The metazoan parasite communities of flounders as indicators of chemical pollution in the southern Gulf of Mexico: taxonomic and trait-based approaches

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Abstract

Metazoan parasite species composition changes with an increase in geographical distance, but not their functional traits. We compared the performance of the taxonomy and functional traits of metazoan parasites as bioindicators of chemical pollution along the continental shelf of the South Gulf of Mexico (sGOM). Parasites were obtained from *Syacium gunteri* and *Syacium papillosum*, together with hydrocarbons, heavy metals and physicochemical variables from water and sediments during 12 oceanographic cruises. We compared regions without oil extraction: Veracruz-Tamaulipas (VT) and Yucatan shelf (YS) and one with it, north-western end of YS to Coatzacoalcos, Veracruz (NYV). In addition to taxonomy, the trait-based approach community-weighted means (CWM) was performed. Species richness was compared between regions using rarefaction curves, and the taxonomic and trait composition between regions were compared using multivariate analyses. The relationships between taxonomy, traits and environmental variables were tested using BIOENV. We identified 57 species and body tegument, transmission, feeding mode, life stage and attachment organs as informative traits. By rarefaction, VT and YS had higher species richness than NYV for both hosts, but there were questionable values due to the small fish sample size. Except for YS, significant parasite community variability was detected by PERMANOVA using taxonomy and CWM for all regions, hosts and interactions. The same group of larval species was detected by SIMPER for regions and hosts, suggesting low functional turnover. BIOENV showed that polyaromatic hydrocarbons explained most of the variability. Taxonomy and functional traits produced similar explained variance, but the latter saved time analysis and financial resources.

