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The pink shrimp *Farfantepenaeus duorarum*, its symbionts and helminths as bioindicators of chemical pollution in Campeche Sound, Mexico

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Abstract

The pink shrimp *Farfantepenaeus duorarum* may acquire pollutants, helminths and symbionts from their environment. Statistical associations were studied between the symbionts and helminths of *F. duorarum* and pollutants in sediments, water and shrimps in Campeche Sound, Mexico. The study area spatially overlapped between offshore oil platforms and natural shrimp mating grounds. Spatial autocorrelation of data was controlled with spatial analysis using distance indices (SADIE) which identifies parasite or pollutant patches (high levels) and gaps (low levels), expressing them as clustering indices compared at each point to produce a measure of spatial association. Symbionts included the peritrich ciliates *Epistylis* sp. and *Zoothamnium penaei* and all symbionts were pooled. Helminths included *Hysterothylacium* sp., *Opecoeloides fimbriatus*, *Prochristianella penaei* and an unidentified cestode. Thirty-five pollutants were identified, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and heavy metals. The PAHs (2–3 ring) in water, unresolved complex mixture (UCM), Ni and V in sediments, and Zn, Cr and heptachlor in shrimps were significantly clustered. The remaining pollutants were randomly distributed in the study area. Juvenile shrimps acquired pesticides, PAHs (2–3 rings) and Zn, while adults acquired PAHs (4–5 rings), Cu and V. Results suggest natural PAH spillovers, and continental runoff of dichlorodiphenyltrichloroethane (DDT), PCBs and PAHs (2–3 ring). There were no significant associations between pollutants and helminths. However, there were significant negative associations of pesticides, UCM and PCBs

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with symbiont numbers after controlling shrimp size and spatial autocorrelation. Shrimps and their symbionts appear to be promising bioindicators of organic chemical pollution in Campeche Sound.

Introduction

It is a matter of current debate whether parasites (metazoans and protozoans) are effective indicators of anthropogenic environmental impact in aquatic ecosystems (Kennedy, 1997; Lafferty, 1997; Yeomans *et al.*, 1997; Marcogliese & Cone, 2001; Vidal-Martinez *et al.*, 2003; Khan, 2004; Sures, 2004a). Theoretical thinking on this question is based on field evidence lacking experimental support (Vidal-Martinez *et al.*, 2003; Khan, 2004; but see Khan & Payne, 2004), or laboratory evidence with no supporting field data (Siddall & des Clers, 1995; Marcogliese *et al.*, 1998; Morley *et al.*, 2001), and data on metazoan parasites as heavy metal sinks (Zimmermann *et al.*, 1999, 2005; Qian & Pin, 2000; Sures, 2003, 2004a,b; Sures *et al.*, 2005).

The debate has centered on whether parasites of fish are at least as useful for environmental monitoring as free-living organisms or other kinds of biochemical bioindicators (Kennedy, 1997; Schmidt *et al.*, 2003). Recent reports, however, support the view that aquatic hosts such as fish, crabs and shrimps, as well as their symbionts and metazoan parasites, are good indicators of environmental impact (Schuwerack *et al.*, 2001a,b; Bergey *et al.*, 2002; Schuwerack & Lewis, 2003a,b; Lewis *et al.*, 2003 and references therein; Schmidt *et al.*, 2003; Williams & MacKenzie, 2003; Sures, 2004a).

One as yet unresolved problem with using parasites as pollution bioindicators is the high temporal and spatial variability of parasite communities in aquatic organisms (Kennedy, 1997). This author pointed out that even within a single locality a parasite species can appear and disappear over time without manifesting an apparent trend, or a species can be present in one locality but absent in a neighbouring one, i.e. a lack of temporal or spatial repeatability. This caveat is linked to the influence of geographical distance on the repeatability of parasite community composition between different localities (see Vidal-Martínez & Poulin, 2003). Recent research has addressed the importance of geographical distance as a factor affecting structure and similarity (and thus repeatability) in the parasite communities of freshwater and marine hosts (Poulin, 1997, 2003; Poulin & Morand, 1999; Karvonen & Valtonen, 2004). Theory suggests that repeatability increases as geographical distance between sampling points decreases. However, it also means that samples collected from one place are not necessarily independent from samples collected in neighbouring localities, i.e. they are spatially autocorrelated. With one exception (Bengtsson & Ebert, 1998), the possible influence of autocorrelation in aquatic parasitological data has not been addressed. This is a vital aspect for mobile coastal or marine species, like fish or shrimps, since they naturally migrate within their home ranges.

