rather than a lack of interest on this topic.

Several shark species utilize specific inshore locations (coastal embayments, estuaries, river mouths) as nursery areas, but only a few elasmobranchs are euryhaline, able to transition between marine and freshwater environments for prolonged periods (Thorson 1972; Pillans et al. 2009). *Carcharhinus leucas* (Valenciennes, 1839), the bull shark, is a circumglobal, euryhaline apex predator widespread in the coastal areas of the tropical, subtropical, and warm-temperate regions of all ocean basins (Compagno 1984; Last and Stevens 2009; Ebert et al. 2021; Gausmann 2021). This species relies on low salinity habitats for reproduction and in the early stages of its life cycle (Thorson 1976). Rivers and river mouths can be considered as important nursery grounds for neonate, young-of-the-year, and juvenile bull sharks, as they provide low-mortality habitats and large amounts of suitable food items (Heupel and Simpfendorfer 2011; Matich and Heithaus 2015; Pillans et al. 2020). *Carcharhinus leucas* has been reported historically from major streams of the world thousands of kilometers inland (Gausmann 2021). Thus, the bull shark is currently known as one of the few shark species that penetrates freshwater for extended periods due to its osmoregulatory competencies (Pillans et al. 2005). Grant et al. (2019) reviewed the use of non-marine habitats by elasmobranchs and produced a classification of elasmobranchs using freshwater based on the importance of freshwater habitats on the life history of each species. According to these authors, only 4 shark species, 3 *Glyphis* spp. and *C. leucas*, can be considered truly euryhaline. In the Indo-Pacific region, bull sharks are born at 60 to 75 cm total length (TL), both males and females reaching maturity at ~ 10-20 years and 180 to 230 cm TL and reaching a maximum recorded size of 400 cm TL (Wintner et al. 2002; Last and Stevens 2009; McCord and Lamberth 2009). *Carcharhinus leucas* is assessed as Vulnerable (VU) on a global scale in the IUCN Red List (Rigby et al. 2021).

Carcharhinus leucas is currently recognized from Sumatra in available distribution maps (Ebert et al. 2021; Gausmann 2021; Rigby et al. 2021). Only few verified inland records of juveniles and subadults from Sumatran freshwater environments had been previously reported (Batang Hari River Basin: Tan and Lim 1998; Musi River: Iqbal et al. 2019). *Carcharhinus leucas* is know from both marine and freshwater Indonesian habitats (Gausmann 2021), but there are gaps in the distribution due to a lack of verified records for many parts of Indonesia. Some of the freshwater records of *C. leucas* from Indonesia are quite old (Boeseman 1964) and require verification. In summary, distributional information on *C. leucas* in Indonesia and Southeast Asia is scarce (Kottelat 2013; Hasan et al. 2021), and better information is needed on specific localities for better management and conservation planning for this species. The present study aims to report hints on new potential nursery areas of *C. leucas* for conservation purposes, to fill in gaps in the distribution of this species in Indonesia, and to outline the benefits of both artisanal and recreational fisheries data to scientific studies.

Herein, catch data on *C. leucas* from Sumatra Island, Indonesia, a data-poor area of Southeast Asia, are summarized (Table 1). Moreover, distributional data of immature bull sharks from Sumatra are provided from alternative and inexpensive existing sources (Figures 1 and 2). The second author of the present work started a call targeting Sumatran fishermen to report catches of *C. leucas* from riverine habitats for scientific investigation and to gain distributional data for this species from a remote region of Indonesia. A systematic survey of entire towns or regions was not conducted. However, six juvenile to subadult specimens of *C. leucas* were landed and photographed by artisanal and recreational fishers in the period between 2013 and 2019, from five river basins on Sumatra Island. These sites were located between ~ 4 and ~ 195 km inland from the mouths of these rivers. Distances of catch sites to the sea were

Table 1. Inland records of *Carcharhinus leucas* in Sumatran river basins in the period 2013-2019. Numbers refer to locations in Figure 1.