Introduction

The parasite communities of aquatic organisms are good indicators of environmental impact. This has been demonstrated in freshwater, coastal and marine environments (Lafferty, 1997; Vidal-Martínez et al., 2010; Sures et al., 2017; García-Teh et al., 2022). Indeed, it is possible to demonstrate environmental impact in one place by changes in the taxonomic species composition and relative abundance of the parasite communities of marine organisms compared to nearby sites (a few dozen to hundreds of kilometers (km)) where such an impact is not occurring. However, as pointed out by Vidal-Martínez et al. (2022) and Ocaña et al. (2021), with an increase in geographical distance between sites, the usefulness of the parasite communities as indicators of environmental impact decreases. This is related to Tobler’s first law of geography: “things that are closer to each other are more similar than those further away”. This is especially true when we use a taxonomic diversity approach, where community metrics such as species composition or relative abundance are used. As stressed by these authors, the practical problem for parasite ecologists is that decision makers in different fields (e.g., fisheries, conservation and oil extraction) require information at large spatial scales from sites or regions located thousands of km apart. This makes it difficult to compare the parasite communities of different areas or regions, simply because the species composition or relative abundance of the species change with distance.
It is also a fact that with an increase in geographical distance (thousands of km apart), the host species composition and their local abundances also change. This is the case for the shoal flounder (*Syacium gunteri*) and the dusky flounder (*Syacium papillosum*) in the southern Gulf of Mexico. The dusky flounder is abundant in the Yucatan Peninsula but scarce in the Campeche sound or in the Tamaulipas-Veracruz region; in contrast, *S. gunteri* is abundant in the Campeche sound, but scarce in the Yucatan shelf (Yáñez-Arancibia et al., 1985; Yáñez-Arancibia and Sánchez-Gil, 1986; Castillo-Rivera, 1999; Torruco et al., 2018; Vidal-Martínez et al., 2021). These two flounder species and their parasites have been used as indicators of the environmental quality in specific regions of the southern Gulf of Mexico (Quintanilla-Mena et al., 2020; Vidal-Martínez et al., 2014; Vidal-Martínez et al., 2019). In fact, Vidal-Martínez et al. (2019) found significant differences between the parasite infracommunity metrics of the dusky flounder from the Yucatan Shelf and those of the shoal flounder from the Campeche Sound related to the environmental quality of these regions. One interesting pattern of the metazoan parasite fauna of both flatfish species (see Vidal-Martínez et al., 2014 and Vidal-Martínez et al., 2019) is that the parasite species composition of the shoal flounder in the Campeche Sound appears to be a subset of the parasite species composition of both host species in the Yucatan shelf. Thus, it is likely that both fish species are sharing the same pool of metazoan parasites along their geographical distribution in the southern Gulf of Mexico. If this is the case, and based on the idea that a healthy environment provides the conditions for high transmission of helminths for the completion of their life cycles (Hudson et al., 2006), then the differences in the number of species and individuals could be indicative of the environmental quality of each region. However, the environmental information required by the oil extraction industry includes even larger comparisons of regions along the continental platform of the southern Gulf of Mexico (sGOM) and the Tamaulipas region had not been studied for metazoan parasites of flounders. Then, a comparison among these three regions of the sGOM (Coatzacoalcos, Veracruz to Tamaulipas and the marine border with the United States (VT), the north-western end of Yucatan shelf to Coatzacoalcos, Veracruz (NYV), and the Yucatan shelf (YS)) has not been possible until now. In 2015, a major research project was carried out to determine the baseline for environmental and biological statuses of the pelagic and benthic zone of the Tamaulipas region prior to oil-extraction activities (Suárez-Mozo et al., 2021; Soler-Jiménez et al., 2022). As part of this effort, we obtained data on the metazoan parasite communities of *S. gunteri* and *S. papillosum* for Tamaulipas. This information gave us the opportunity to compare the parasite communities of these two host species among the three regions mentioned above which are thousands of km apart (Fig. 1). One way to compare parasite communities under different environmental conditions is species richness. Unfortunately, it is difficult to use this metric when there are large differences in the number of sample units being compared (Magurran, 2004). However, current tools such as INext (Chao et al., 2014; Hsieh and Chao, 2016) allow the bias to be decreased in the calculation of species richness based on the use of rarefaction for samples with unequal sample size. Thus, based on the use of these tools, our first hypothesis was that the species richness of the metazoan parasite communities of both flatfish species in the regions without oil extraction activities (VT and YS) would be significantly higher than those of the region with oil extraction activities (NYV).
A faster and cheaper alternative to overcome the difficulty in comparing the parasite communities of distant areas or regions is by using functional traits. These traits can be biological (e.g., weight, total length, or the presence of hard body parts) or ecological (e.g., invasion strategy, feeding habits, or direct or indirect life cycles) (Chevenet et al., 1994; Beauchard et al., 2017; Llopis-Belenguer et al., 2019). These biological and ecological traits are expected to be at least as informative as the classical taxonomical traits because the species forming the communities in a region, independent of their phylogenetic relatedness, should be responding to the same environmental selective forces in that region (Beauchard et al., 2017). Then, it would be expected that the species in a specific region would develop similar strategies, such as hard shells, attaching structures, the production of many offspring, short life cycles or active infection capacities, to cope with specific selective forces. Recently, Ocaña et al. (2021) and Vidal-Martínez et al. (2022) found that the variance explained by the taxonomic and functional metrics of metazoan parasite communities of the dusky flounder (*Syacium papillosum*) and the shoal flounder (*Syacium gunteri*) as indicators of chemical pollution in the southern Gulf of Mexico (sGOM) was similar (~50%) when comparing temporal and spatial data from two contiguous regions in the sGOM, the Yucatan Peninsula and the Campeche Sound. Now, as explained above, we recently obtained parasitological and environmental information from Tamaulipas prior to oil extraction activities. These data gave us the opportunity to determine the amount of variance explained by the taxonomic and functional metrics of metazoan parasite communities of both flounder species as indicators of chemical pollution in two regions without oil extraction activity (VT and YS) and compare these results with those of a region with heavy oil extraction activity: NYV. Indeed, only the NYV produces an annual release of 250,000 to 1.4 million barrels of oil per year through hydrocarbon seeps (Mitchell et al., 1999; Miranda et al., 2004; MacDonald et al., 2015; Árcega-Cabrera and Dótor-Álmazan, 2021). At the same time, this region has had high oil extraction activities since the 1970s (García-Cuellar et al., 2004; Árcega-Cabrera and Dótor-Álmazan, 2021); in addition, there is also a release of processed hydrocarbons, other organic pollutants (e.g., pesticides, polychlorobipheniles or PCB) and sewage from the coastal cities (Vidal-Martínez et al., 2006). In contrast, aside from the natural hydrocarbon releases from seeps in the border of continental platform of the YS and VT (Love, 2013), there are no other oil extraction activities in these regions (Peters et al., 2021; Árcega-Cabrera and Dótor-Álmazan, 2021). Thus, our second hypothesis was that we would have significant differences in the amount of explained variance on the effect of chemical pollution on the taxonomical and functional traits of the metazoan parasites of both host species for regions with and without oil extraction activity.

Thus, the goals of this study were two-fold. Firstly, to determine whether *S. gunteri* and *S. papillosum* are exposed to the same parasite pool and, if so, whether parasite communities are responding in the same way to the environmental quality of the region in which they inhabit. Secondly, to determine whether there are significant differences in the amount of explained variance on the effect of chemical pollution on the taxonomical and functional traits of the metazoan parasites of two flounder species for the regions with and without oil extraction activity.