During research on shrimp (a mobile species) health status for the Mexican national petroleum company Petroleos Mexicanos (PEMEX) in Campeche Sound (Gulf of Mexico), we collected data on pollutants and parasites in the pink shrimp *Farfantepenaeus duorarum*, as well as on pollutants in water and sediments in areas with and without offshore oil platforms. Data included symbiont and metazoan parasite species and individuals, and pollutants (heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)). However, the sampling design proposed by PEMEX imposed a high degree of contiguity between sampling points, resulting in autocorrelation in the pink shrimp data (Castrejón *et al.*, 2004). To deal with the data's spatial structure and control for autocorrelation we used spatial analysis using distance indices (SADIE) (Perry *et al.*, 1999). Originally used in insect ecology, this technique identifies areas of significant clustering and groups them into patches (in this case, areas of high parasite density or high pollutant concentrations), or gaps (areas of low density or low concentration) by producing an index that quantifies the degree to which each locality contributes to clustering. Furthermore, SADIE determines the extent to which cluster indices 'agree' at each point, thus providing a measure of spatial association.

Given that agriculture and offshore oil extraction are significant economic activities in the state of Campeche, Mexico, high pesticide concentrations in sediments and waters near the coast, and high PAH concentrations in sediments and waters near offshore platform zones can be expected. Consequently, the study objectives were: (i) to determine if the pink shrimp acquires organic pollutants (i.e. PAHs, PCBs, heavy metals and pesticides); and (ii) to determine if the number of symbionts and metazoan parasites in shrimp have significant statistical associations with pollutant concentrations in sediments, water and shrimp pools.

Materials and methods

Study area and sampling techniques

The study area encompassed 20 points in Campeche Sound, Gulf of Mexico (fig. 1). Shrimps were collected between 17 and 22 October 2002 using 0.5–1 h trawls at depths of 12.2 to 50 m undertaken from a ship (Cetmar II) equipped with two shrimp trawl nets. After each trawl (indicated by letters in fig. 1) surface sediments were collected with a 1 m^2 van Veen grab and frozen, while water samples were taken at 5 m depth intervals using 1-gallon amber glass bottles closed under water to avoid contamination with surface mixtures. Water samples were extracted with hexane, and constantly agitated for 5 min. Processed sediment and water samples were transported to CINVESTAV-Merida for further analysis. All trawls are shown in fig. 1, but only those (15) containing pink shrimps are shown in table 1.

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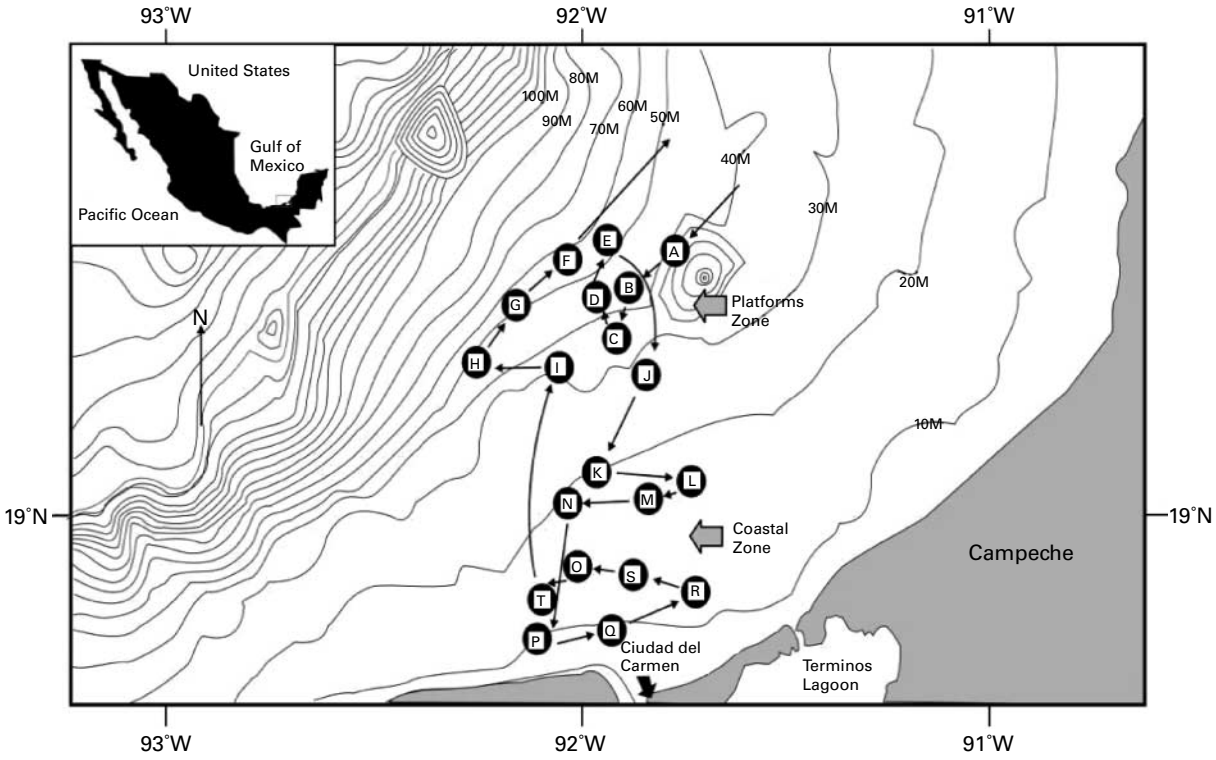