No	Date of catch	River Basin	Water temperature (°C)	Province	Name of village or town	Coordinates	Distance from sea/river mouth (km)	Used fishing gear	Estimated size (cm –total length)	Life history phase and sex
1	08.03.2013	Buluh	29-32	Sumatera Barat (West Sumatra)	Padang	0° 49′ 54.4″ S, 100° 18′ 52.7″ E	~ 4	Small hook (< 7/0)	~ 70	Juvenile, female
2	07.05.2016	Asahan	29-31	Sumatera Utara (North Sumatra)	Pulau Raja	2° 42′ 20.3″ N, 99° 37′ 17.6″ E	~ 80	Casting net	~ 130	Subadult, female
3	21.06.2017	Babalan	29-30	Sumatera Utara (North Sumatra)	Pangkalanbrandan	4° 01′ 47″ N, 98° 15′ 14.0″ E	~ 11	Medium hook (> 7/0)	~ 75	Juvenile
4	29.09.2017	Musi	30-32	Sumatera Selatan (South Sumatra)	Teluk Kijing	2° 58′ 58.1″ S, 104° 07′ 47.1″ E	~ 195	Small hook (< 7/0)	~ 70	Juvenile, male
5	26.08.2019	Asahan	28-31	Sumatera Utara (North Sumatra)	Tanjung Balai	2° 58′ 14.7″ N, 99° 47′ 33.9″ E	~ 16	Medium hook (> 7/0)	~ 120	Subadult, female
6	08.09.2019	Indragiri	30-32	Riau (East Sumatra)	Tembilahan	0° 18′ 11.7″ S, 103° 14′ 32.2" E	~ 44	Gill net	~ 70	Juvenile

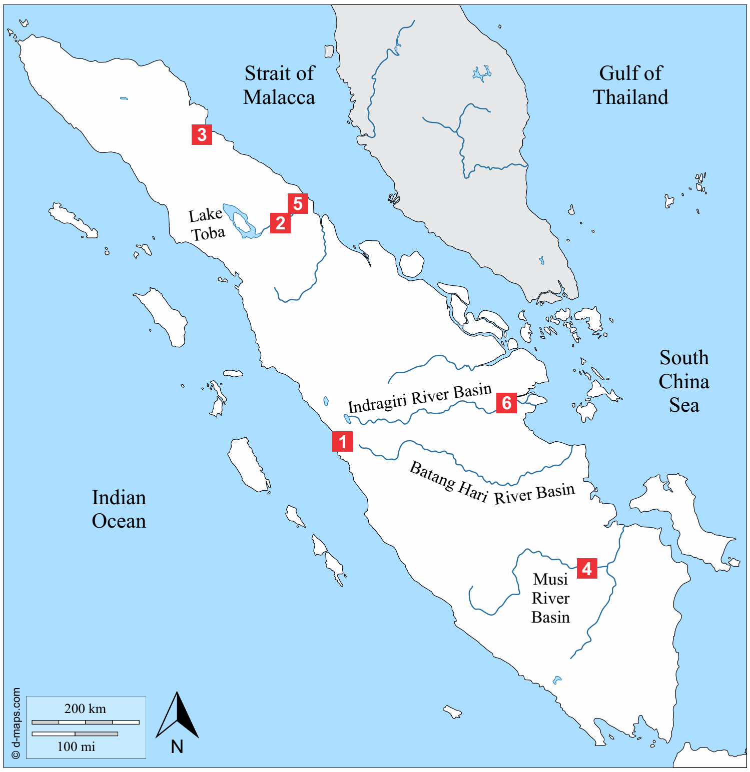

Figure 1. Known records of *Carcharhinus leucas* in river basins of Sumatra from 2013 to 2019. Numbers refer to Table 1.

measured by using a Geographical Information System (GIS), although a small inaccuracy remains. Some of these catches were incidental, as *C. leucas* is not a target species for local small-scale fisheries. Photos of the sharks were voluntarily shared with the authors by the fishers and villagers, who consented to their use in this publication. The sizes of the reported sharks were estimated by the authors from the received photographic material.

While the Asahan, Babalan, Musi, and Indragiri rivers drain into the Strait of Malacca and the South China Sea, the Buluh River drains into the Indian Ocean (Figure 1). Due to the remote location of the catch sites, the only measured water parameter was temperature, which was obtained from nearby measuring stations. The five rivers can be characterized as typical, low- to medium-impacted lowland rivers of the wet tropics, with peak discharges during the rainy monsoon season (November to March) and a period of low flow when rainfall decreases. Due to larger settlements along the larger Musi and Indragiri rivers, these are more polluted than the more pristine Asahan, Buluh, and Babalan rivers. Catch sites with short distance to the sea (Table 1, records 1, 3, and 5) are presumably tidal-influenced but nevertheless low salinity habitats.