**Methods**
Sampling

Shoal flounder (*S. gunteri*) specimens were collected from 19 sampling stations from NYV, 8 sampling stations in the YS and 16 sampling stations from VT. The dusky flounder (*S. papillosum*) specimens were collected from 26 sampling sites from NYV, 33 sampling stations in the YS and 8 sampling stations from VT. Fish collection occurred during 12 oceanographic cruises performed between 2015 and 2018. The sampling sites in which the fish (*S. gunteri* and *S. papillosum*) were captured, were grouped into three regions following Soler-Jiménez et al. (this volume): 1) Yucatan Shelf ([YS] Gomex 1–6 cruises); 2) north-western end of the Yucatan Shelf to Coatzacoalcos, Veracruz ([NYV] CO2015, KAB121-1, KAB121-2, LBA-AC08, LBA-AC18, and LBA050913 cruises); and 3) from Coatzacoalcos, Veracruz to the maritime limit with the United States including Tampico city ([VT] LBA-AC02, LBA-AC32 and PERDIDO 01–04). All of the sampling stations were located along the continental shelf of the southern Gulf of Mexico (Fig. 1). Fish sampling was performed onboard shrimp fishing boats and oceanographic vessels. At each station, fish were collected during 0.5 h trawls using two 20 m commercial shrimp nets at a speed of 2.0–2.3 knots. The physicochemical data, including the presence of heavy metals and hydrocarbons in sediments, were obtained on board *Justo Sierra*, *Riviera Maya* and *Alpha Helix* oceanographic vessels (OVs), using a 0.25 m$^2$ Hessler Sandia MK-III box corer. Flounders are benthic; thus, the water samples included in the analysis were those obtained from the sea bottom. These samples were obtained at depths of 15–250 m in all three regions (Fig. 1) using 20 L plastic Niskin bottles set up as a rosette. These bottles were closed electronically under water to avoid contamination with surface mixtures. A total of 32 environmental variables have been measured in all these oceanographic cruises, including physicochemical variables (e.g., oxygen (mg/L), salinity (UPS), pH, nitrate (µm)), hydrocarbons and heavy metals from water, sediment and organisms and they have been published elsewhere (Vidal-Martínez et al., 2014; Centeno-Chalé, 2015; Vidal-Martínez et al., 2019; Sánchez-Soto et al., 2021). Sediment samples were placed in high-density polythene bags and maintained at 4°C for transportation to CINVESTAV-IPN-Mérida. Hydrocarbon sampling procedures are described elsewhere (Vidal-Martínez et al., 2006). The physicochemical characteristics and the hydrocarbon and heavy metal concentrations of the sediment were determined at the Laboratory of Geochemistry (CINVESTAV-IPN-Mérida) following standard protocols by Sericano (1990) and Wade (1993).

Sampling Procedures For Flatfishes And Helminth Parasites

The collected dusky flounders were placed on a board and the total length, standard length, maximum height (cm ± 0.1 cm) and weight (g ± 0.1 g) of each individual fish were recorded. Each individual was subsequently examined externally for gross pathologies (e.g., tumors, ulcers and rotten fins, among others) before undergoing dissection to obtain samples of the gills, spleen, liver, kidney and muscle for histology (Aquatic Pathology Laboratory) and pollutant analysis (heavy metals and hydrocarbons). Fish tissues (a portion of liver and muscle) for pollutant analysis were collected by the personnel of the Laboratory of Geochemistry (CINVESTAV-IPN-Mérida). The remaining tissues of each individual fish were tagged, maintained in isolated plastic bags at −20°C on board the vessel and transported to the
CINVESTAV-IPN Mérida Unit for parasitological examination. The *S. papillosum* and *S. gunteri* collected were identified by ichthyologists of the Necton Laboratory (CINVESTAV-IPN-Mérida). The body surface, cavities and internal organs were individually examined for parasites using a dissection microscope following the procedures of Vidal-Martínez et al. (2001). All metazoan parasites (monogeneans, digeneans, cestodes, nematodes and parasitic crustaceans) from each individual host were counted *in situ* and preserved in 4% formalin or 96% alcohol in labelled vials for subsequent morphological or molecular taxonomic identification. Digeneans, cestodes and acanthocephalans were stained using Mayer's paracarmine technique and nematodes were cleared using increasing concentrations of glycerin (Vidal-Martínez et al., 2001) for morphological identification. Voucher specimens have been deposited in the National Helminthological Collection, Universidad Nacional Autónoma de México (see Vidal-Martínez et al., 2014 and Vidal-Martínez et al., 2019 for collection numbers). The prevalence and mean abundance of each parasitic species were determined following Bush et al. (1997). Host-specialist metazoan parasite species were those previously reported in fish species of the same genus or family, whereas generalist species were those previously reported in fish species from different families.

**Metrics of the parasite communities of both host species**

The infracommunity was defined as all metazoan parasites infecting an individual fish. At the infracommunity level, the mean ± SD (standard deviation) of the number of species and individual metazoan parasites per fish were calculated for each host species in each of the three regions. The Brillouin diversity index and the Brillouin evenness index were also calculated. The package Tabula in R (Frerebeau 2019) was used to calculate the diversity and evenness indices. For species richness, the package Tabula calculates asymptotic species richness based on the Chao1 and Chao2 indices. However, instead of using diversity indices which are useful for comparison at alpha diversity (species richness and composition at the local level), we preferred to use species richness indices considering variability within a region at beta diversity level (ratio between regional and local species diversity). Thus, we defined a component community as all of the parasite species in a sample of a host species from a sampling site. Posteriorly, following the proposal of Aho and Bush (1993), we defined a region as all of the component communities that were in each of the geographic areas studied, namely NYV, VT and YS. Thus, we calculated asymptotic species richness for each region by rarefaction using iNEXT online (Hsieh and Chao, 2016; Chao et al., 2014). It is important to note that the number of samples of *S. gunteri* in the YS was low (n = 11), basically because this species is relatively rare in this region. Despite this, we decided to keep these samples in the analysis because they are extremely difficult (= costly) to obtain and because the data at the infracommunity level are useful for comparison at this specific hierarchical level. However, please be aware that the comparison of species richness of the parasite component community of *S. gunteri* from the YS with all other regions and with *S. papillosum* from the same region was only for demonstrative purposes.