Fig. 1. Sampling points in Campeche Sound, Gulf of Mexico, where sediment and water samples were taken. Shrimps were caught in trawls between each sampling point. Points A–J were near offshore platforms, and points K–T near the coast.

A total of 248 pink shrimps were obtained from all trawls. These were identified on board and dissected sagittally. The right half of each specimen was stored for determination of pollutant concentrations. The left half was fixed in Davidson's AFA solution for four days then transferred to 70% ethanol for histological examination. These samples were then embedded in paraffin, and three sagittal sections, 5 μm thick, cut from each specimen. Tissue slides were stained with hematoxylin and eosin, and fixed to glass slides. The left sagittal slides of individual shrimps were examined to determine the number of individuals for each symbiont or metazoan parasite species, following the procedures of Khan (2004) for fish.

Chemical analysis

Hydrocarbon, pesticide and PCB levels were determined according to Sericano *et al.* (1990) and Wade *et al.* (1993). Shrimp samples were extracted in a Soxhlet apparatus for 8 h with hexane, and for another 8 h with dichloromethane. These extracts were concentrated in a Kuderna-Danish system, purified and divided into two fractions using column chromatography with alumina-silica gel. Lipids were removed from the second fraction in an HPLC system with a size-exclusion column, and organic compounds were quantified using gas chromatography (Hewlett-Packard 5890 Series II) with flame ionization detection for hydrocarbons, and electron capture detection for organochlorines. Internal standards (PCBs 103 and 198 for organochlorines and n-C_{23} and dihydroanthracene for hydrocarbons) were added for quality control. Concentrations of these pollutants in sediments and water were measured using the same process. Metals in water were extracted using Chelex 100 resin, and sediments were extracted with 0.1 M HCl (simultaneously extracted metals fraction). Organisms were digested with nitric acid and hydrogen peroxide. Final measurements were taken in a Perkin Elmer inductively coupled plasma (ICP) spectrometer.

Statistical analysis

Rather than describing parasite and symbiont infection parameters, our interest was in determining any possible associations between the number of symbionts and parasites identified in the shrimp slides with sediment and water pollutant concentrations. Consequently, the terms prevalence and mean abundance as suggested by Bush *et al.* (1997) were not used. Instead, the percentage of infected shrimp (number of shrimps infected with symbionts or a metazoan parasite species/number of shrimps examined per trawl) and the mean number of individuals (number of individual symbionts or individuals of a metazoan parasite species/ number of shrimps examined per trawl) were calculated. An 'all parasites' category was created to include all symbionts and parasites in the shrimps per trawl. Because symbionts

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Table 1. Helminths and symbionts of the pink shrimp *Farfantepenaeus duorarum* ($n = 248$) from 15 trawls in Campeche Sound, Gulf of Mexico between 17 and 22 October, 2002.