Specimens recovered by fishers were in acceptable condition, allowing for their identification using visible features (blunt snout, small eyes, lack of an interdorsal ridge, and typical first to second dorsal fin ratio) cross-referenced with information in the literature (Garrick 1982; Compagno 1984; Ebert et al. 2013). Similar looking carcharhinids, such as members of the genus *Glyphis* and *Lamiopsis* were excluded by their relatively large size of the second dorsal fin in

Figure 2. Selection of photographs of bull shark specimens caught by local fishers in Sumatra. A) Subadult female of *Carcharhinus* from the Asahan River, North Sumatra (Table 1: record 2). B) Juvenile of *C. leucas* from the Babalan River, North Sumatra (Table 1: record 3). C) Local fisher holding a subadult female *C. leucas* from the Asahan River, North Sumatra (Table 1: record 5). D) Underside of *C. leucas* specimen in D. E) Juvenile male *C. leucas* from the Musi River, South Sumatra (Table 1: record 4). F) Recreational fisherman holding juvenile *C. leucas* was captured in the Indragiri River, Riau, East Sumatra (Table 1: record 6).

comparison to the first dorsal fin. The similar *C. amboinensis* was also excluded by the ratio of the first to the second dorsal fins that were used to separate *C. leucas* (< 3.1:1) from *C. amboinensis* (> 3.1:1). Estimated sizes suggest that specimens recorded herein were juveniles (~ 70-75 cm TL) and subadults (~ 120-130 cm TL). Sex was determined by the presence/absence of claspers. No specimens were preserved. Some of the captured specimens were later on sold on the local markets and some were directly consumed, with no specimens released again.

Sites recorded herein (Figure 1; Table 1) include the Musi River Basin (n = 1), from where *C. leucas* had been previously reported by Iqbal et al. (2019), whereas records from the Asahan (n = 2), Buluh (n = 1), Babalang (n = 1) and Indragiri (n = 1) rivers are putative new records.

Moreover, the present records of *C. leucas* from North Sumatra (Asahan River) and West Sumatra (Buluh River) represent first records for these regions. Reported specimens were representative of different life history stages of *C. leucas* from juveniles to subadults, with juveniles dominating (Figure 2). The farthest freshwater penetration was that in the Musi River, the largest of the five rivers (~ 750 km length), at approximately 195 km from the sea (Figure 1; Table 1).

Reports by fishers to scientists can be a valuable toolkit for the identification of crucial habitats for sharks in data-poor regions. The use for scientific purposes of animals caught by artisanal and commercial fishers can be an effective tool for the analysis of fish distributions, including those of elasmobranchs (Giareta et al. 2021). The confirmed presence of *C. leucas* in the Musi River indicates that juvenile and subadult bull sharks utilize this river as a freshwater habitat, presumably as a nursery area. This was already suggested by previous records of *C. leucas* from the Musi River, therefore fulfilling the repeated use criterion for nursery areas as outlined by Heupel et al. (2007). The present data suggests that numerous river basins on Sumatra may be utilized as nursery areas by immature bull sharks.

Present records from four rivers (Buluh, Asahan, Babalan, Indragiri), in addition to the Musi and Batang Hari rivers, increase the number of known Sumatran river basins with occurrences of *C. leucas* to six. Moreover, our data show that the rivers of Sumatra likely represent an important habitat in the life-history of this species in the area, and that they therefore require management alongside the coastal areas of Indonesia. Catch dates indicate that these Sumatran rivers are utilized by immature *C. leucas* at least between March and September (Table 1). Our results suggest that additional river basins of Sumatra and Indonesia may also function as nursery areas for bull sharks, so future records of *C. leucas* from Indonesian and adjacent river basins in Southeast Asia can be expected.

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In Memoriam
Enrique Eduardo Boschi
(27 de julio de 1928, Buenos Aires, Argentina - 23 de mayo de 2022, Mar del Plata, Argentina)

Este año nos ha dejado un pionero de las ciencias marinas de nuestro país. Con una vida profesional prolífica y amplia, Enrique se dedicó tanto a la investigación y la docencia, como a labores de construcción y consolidación institucional que supo desarrollar tanto en la Argentina como en el ámbito internacional, principalmente Latinoamericano.

En 1958 obtuvo la Licenciatura en Ciencias Biológicas en la Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires (FCEyN-UBA) y se doctoró en la misma casa de estudios en 1962. Mientras estudiaba y antes de obtener su título universitario, comenzó a trabajar en biología pesquera marina y de agua dulce en el entonces Departamento de Investigaciones Pesqueras de la Nación junto al Dr. Víctor Angelescu, con quien luego compartiera su vida institucional durante décadas.

Estudios especializados, becas y viajes internacionales perfilaron su formación profesional. En esos años de juventud y aún como estudiante comenzó a publicar trabajos de investigación así como de divulgación. La nómina de sus trabajos científicos abarca unos cien títulos y los aportes que hizo a la divulgación prosiguieron a lo largo de toda su vida profesional.