**Biological traits and trait approach**
For each of the species in Online Resource 1, we determined the state of each of the seven trait categories proposed by Ocaña et al. (2021): body tegument, life cycle, transmission, feeding mode, life stage, specificity and attachment organs. We did this because most of the metazoan parasites of both flatfish species (*S. gunteri* and *S. papillosum*) were already classified into functional groups based on these trait categories for the NYV and the YS (see Ocaña et al. 2021 and Vidal-Martínez et al. 2022). However, this has not been done for the VT region. As an example, we illustrate the selection process with some of these trait categories for the adult digenean *Helicometrina nimia*. The two categories of body tegument in Ocaña et al. (2021) classification could be soft or hard. Since adult digeneans such as *H. nimia* have a soft tegument, that was the chosen trait category. In the case of the life cycle, this could be direct or indirect, but *H. nimia* has several intermediate hosts. Thus, the selection was an indirect life cycle. In the case of transmission, this could be active or passive. Since adult digeneans develop in the intestine of vertebrates after the ingestion of infected intermediate hosts, the transmission process is passive. The whole classification of each of the metazoan parasite species in Online Resource 1 into each of the seven categories mentioned above is presented in Online Resource 2. Posteriorly, once all of the parasite species recovered were assigned to a particular trait category. Ascribing a parasite species to a particular trait category was based on microscopy observations (e.g., attachment organs), a literature review of the species or closely related taxa (i.e., genus or family) and expert consulting.

Ocaña et al. (2021) assessed the performance of taxonomic and four functional trait-based approaches for the dusky flounder (*S. papillosum*) parasite assemblages along the YS to determine their potential as bioindicators of marine chemical pollution. These functional trait-based approaches were community-weighted means (CWM), functional trait niche (FTN), functional groups (FGs) and Rao's functional diversity (FD). The authors found that CWM was the approach that is best suited to detecting spatio-temporal variation and pollution gradients. For this reason, in addition to the classical taxonomic approach, we used CWM for data analysis. In this analysis, the ‘taxa by trait matrix’ is multiplied by the ‘species abundance by sample matrix’ to obtain a ‘trait by sample matrix’ weighted by parasite abundance per sample (Garnier et al., 2007). Then, the matrix is standardized according to total abundance per sample. Detailed illustrations of this approach can be seen in Beauchard et al. (2017) and Ocaña et al. (2021).

**Data analysis**

All environmental variables from water and sediments were Log (x + 1) transformed and correlations between variables were explored using the Pearson correlation. If collinearity was present between pairs of variables, the variable with the lowest biological relevance (e.g., Al) was removed from subsequent analyses. All of the remaining environmental variables were used to build a similarity matrix between sampling sites based on Euclidean distance after normalization. The taxa abundance and the trait-based matrices were transformed (fourth root) and their respective triangular resemblance matrices were produced using the Euclidean distances between samples. Permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was performed to test spatial variations in the taxonomic and functional compositions of parasites of each host species (*S. gunteri* and *S. papillosum*) in the study.
area. The following sources of variations were considered: hosts (S. gunteri and S. papillosum) and regions (VT, NYV and YS). Regions and hosts were treated as fixed factors. The method used was the permutation of residuals under a reduced model and Type I sum of squares suited for unbalanced (i.e., variable number of hosts per sampling site) designs (Anderson et al., 2008). PERMANOVA t-tests were used to test the significance of pairwise comparisons of the effects of fixed factors.

Resemblance patterns between regions were displayed using non-metric multidimensional scaling (NMDS), with a centroid analysis with 50 bootstraps and 999 permutations. SIMPER analysis was also used to determine which species contributed the most to the observed patterns of infracommunity similarity. Those species with fewer than three individuals were removed from the analysis. The relationship between Aliphatic Hydrocarbons (Hc), Aromatic Hc and Total Hc was analyzed using the BIOENV routine to determine the best (BEST) set of explanatory variables with a Spearman correlation (Clarke and Ainsworth, 1993). After screening, the BEST procedure only retained the environmental variables listed above. All multivariate statistical tests were performed using 999 permutations of Primer-e v7/PERMANOVA + software (Anderson et al., 2008; Clarke and Gorley, 2015).