Trawl	Initial latitude longitude	Final latitude longitude	Number of shrimps	Total length (0±SD)	Total number of parasites	Helminths and symbionts				
						Cest	Hyst	Opec	Proc	Symb*
A	19° 31′ 098 91° 46′ 228	19° 31′ 137 91° 48′ 140	13	11.38 ± 3.19	18	–	–	–	12 0.12 ± 0.34	6 0.44 ± 1.71
B	19° 26′ 035 91° 53′ 981	19° 26′ 743 91° 51′ 570	17	12.71 ± 3.28	6	–	–	–	17 0.83 ± 2.18	18 0.82 ± 2.13
C	19° 20′ 873 91° 55′ 673	19° 21′ 041 91° 54′ 635	5	11.84 ± 3.8	60	–	–	–	60 0.6 ± 0.55	–
D	19° 25′ 460 91° 57′ 487	19° 26′ 689 91° 54′ 242	31	13.3 ± 2.78	17	3 0.74 ± 3.09	–	–	13 0.45 ± 1.31	6 1.70 ± 7.47
E	19° 32′ 418 91° 56′ 051	19° 30′ 139 92° 02′ 043	23	15.17 ± 2.98	9	–	–	–	22 0.43 ± 0.94	4 0.09 ± 0.42
F	19° 30′ 139 92° 02′ 043	19° 25′ 476 92° 07′ 874	22	12.04 ± 4.11	14	4 0.27 ± 1.28	–	–	14 0.36 ± 1.14	4 0.04 ± 0.21
G	19° 25′ 476 92° 07′ 874	19° 23′ 629 92° 10′ 378	26	12.47 ± 3.29	19	–	–	–	15 0.65 ± 1.80	4 0.08 ± 0.39
H	19° 19′ 322 92° 14′ 837	19° 18′ 437 92° 15′ 385	24	14.63 ± 3.37	8	–	4 0.08 ± 0.4	–	20 0.6 ± 1.44	12 0.68 ± 2.81
J	19° 17′ 378 91° 50′ 435	19° 16′ 66 91° 53′ 589	8	10.58 ± 1.46	37	–	12 0.12 ± 0.33	–	12 0.5 ± 1.33	–
K	19° 05′ 488 91° 57′ 535	19° 04′ 235 92° 00′ 799	10	10.52 ± 1.91	40	–	–	–	40 3.1 ± 4.95	10 0.5 ± 1.58
L	19° 04′ 296 91° 47′ 670	19° 04′ 296 91° 44′ 839	27	10.33 ± 1.18	13	–	–	–	10 0.33 ± 1.09	3 0.03 ± 0.18
M	19° 02′ 062 91° 51′ 660	19° 00′ 604 91° 54′ 320	15	10.02 ± 1.33	19	–	–	–	6 0.19 ± 0.75	6 0.19 ± 0.75
N	19° 00′ 956 91° 58′ 695	19° 01′ 673 92° 01′ 677	13	9.59 ± 1.39	23	–	0.08 ± 0.28	8 0.08 ± 0.28	23 0.61 ± 1.32	8 0.08 ± 0.28
R	18° 51′ 500 91° 44′ 753	18° 52′ 262 91° 47′ 753	5	8.8 ± 0.54	1	–	–	–	100 1.2 ± 0.45	40 0.8 ± 1.09
S	18° 52′ 513 91° 48′ 439	18° 52′ 958 91° 51′ 544	9	9.87 ± 1.4	11	–	–	–	11 0.22 ± 0.67	11 2.44 ± 7.33

Trawls with no pink shrimps are not shown. Entries for parasites are the percentage of infected shrimps (upper value) and mean number of individuals (lower values).

Cest, unidentified intestinal cestode; Hyst, *Hysterothylacium* sp.; Opec, *Opecoeloides fimbriatus*; Proc, *Prochristianella penaei*; Symb, the sum of all symbionts found; independent of species. The most probable species in this group were *Zoothamnium penaei* and *Epistylis* sp., based on Vidal-Martínez *et al.* (2002).

could not be identified to species level, they were grouped in a single 'symbionts' category.

Possible associations between the percentage and mean number of individuals of symbionts or metazoan parasite species and pollutant concentrations in shrimp tissue from the same trawl were determined. However, due to technical restrictions on the amount of shrimp tissue needed for pollutant analysis, the sagittal right halves of every 3–5 shrimps from each trawl were pooled. For each pool associations between the percentage, total and mean number of symbionts and parasites identified in the slides of the left half of the shrimps were determined, and the pollutant concentrations recorded in the right half of the same shrimps. A total of 39 pools were obtained from all trawls. Associations between the percentage and mean number of symbionts and parasites in the shrimps, and the sediment and water pollutant concentrations from each of the 15 trawls in which shrimps were caught were also studied. Data on the potential associations between pollutants and shrimp histology produced from these samples are to be published elsewhere.