Aunque se inició trabajando en biología pesquera de peces de agua dulce, pronto se orientó a los crustáceos marinos, área en la que desarrolló su especialidad y en la que realizó investigaciones sobre morfología, biología, taxonomía y desarrollos larvales en cultivo. Dedicó un especial énfasis a estudios de biología pesquera de especies comerciales de la familia Penaeidae –camarones y langostinos del litoral bonaerense y patagónico (*Artemesia longinaris* y *Pleoticus muelleri*)–, y de la centolla del Canal Beagle (*Lithodes santolla*), así como al desarrollo larval de varias especies de cangrejos. Uno de sus aportes más significativos y en el que pudo sintetizar los conocimientos y experiencia adquiridos a lo largo de décadas fue el inventario y biogeografía de las especies de decápodos de las costas del Atlántico y Pacífico del continente americano (Boschi EE. 2000. *Species of Decapod Crustaceans and their distribution in the* *american marine zoogeographic provinces*. Revista de Investigación y Desarrollo Pesquero 13, 136 p; https://aquadocs.org/handle/1834/2606).

A la publicación de trabajos sumó una labor editorial, un segundo aspecto que cultivó con verdadera pasión y que iniciara en el ex Instituto de Biología Marina de Mar del Plata (IBM) poco después de su creación a comienzos de la década de 1960. En este sentido, sus ideas y dedicación permitieron crear un sistema de publicaciones propio en los albores de la institución desempeñándose como editor del Boletín del IBM hasta 1976. Mucho después y reuniendo su extensa experiencia de décadas retoma esta labor para liderar como editor general los seis volúmenes de la obra *El Mar Argentino y sus recursos pesqueros* (https://www.argentina.gob.ar/inidep/biblioteca/publicaciones-especiales).

Enrique fue miembro de la Carrera del Investigador Científico del Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) desde 1962 hasta su retiro, fue director del IBM entre 1966 y 1973 y director del Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) en el período 1984-1985, institución en la que fue investigador desde su creación en 1977 y de la que fuera designado Director Consulto a partir de 1991.

Enrique desplegó un abanico de actividades que abarcaron la coordinación de proyectos y de las campañas de investigación pesquera durante el Proyecto de Desarrollo Pesquero de la FAO implementado en nuestro país durante una década desde mediados de los años ’60 y la coordinación de las campañas de los buques “Walther Herwig” (Alemania) y “Shinkai-Maru” (Japón) en la década de 1970. Asimismo, participó en numerosas campañas exploratorias y de evaluación en buques de investigación de bandera nacional a fin de recomendar pautas para una explotación racional de los recursos pesqueros.

A lo largo de su vida profesional participó en numerosos simposios y reuniones, presidiendo algunos de ellos, y fue miembro de varias sociedades científicas, recibiendo algunos premios y distinciones.

Y sin dudar, debemos resaltar como uno de sus rasgos más sobresalientes el ejercicio de la docencia, actividad que mantuvo durante gran parte de su vida. Iniciándose como ayudante-alumno en cursos dictados en la FCEyN-UBA, desde 1968 y hasta su retiro de la universidad, sumó el cargo de Profesor de Oceanografía Biológica en la cátedra veraniega que esa facultad mantuvo en Mar del Plata, primero en el IBM y luego en el INIDEP. Su actividad en la enseñanza se desplegó desde un inicio con el dictado de clases en temas de su especialidad durante seminarios y cursos regionales, a lo que sumó una importante labor como director de seminarios de licenciatura, becas de iniciación y de perfeccionamiento y tesis doctorales de numerosos investigadores argentinos y la dirección de graduados latinoamericanos.

Finalmente, no podemos dejar de destacar que aspectos de su personalidad como trabajador incansable, sumó siempre un buen humor y optimismo hasta en situaciones difíciles, dispuesto siempre a ayudar a estudiantes, colegas, miembros del CONICET y a becarios que acudían para referencias.

Este breve resumen sobre su trayectoria y aportes en investigación, docencia y consolidación institucional precede a la próxima aparición de un trabajo en el que trataremos en detalle estos aspectos en conjunto con el listado de sus publicaciones y de las obras de las que fue editor.

Rut Akselman-Cardella y Martín D. Ehrlich

In Memoriam
Enrique Eduardo Boschi
(July 27, 1928, Buenos Aires, Argentina - May 23, 2022, Mar del Plata, Argentina)

This year a pioneer of marine sciences in our country has left us. With a prolific and extensive professional life, Enrique dedicated himself to research and teaching, as well as to an institutional construction and consolidation work both in Argentina and internationally, mainly in Latin America.