Results

Online Resource 1 shows the list of 57 metazoan parasites recovered for S. gunteri and S. papillosum from the three regions: VT, NYV and YS. The proportion of species in larval stage was 67.21% versus 32.78% of species in the adult stage. The proportion of generalists, specialists and unknown status species was 91.81%, 3.27% and 4.9%, respectively. The proportion of the most frequent and abundant species was 47.45% of which 89.28% were larval stages and only 10.71% were adults (Online Resource 1). The descriptive data at sampling site level on the metazoan parasite communities of S. gunteri and S. papillosum for the YS and NYV have been published elsewhere (Vidal-Martínez et al., 2014; Vidal-Martínez et al., 2019). However, the descriptive data of the metazoan parasite communities of S. gunteri and S. papillosum for VT are presented for the first time. The data on the parasite infracommunities of these host species together with data on the infracommunities of the same host species for the NYV and YS regions are provided for comparison in Online Resource 3. The most relevant pattern in Online Resource 3 was that the YS remains the region with the largest number of metazoan parasite species and individuals for both S. gunteri and S. papillosum. However, the largest value of the Brillouin’s diversity index was for VT, which was because the evenness value for this region was also the largest. It was also evident that S. papillosum acquired more parasite species than S. gunteri, with the exception of YS, where both host species had similar mean numbers of species (Online Resource 3). In fact, we found significant differences between regions for the values of the Brillouin’s diversity index for S. gunteri and S. papillosum (ONE-WAY ANOVA; F\textsubscript{5,407} = 79.97; P < 0.0001). However, even when both host species had a similar number of species in this region, the sample size was quite different as was the number of individuals of metazoan parasites, casting doubts about these comparisons. Thus, even when these data are shown here with descriptive purposes, explanations about the patterns of the Brillouin index or any other analysis at the infracommunity level were not considered any further. Instead, we decided to use a
comparison of species richness (rarefaction) at component community level between species and regions based on 5000 iterations. Figure 2 shows the medians and 95% confidence intervals of the rarefacted values of species richness for each host species in each region. Clearly, *S. papillosum* in the YS was the host species with the largest median values of species richness, followed by *S. gunteri* and *S. papillosum* from VT. These patterns can be corroborated in Online Resource 4 which shows the asymptotic rarefacted values of species richness for each host species in each region. Online Resource 5 shows the significant differences between specific combinations of hosts and regions. Since there were significant differences in the number of metazoan parasite species that each host can harbor, the most logical comparison would be for each host species between regions. In the case of *S. gunteri*, the parasite species richness of this host species from NYV was significantly smaller than that of VT (Fig. 2, Online Resource 4). In contrast, there were no significant differences in parasite species richness between NYV and YS (Fig. 2, Online Resource 4), most probably due to the small sample size of this host species in YS. The same applies for species richness between *S. gunteri* of VT and YS. With respect to *S. papillosum*, there were no significant differences in species richness between NYV with respect to VT. However, there was a significant difference between the parasite species richness of *S. papillosum* from NYV with respect to YS. Finally, there were no significant difference in species richness between *S. gunteri* of VT and YS. Now, if we compare the species richness of both host species by region, there were also large differences. In this case, *S. gunteri* from NYV fish had a significantly smaller species richness than that of *S. papillosum* from NYV, VT and the YS. In contrast, there were no significant differences in parasite species richness of *S. gunteri* from VT with respect to that of *S. papillosum* from NYV and VT. However, the parasite species richness of *S. gunteri* from VT was significantly smaller than that of *S. papillosum* from the YS. Finally, there were no significant differences in species richness between *S. gunteri* from YS and *S. papillosum* from NYV. However, there were strong significant differences in species richness between *S. gunteri* from YS and *S. papillosum* from VT and YS (Fig. 2, Online Resource 4).

The taxonomic and functional composition of metazoan parasites of flounders showed significant variations along the sGOM, by region and host species (Table 1). In addition, there was an interaction of region by host. The posteriori analysis showed that there were differences between all regions and hosts, with the exception of YS, where the host had no differences at the species or functional attribute levels. The similarity patterns using non-metric multidimensional scaling (NMDS) for species and traits for the three regions are shown in Online Resource 6.
Table 1
PERMANOVA results showing taxonomic (upper) and functional trait (lower) variation in metazoan parasite communities of *S. gunteri* and *S. papillosum* along the continental shelf of the southern Gulf of Mexico.

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<th>Source</th>
<th>df</th>
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<th>MS</th>
<th>Pseudo-F</th>
<th>P(perm)</th>
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<td>0.001</td>
<td>998</td>
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<td>996</td>
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<tr>
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<td>16185</td>
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**Percentage of similarity analysis (SIMPER)**

The SIMPER analysis identified the species that made the largest contribution to the total number of individuals and dissimilarity within each host species (Online Resource 7) and region (Online Resource 8). Comparing the host species in terms of their parasite species composition, we had two patterns (Online Resource 7). First, the parasite species composition between both fish species is similar since the most frequent and abundant species were practically the same. However, the second pattern indicates that the mean abundance of each parasite species is different and it should certainly be different between regions. In fact, the average dissimilarity between both host species was 80.07%.