The sampling design originally proposed by PEMEX produced autocorrelation in the data, and a clear bathymetric pattern in pink shrimp size (fig. 1) (Castrejón *et al.*, 2004), with shrimp size increasing with depth. The technique of Moles & Wade (2001) was used to compensate for the effect of shrimp size and then the number of parasites in each shrimp was divided by that shrimp individual's length.

SADIE (Perry *et al.*, 1999) was used to determine if pollutants and parasites were randomly distributed or significantly clustered in the study area. SADIE detects spatial patterns in the form of patch clusters, rather than characteristics of local population densities or concentrations. In this sense, its purpose differs from geostatistics, which estimates local density (Perry & Dixon, 2002). Mathematical details of SADIE are presented at http://www.rothamsted.bbsrc.ac.uk/pie/sadie. Briefly, spatial patterning in SADIE is measured at each sampled unit, using a clustering index. This algorithm is based on the movement of individuals to a new location (similar to transmission). In any case, an individual's movements between sampling units are expressed as distances. A randomization procedure randomly and independently reassigns each of the total individuals in the sample to one of the n sample units. The randomization process is then repeated 5000 times to build a frequency distribution of these values that relates to an initially random spatial distribution of individuals in the sample. The computed value of the sample is compared with this frequency distribution, and the proportion (P_r) of the values that are equal to, greater or smaller than the observed value is recorded (Perry, 1995). Therefore, the null hypothesis that the observed sample originated in a spatially random population is rejected at a 0.05 level for a two-tailed test if the indices values are equal to, greater or smaller than the observed value.

The I_r index, based on the average distance of randomized rearrangements, is $I_r = D/E_r$ where D is the distance of the actual sample, and E_r is the distance of random rearrangements. A unity value represents a spatially random sample. Each unit with a count greater or smaller than the overall mean is assigned to a patch (positive) or gap (negative) cluster index. Cluster indices for each sample were contoured in maps using interpolation procedures (e.g. kriging). We used Surfer ver. 7 software to produce maps with three region types: values near zero were random; areas with indices ≥ 1 were patch clusters; and areas with an indices ≤ 1 were gap clusters.

To determine spatial association using SADIE, it was assumed that parasite and pollutant cluster indices at each sample unit are measures of their local spatial pattern (patches or gaps) at that point. The extent to which the parasite and pollutant cluster indices in shrimps, sediments or water 'agree' or 'disagree' at each point provide a measure of local spatial association or dissociation. For the two data sets and clustering indices of set one z_{k1} (combining patch and gap data) with mean q_1, and those of set two z_{k2}, with mean q_2, the measure of local spatial association for unit k is:

$$X_k = n(z_{k1} - q_1)(z_{k2} - q_2)/[\Sigma_k(z_{k1} - q_1)^2\Sigma_k(z_{k2} - q_2)^2]^{1/2}$$

The overall spatial association is the mean of these local values, $X = \Sigma_{iXi}$, where X is the correlation coefficient between the clustering indices of each set. Assessment of the significance of X was tested against X_{rand} with a randomization test in which the clustering indices of each set are randomly reassigned to each set of units: $K = 1, 2, 3 \ldots n$. Critical values for the test statistic were estimated using the X_{rand} randomization distribution quantiles, derived from the null hypothesis of no association. Local spatial association values were also mapped and contoured.

When a set of cluster indices is spatially autocorrelated, then the amount of information in that set is reduced in proportion to the degree of autocorrelation. Hence, when testing a statistic that estimates the association between two data sets, such as X, the test and critical values must be adjusted to allow for any reduction in effective sample size caused by the autocorrelation. SADIE employs the adjustment proposed by Dutilleul (1993). This functions by computing the effective size of the combined sets, allowing the test's degrees of freedom to be adjusted. Since SADIE uses randomizations to build frequency distributions, it is essentially a distribution-free procedure. The significance of all statistical analyses was established at $P = 0.05$, unless otherwise stated.