He graduated in Biological Sciences from the Faculty of Exact and Natural Sciences of the University of Buenos Aires (FCEyN-UBA) in 1958 and received his doctorate from the same university in 1962. While studying and before obtaining his university degree, he began working in marine and freshwater fisheries biology at the then Department of Fisheries Research with Dr. Víctor Angelescu, with whom he later shared his institutional life for decades.

Specialized studies, scholarships and international trips shaped his professional training. In those years of youth and still as a student, he began to publish research and dissemination works. The list of his scientific works includes nearly a hundred titles and his contribution to the dissemination of science continued throughout his professional life.

Although he began working in fisheries biology of freshwater fish, he soon turned to marine crustaceans, an area in which he developed his specialty and conducted research on morphology, biology, taxonomy and larval development in laboratory. He devoted special emphasis to the studies of fishery biology of commercial species of the Penaeidae family –shrimps and prawns from Buenos Aires and Patagonian coast (*Artemesia longinaris* and *Pleoticus muelleri*)– and spider crab from the Beagle Channel (*Lithodes santolla*), as well as larval development of several species of crabs. One of his most significant contributions, in which he was able to synthesize the knowledge and experience acquired over decades, was the inventory and biogeography of decapod species of the Atlantic and Pacific coasts of the American continent (Boschi EE. 2000. *Species of Decapod Crustaceans and their distribution in the American marine zoogeographic provinces*. Journal of Fisheries Research and Development 13, 136 p; https://aquadocs.org/handle/1834/2606).

In addition to publishing articles, he was also involved in editorial work, a second aspect that he cultivated with true passion and that he began at the former Institute of Marine Biology of Mar del Plata (IBM) shortly after its creation in the early 1960s. In this sense, his ideas and dedication allowed him to create his own publication system in the early days of the institution, serving as editor of the IBM Bulletin until 1976. Much later, and gathering his extensive experience of decades, he returned to this work to lead as general editor the six volumes of the *El Mar Argentino y sus recursos pesqueros* (https://www.argentina.gob.ar/inidep/biblioteca/publicaciones-especiales).

Enrique was a member of the Scientific Researcher Career of the National Council for Scientific and Technical Research (CONICET) from 1962 until his retirement. He was Director of the IBM from 1966 to 1973 and Director of the National Institute for Fisheries Research and Development (INIDEP) between 1984-1985, an institution in which he was a scientific researcher since its creation in 1977 and of which he was also appointed Consulting Director since 1991.

Enrique carried out a range of activities that included the coordination of exploratory fisheries research projects and campaigns during the FAO Fisheries Development Project implemented in Argentina for a decade since the mid-1960s, and the coordination of campaigns of 'Walther Herwig' (Germany) and 'Shinkai-Maru' (Japan) ships in the 1970s. He also participated in various exploratory and evaluation surveys on national research vessels in order to recommend guidelines for a rational exploitation of fishery resources.

Throughout his professional life, he participated in numerous symposia and meetings, chairing some of them, and was a member of several scientific societies, receiving some awards and distinctions.

As an outstanding point, we must highlight his teaching activities for most of his life. Starting as a student-assistant in courses taught at the FCEyN-UBA, from 1968 until his retirement from the university, he held the position of Professor of Biological Oceanography in the summer professorship that this faculty held in Mar del Plata, first at the IBM and then at INIDEP. His teaching activities was deployed through the teaching of classes on topics of his specialty in seminars and regional courses, to which he added an important work as director of undergraduate seminars, introductory and advanced scholarships and doctoral theses of numerous Argentine researchers and of the direction of Latin American graduates.

Finally, we cannot fail to emphasize that in addition to his personality as a tireless worker, he always had a good sense of humor and optimism even in difficult situations, always willing to help students, colleagues, members of CONICET and fellowship holders who came to him for references.

This brief summary of his trajectory and contributions in research, teaching and institutional consolidation precedes a forthcoming publication of a paper in which we will deal in detail all these aspects together with a list of his publications and the works of which he was the editor.

Rut Akselman-Cardella and Martín D. Ehrlich

AUTHOR GUIDELINES

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This Journal operates a double blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to three independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final.

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For the use of abbreviations and units the Typographic Code adopted by the FAO and the International System of Units (SI) must be followed (http://physics.nist.gov/cuu/Units/units.html).

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REFERENCES

The Council of Scientific Editors (CSE) citation style should be followed: *Name-Year (N-Y)* system (*Scientific style and format: the CSE manual for authors, editors, and publishers. 2014. 8th ed. Chicago (IL): University of Chicago Press*).

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