Online Resource 8 shows the species that made the largest contribution to the total number of individuals and dissimilarity comparing all regions. First, the level of dissimilarity in the parasite species composition between NYV and the YS was the lowest in the comparison among regions with 66.62%, which means that the most frequent and abundant species were similar for both regions. However, the reason for the still high level of dissimilarity is related to the fact that the mean abundance of each parasite species was different between regions. Here, with exception of *L. floridensis*, a generalist digenean, and *S. chetumalensis*, a generalist adult nematode, all other species were in the larval stage. In the case of NYV versus VT, the average dissimilarity was 86.57%. Again, the species composition of those species
contributing the most to the number of individuals between both regions was similar. However, except for *L. floridensis*, the dissimilarity was again based on the different number of individuals of species in the larval stage. Finally, the average dissimilarity between VT and YS was 84.43%; again, with exception of *L. floridensis*, the larval stages of helminths were those contributing the most to the average abundance and dissimilarity. Something that is remarkable is that the species composition for the three regions is basically the same, but the difference was in the abundance of the larval stages.

Online Resources 9 and 10 show the trait profiles of the flounder hosts *S. gunteri* and *S. papillosum* for VT, NYV and YS. In the case of body covering, there were more metazoan parasites with soft body covering in the YS compared with the other two regions (Online Resource 9A-B and 10A-B). In the case of the transmission strategy, there were more metazoan parasites transmitted actively in the YS compared with the other two regions (Online Resource 9C-D and 10C-D). For the feeding mode, there were more absorbers than predators in the three regions (Online Resource 9E-F and 10E-F). With respect to the proportion of life stages, there were more larval stages than adult stages in the three regions (Online Resource 9G-H and 10G-H). However, the number of larvae was more variable in the YS compared with the other two regions, where there were far more larvae. Finally, with respect to the proportion of attachment organs, there was a higher proportion of soft attachment organs in the NYV and YS regions, compared with the VT region. In contrast, there was a much higher proportion of hard attachment organs in VT and NYV regions compared with the YS (Online Resource 9I-K and 10I-K). The BIOENV test determined that the best explanatory variable of the variation for species and functional assemblages was aromatic hydrocarbons with a significant correlation. In the case of species, the correlation was $R = 0.14 \ p < 0.05$, while for functional assemblages, it was $R = 0.17 \ p < 0.05$.

**Discussion**

In this study, our first hypothesis was that the species richness of the metazoan parasite communities of both flatfish species in the regions without oil extraction activities [Tamaulipas (VT) and Yucatan shelf (YS)] would be significantly higher than those of the region with oil extraction activities [Northern Yucatan shelf, (NYV)]. Since there were significant differences in the asymptotic species richness of both host species, it was necessary to interpret their results separately. In the case of *S. gunteri*, this hypothesis was accepted because its parasite species richness in the NYV region was significantly lower than that of VT (Fig. 2). This suggests that the life cycles of the metazoan parasites in the NYV region were negatively affected and could not be completed. Apparently, the species composition of the metazoan parasite fauna of *S. gunteri* in NYV suffered changes from pollution-sensitive species to pollution-resistant species. This could be the case of species that were only in the larval stage, such as *Stephanostomum* sp. 1, *Lecanicephalum* sp., *Trypanorhyncha* gen. sp., which presented a rather large number of individuals in the NYV compared with VT. This high number of individuals suggests high levels of transmission of larval stages, which implies a large number of intermediate hosts being infected. This number of infected intermediate hosts could be possible only if the amount of nutrients and or hydrocarbons are higher in the NYV region compared with VT. This seems to be the case since three coastal cities (Ciudad del Carmen, Campeche and Coatzacoalcos and Veracruz port, Veracruz) with bad poor sewage management
are present in this region (Ponce-Vélez and Botello, 2005; Barrera-Escorcia et al., 2014; Sericano et al., 2019). The number of adult metazoan parasite species was very similar between both the NY-V and V-T regions, which suggest that this metric was not consistent to indicate a potential effect of the environmental impact produced by the hydrocarbon extraction activities. Similar suggestions were made by Vidal-Martínez et al. (2014). These authors divided the Campeche Sound region into three regions. In the region with the highest concentration of pollutants (e.g., hydrocarbons and heavy metals) they noticed an increase in the number of metazoan parasite species in the larval stage in the same host (S. gunteri). We did not include the data of S. gunteri for the Yucatan shelf region for comparisons because, even using rarefaction, it was evident that the number of fish sampled for this region was so low that the parasitological results became biased. However, if we compare the number of adult species of S. papillosum (17), this is three times the number of species reported for S. gunteri in the NYV region (6) (Online Resource 1). If this is the case and the number of adult parasites is less due to contamination in the NYV region, then the argument about the negative effect on the intermediate host mentioned above applies. Other authors such as Schmidt et al. (2003) and Vidal-Martínez et al. (2003) have reported that in environments with high level of contamination, there is a decrease in the number of species in flounder (Platichthys flesus) and of the infection parameters of digeneans in Mayan Sea catfish (Ariopsis assimilis) respectively. Additionally, similar patterns for marine benthic organisms exposed to oil spill, organic matter and sewage suffer changes in species composition from contaminant-sensitive species to contaminant-resistant species (Dauvin et al., 2012; Rombouts et al., 2013).