Results

From 15 trawls in which pink shrimps were recovered (table 1), a total of four metazoan parasite species were found, namely an unidentified intestinal cestode, the nematode *Hysterothylacium* sp. in muscle, metacercariae of *Opeocoeloides fimbriatus* in muscle, and the cestode *Prochristianella penaei* in hepatopancreas. All recorded symbionts were found in the gills of the pink shrimp. Use of histology, rather than full parasitological examination of fresh specimens, precluded the identification of symbionts to species or detection of low-prevalence species like gregarines. However, it is highly probable that symbionts in the histological slides were *Zoothamnium penaei* and *Epistylis* sp. as these are the most frequent and abundant symbiont species on the Yucatan Peninsula

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(Vidal-Martinez *et al.*, 2002), the Texas and Georgia coasts and in the Mississippi Sound (Overstreet, 1973). All metazoan parasites were at the larval stage, and all symbionts were adults. *Prochristianella penaei* was the most frequent and abundant parasite, followed by the symbionts (table 1).

Detected sediment and water pollutant concentrations from the 15 trawls showed that significantly clustered pollutants were the 2–3 ring PAHs in water, the unresolved complex mixture (UCM), and the heavy metals Ni (nickel) and V (vanadium) in sediments (table 2). The remaining pollutants were randomly distributed in the study area.

Pollutant concentrations from 39 pink shrimp pools from all trawls which had both pollutants and parasites shows the presence of four PAHs metabolites, five heavy metals, 11 pesticides, and seven PCBs (table 3). Most pollutants in table 3 were not significantly clustered in the study area, for example, all the PAHs. Also, apart from Cu (copper) and Fe (iron), all heavy metals were randomly distributed. Of the pesticides, only heptachlor was significantly clustered. None of the PCBs was significantly clustered in the SADIE randomization process. Also, none of the parasites, symbionts, or the sum of both was significantly clustered in the shrimp pools. Finally, the sum of *P. penaei*, the symbionts plus all less-abundant parasites did not cluster significantly.

Figures 2 and 3 show the concentrations of selected pollutants from tables 2 and 3 that were significantly clustered in the study area. Each map shows the cluster indices for each pollutant in individual shrimps, sediments and water. In fig. 2A heptachlor in shrimp had significantly higher concentrations in the coastal zone than in the platforms zone, while the clustering values of Cu, UCM (fig. 2B and C), Ni and V in sediments (fig. 3A and B) were higher in the platforms area. Finally, the overall distribution for 2–3 ring PAH concentrations in water, even when the local cluster values were not significantly different from a random distribution, was significantly clustered in the coastal zone in comparison with the platforms zone (table 2; fig. 3C).

Shrimp size had a substantial influence on the data. Shrimps from trawls around offshore platforms were larger than those from near the coast (one-way ANOVA; $F_{15,510} = 17.68$; $P < 0.0001$). There were also significant correlations between mean total length and depth (Kendall's rank correlation Tau $= 0.70$; $n = 15$; $P < 0.01$), and between individual shrimp size and total number of parasites (Kendall's Tau $= 0.19$; $n = 37$; $P = 0.04$). There was a negative but statistically non-significant correlation between the number of *P. penaei* and shrimp size (Kendall's Tau $= -0.11$; $n = 37$; $P > 0.10$). This is why *P. penaei*, and other parasite species in table 1 that were not abundant enough for correlation analysis, were not included in further analyses. There were also no correlations between the percentage of shrimps infected with symbionts or a metazoan parasite species and pollutants from water, sediments or the shrimp hosts. Consequently, they were also not considered for further analyses. Symbionts numerically dominated in the histological slides throughout the study area. There was a significant positive correlation between symbiont clustering values and the total number of parasites ($X = 0.87$; $n = 37$; $P = 0.001$), and thus all the following figures showed associations between symbionts and pollutants.

Figures 4A to C and 5A to C show local values of the correlation coefficients (X_k) of the cluster indices of symbionts and pollutants from table 2, after shrimp size and autocorrelation were controlled for in the trawl data. In fig. 4A and B, negative correlation values mean that the higher the pesticide concentration, the lower the number of symbionts. Furthermore, there were overall significant negative associations between the clustering indices of symbionts and the pesticides alpha-HCH ($X = -0.69$; $n = 15$; $P = 0.99$) and HCH ($X = -0.52$; $n = 15$; $P = 0.98$). In contrast, there was a significant positive correlation between symbionts and heptachlor ($X = 0.53$; $n = 15$; $P = 0.03$), especially near the coast (fig. 4C). In fig. 5A and B, the higher the clustering indices values of UCM in sediments from the platforms zone ($X = -0.54$; $n = 15$; $P = 0.98$) and water ($X = -0.59$; $n = 15$; $P = 0.99$), the lower the symbiont values become. Finally, fig. 5C shows high Ni clustering indices where high symbiont clustering indices ($X = 0.51$; $n = 15$; $P = 0.03$) were recorded, especially near the coast.

Figures 6A to C and 7A to C show the correlation coefficients (X) of the symbiont clustering indices with selected pollutants from table 3 after controlling for shrimp size and autocorrelation for the 39 shrimp pools. Figure 6A shows a positive significant correlation between symbionts and phenanthrene ($X = 0.38$; $n = 39$; $P = 0.008$), with higher correlations in the coastal area. In contrast, fig. 6B has a significant negative correlation between symbionts and alpha-HCH ($X = -0.39$; $n = 39$; $P = 0.9918$) with positive correlations in the platforms area (top of the figure). In fig. 6C, high local and overall negative correlations between symbionts and heptachlor ($X = -0.76$; $n = 39$; $P = 0.9999$) were found in the coastal area. Figure 7A shows significant negative local correlation values for both symbionts and PCB1016, which in turn produced a positive overall correlation ($X = 0.64$; $n = 39$; $P = 0.0001$). In contrast, there were significant negative local and overall correlation values between symbionts and PCB1242 ($X = -0.77$; $n = 39$; $P = 0.9999$) (fig. 7B). Finally, significant positive local and overall correlation values between symbionts and zinc ($X = 0.32$; $n = 39$; $P = 0.0244$) were found near the coastal area (fig. 7C).

Discussion

The results show that the pink shrimp acquired pollutants from the environment, and that there were significant statistical associations between chemical pollutants and symbionts, once the effect of shrimp length and autocorrelation were controlled. However, there were unexpected results such as the random distribution of some pollutants. No correlations were observed between metazoan parasites or their percentages of infection and pollutants from water, sediments or the shrimp hosts. The results suggest that histological slides were useful in detecting statistical associations between symbionts and pollutants and that this is a useful technique when dealing with mobile hosts as bioindicators of environmental impact.

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Table 2. Pollutants detected in sediments and water for each of 15 trawls in Campeche Sound, Gulf of Mexico.