In the case of S. papillosum, its parasite species richness for the YS was significantly higher than that from NYV region. In addition, the concentrations of hydrocarbons, heavy metals, hydrocarbon metabolites and histological damage index in flatfishes from the YS were lower compared with those of the NYV region (Vidal-Martínez et al., 2022). Thus, we can at least partially accept our first hypothesis since the species richness of the metazoan parasite communities of one of the flatfish species (S. papillosum) was higher in the region without oil extraction activities (YS) compared to the region with oil extraction activities (NYV). However, since there were no significant differences between the species richness of the parasite communities of S. papillosum from NYV and those from VT, the hypothesis could not be totally accepted. The concentrations of hydrocarbons and heavy metals, hydrocarbon metabolites and histological damage index in flatfishes from the VT region were not as high as those from flatfishes from the NYV region (Vidal-Martínez et al., 2022). Then, a significant difference in species richness, similar to that seen in the comparison with the YS could be expected. This was not the case; therefore, we can conclude that these results suggest that asymptotic species richness was not a consistent metric to indicate a potential effect of the environmental impact produced by the hydrocarbon extraction activities. This is important, because, if after the process of identification of the parasites at species level they do not indicate clear changes even under strongly adverse environmental impact conditions, then the investment of time and money in those molecular and morphological identifications is not worth for environmental impact studies. The literature also suggests variable results with respect to the usefulness of species richness as an indicator of environmental impact. This is the case of Dzikowski et al. (2003) for the marine rabbitfish Siganus rivulatus in the Gulf of Aqaba, Red Sea, Gelnar et al. (1997) in parasites
of freshwater fishes in Europe and Schmidt et al. (2003) for the parasite component communities of *Platichthys flesus* from the German Bight, North Sea.

Our second hypothesis was the presence of significant differences in the amount of explained variance on the effect of chemical pollution on the taxonomical and functional traits of the metazoan parasites of both host species for regions with and without oil extraction activity. Indeed, the trait-based approach varied as much as the taxonomic approach in the three regions studied in the sGOM, suggesting that changes in taxa composition also resulted in functional composition shifts. According to the PERMANOVA, the taxonomic and functional composition of metazoan parasites of the dusky flounder and the shoal flounder showed significant spatial variations along the sGOM, by region (Table 1). An important implication of these results is that either matrix (species or functional traits) could be used as a bioindicator. Consequently, the biological and ecological traits of the metazoan parasites of *S. gunteri* and *S. papillosum* function as bioindicators of environmental health as much as the parasite taxonomical component. Therefore, we accepted our second hypothesis. Our results concur with those published by Vidal-Martínez et al. (2022) and Ocaña et al. (2021) who demonstrated that the biological and ecological traits of the metazoan parasites of *S. papillosum* produce the same amount of explained variance as the traditional taxonomic approach for assessing the relationship between parasite community metrics and environmental and pollutant variables in the Yucatan shelf. This is an important result for parasite ecologists working on environmental impact studies because they can use these functional traits instead of detailed taxonomical identifications at the species level for rapid environmental assessments. Similar usefulness of functional traits of fishes and benthic organisms as bioindicators of environmental impact have been published elsewhere (Aguilar-Medrano and Vega-Cendejas, 2020; González-Gandara 2020; Aguilar-Medrano et al., 2021; Hernández-Avila et al., 2021). Additionally, we do not need to spend as much time and resources on the identification of functional traits for bioassessments as we do on the identification at species level. In this sense, using functional traits will save time and money.

The PERMANOVA also indicated that significant variation between hosts and the significant interaction between hosts and regions (Table 1) are apparently explained by the same results. These variations were apparently related to the relative abundance of the species contributing the most to dissimilarity (Online Resource 7 and 8). In fact, the information in these Tables and the NMDS results (Online Resource 6) show that both host species are essentially exposed to the same pool of parasites in the sGOM. Clearly, the most frequent and abundant species of metazoan parasites in both host species (*Lecanicephalum* sp., *K. pronosoma* and *Lecithochirium oridense*) were present in all of the regions (Online Resource 8). It is important to remark that two of this species were larval stages of cestodes with elasmobranchs as definitive hosts and very frequent in sGOM (see Vidal-Martínez et al., 2014; Vidal-Martínez et al., 2019). In the case of *L. floridense*, it is a generalist digenean which is widely distributed in the sGOM in fish species of several families (Hernández-Mena et al., 2023). This repeatability on the composition of the dominant species among regions suggest nestedness, since the most frequent and abundant species of metazoan parasites of *S. gunteri* are a subset of the parasite fauna of *S. papillosum* (Online Resource 7 and 8). This means that both host species are exposed to the same pool of parasites in the sGOM, but they become
infected differentially by these metazoan parasites in the three regions probably due to slight biological differences in their feeding habits or specific habitat location. Certainly, a formal study of nestedness in the metazoan parasite communities of *S. gunteri* and *S. papillosum* will be necessary. Our results concur with those of González and Poulin (2005) for the parasite component communities of the rockfish *Sebastes capensis* from the Pacific coast of South America. These authors found the presence of nestedness in the parasite communities of this fish consistently along the coast of Chile for endoparasites and attributed its variability to the feeding habits and characteristics of the habitat of the rockfish along its geographical distribution. Similar results were also found by Aparicio-Rizzo and González (2015) for five intertidal fish species from Chile. These authors found that those fish species with the highest number of species and individuals presented a nested pattern. This was certainly the case for the parasite communities of the two flounder species in the present study.

Why is so important for the study of the parasite communities that the functional traits are providing a certain amount of explained variance in relation to environmental variables? Basically, because these traits express the way the species are responding to selection pressures at local or regional levels. This means that the life cycles of the parasites in some ways are being affected by an excess of nutrients or the absence of some intermediate hosts. In short, the parasites respond to these environmental pressures in a short time (weeks to months). It is important to note that this time interval is faster than that proposed by Adams (2002) as being adequate to reflect the ecological relevance of the data. Thus, an important question here is if the species in the polluted place are responding to the specific pressure of a higher concentration of nutrients and hydrocarbons in the environment and this is producing more larval stages that accumulate in the tissues of the hosts; does this increase the probability of mortality for the fishes in this region? We do not know, but it is an important line of research to establish the relevance of metazoan parasites as bioindicators of environmental impact.

Another important point is that our study was performed at β-diversity level, comparing regions in the sGOM. β-diversity can be decomposed into two terms: functional nestedness (if the species in a community present only a small subset of the functional strategies present in another community) and functional turnover (if the communities present different functional strategies) (Villeger et al., 2013). In our case, high levels of functional nestedness were present for the functional traits of the parasites of both species (Online Resource 9 and 10) because the same strategies in the species rich communities (YS) were present in both the impacted region (NYV) and the VT region. Also, we found a low level of functional turnover, because the most frequent and abundant metazoan parasite species in the larval stage (e.g., *Stephanostomum* sp. 1, *Lecanicephalum* sp., Trypanorhyncha gen. sp.) with similar functional traits were present in all regions in this case (Online Resource 9 and 10). The point here is that the high number of larvae exacerbated the effect of this low level of turnover with the unknown but possibly deleterious consequences of a high accumulation of larvae in both host species. Similar results on the increase in the number of heteroxenous parasites (as a trait) after the Prestige-oil spill on the Iberic Peninsula were reported by Pérez-del Olmo (2007b) and Pérez del Olmo (2009).
Conclusions

Species richness did not result a reliable indicator of the impact of pollution over the metazoan parasite communities of both flatfish species. In contrast, functional traits provided very consistent and easy to use indicators of the effect of pollution over these parasite communities. The larval stages of cestodes and digeneans were the parasites that presented characteristics of species that are resistant to pollution and there were marked differences in the number of individuals between polluted and non-polluted places. Since these larval species were present in the three regions studied, they produced a high level of functional nestedness and a low level of functional turnover due to the presence of species with similar traits between regions. Certainly, the nestedness and turnover patterns and response to environmental variables found in these parasite communities should be compared with those of other groups such as fishes or benthic invertebrates to determine whether or not we can generalize the response of functional diversity to environmental impact in the southern Gulf of Mexico. However, functional groups in other groups of vertebrates such as fishes (Aguilar-Medrano et al., 2021) and metazoan parasites (Vidal-Martínez et al., 2019) also suggest that the YS is a pristine place and that the NYV region is highly impacted.

Declarations

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Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Center for Research and Advanced Studies (CINVESTAV) of the National Politechnical Institute (Protocol number: 0138-15).

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.
VMVM conceptualized the study, analyzed data and draft the manuscript. All authors read and approved the final manuscript. FAO help with methodology performed the statistical analyzes and helped to review and edit the manuscript. LCSJ participated in the design of the study, data curation. ALMT did data curation performed analysis and helped to review and edit the manuscript. OACC data curation and performed the maps. MLAM Funding acquisition, project administration, contributed to conceptualize the study, review original draft of the manuscript.

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Figures
Figure 1

The three regions in which the whole southern Gulf of Mexico was divided: Northwestern Yucatan shelf to Coatzacoalcos, Veracruz (NYV), Veracruz-Tamaulipas (VT), and the Yucatan shelf (YS). The marks in different colors present the geographical positions of the sampling sites.
Figure 2

Asymptotic species richness values for the different combination of hosts *Syacium gunteri* (Sg) and *Syacium papillosum* (Sp) for Northwestern Yucatan shelf to Coatzacoalcos, Veracruz (NYV), Veracruz-Tamaulipas (VT), and the Yucatan shelf (YS)

**Supplementary Files**

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