		Polycyclic aromatic hydrocarbons				Heavy metals						Pesticides			Polychlorinated biphenyls
		Alipha	PAH 2–3	PAH 4–5	UCM	Ba	Fe	Ni	V	Pb	Zn	HCHs	Metox	DDTs	PCBs
I_r Sedim		1.12	1.29	0.99	1.95	1.13	1.12	1.58	2.07	–	1.04	–	–	0.99	0.96
P value		0.27	0.09	0.48	0.0008	0.25	0.3	0.03	0.001	–	0.42	–	–	0.48	0.46
I_r Water		–	1.38	–	0.69	–	0.81	0.56	1	1.17	1.29	0.93	0.86	–	0.51
P value		–	0.03	–	0.94	–	0.74	0.97	0.41	0.25	0.14	0.61	0.68	–	0.98
Trawl															
A	S	0.95	0.11	0.18	26.90	2.52	2.54	12.72	45.074	ND	ND	ND	ND	0.47	1.80
	W	ND	ND	ND	4.90	ND	28.74	3.98	4.73	0.17	6.40	–	0.28	ND	0.9
B	S	0.83	ND	5.03	34.72	ND	8.80	15.15	49.42	ND	ND	ND	ND	ND	63.06
	W	ND	ND	ND	2.33	ND	91.10	3.78	57.09	0.23	6.39	0.75	ND	ND	0.9
C	S	0.69	ND	6.20	38.21	2.57	ND	13.22	47.38	ND	ND	ND	ND	ND	46.56
	W	ND	ND	ND	5.40	ND	110.34	4.17	49.75	0.60	15.93	0.75	0.50	ND	0.9
D	S	0.58	ND	0.10	23.65	3.23	51.70	14.32	57.10	ND	ND	ND	ND	ND	1.8
	W	ND	0.06	ND	6.82	ND	72.74	3.82	49.46	0.22	15.58	9.00	ND	ND	1.25
E	S	0.50	ND	0.67	22.36	ND	6.44	13.34	55.77	ND	ND	ND	ND	ND	35.92
	W	ND	0.20	ND	3.57	ND	49.23	4.29	50.84	0.24	8.55	ND	ND	ND	0.9
F	S	1.21	0.03	0.40	32.74	3.02	151.12	16.52	43.18	ND	ND	ND	ND	ND	ND
	W	ND	ND	ND	2.64	ND	58.15	4.17	38.09	0.14	13.62	ND	0.46	ND	0.9
G	S	0.41	0.01	0.04	20.94	2.92	198.93	15.47	56.23	ND	ND	ND	ND	ND	1.8
	W	ND	ND	ND	48.76	ND	34.77	3.98	38.14	0.13	2.87	ND	0.32	ND	0.9
H	S	1.38	0.02	0.44	81.36	3.50	730.10	19.39	55.44	ND	3.36	ND	ND	ND	0.90
	W	ND	ND	ND	36.88	ND	39.05	4.05	54.07	0.14	6.90	ND	ND	ND	0.9
J	S	0.90	0.01	0.67	10.05	ND	ND	9.97	43.23	ND	ND	ND	ND	ND	8.10
	W	ND	ND	ND	177.29	ND	27.77	3.77	39.97	0.53	6.34	ND	0.37	ND	ND
K	S	0.52	ND	0.89	0.03	2.70	ND	6.91	35.52	ND	ND	ND	ND	ND	26.65
	W	ND	ND	ND	22.38	ND	20.96	4.50	49.89	0.12	7.47	ND	0.30	ND	0.9
L	S	0.35	ND	−0.05	3.99	3.02	3.52	1.58	34.03	ND	ND	ND	ND	ND	32.34
	W	ND	ND	ND	24.21	ND	78.76	4.20	48.07	0.18	8.78	ND	0.28	ND	1.80
M	S	0.90	ND	0.98	ND	3.15	69.98	6.54	32.72	ND	ND	ND	ND	ND	ND
	W	ND	ND	ND	23.57	ND	55.56	3.84	48.89	0.11	7.81	ND	0.36	ND	ND
N	S	0.55	ND	0.93	ND	2.64	11.32	8.54	37.03	ND	ND	ND	ND	ND	2.00
	W	ND	ND	ND	18.68	ND	32.67	3.69	54.58	0.16	3.86	ND	0.25	ND	0.9
R	S	0.26	ND	ND	ND	2.59	8.07	8.57	36.91	ND	ND	ND	ND	0.43	3.81
	W	ND	3.94	ND	24.88	ND	48.59	3.97	33.65	0.12	3.08	ND	ND	ND	0.9
S	S	0.96	ND	0.36	ND	3.18	584.20	17.98	41.65	ND	2.40	ND	ND	ND	2.22
	W	ND	0.13	ND	23.09	ND	65.03	4.17	40.03	0.10	5.49	ND	ND	ND	0.9

Upper values are sediment (S) concentrations ($\mu g\ g^{-1}$ for polycyclic aromatic hydrocarbons, heavy metals and pesticides, $pg\ g^{-1}$ for polychlorinated biphenyls) and lower values are water (w) concentrations ($\mu g\ l^{-1}$). I_r Sedim, sediments clustering index value; I_r Water, water clustering index value; *P* value, significance level; ND, not detected.

Table 3. Pollutants, helminths and symbionts of 39 pink shrimp *Farfantepenaeus duorarum* pools from Campeche Sound, Gulf of Mexico.

	Polycyclic aromatic hydrocarbons ($\mu g\ g^{-1}$)				Heavy metals ($\mu g\ g^{-1}$)					Pesticides ($\mu g\ g^{-1}$)		Polychlorinated biphenyls ($\mu g\ g^{-1}$)	Helminths and symbionts (number of individuals)		
	BAPir	Pir	Naph	Phena	Cu	Fe	Ni	V	Zn	Hepta	Pesti	PCBs	Apro	Asymb	All
I_r	0.84	0.76	0.80	0.14	1.76	1.37	1.23	1.22	0.58	1.45	0.89	0.84	1.00	1.20	1.11
P value	0.69	0.84	0.78	0.28	0.01	0.04	0.18	0.18	0.98	0.02	0.64	0.67	0.47	0.25	0.27
Shrimp															
A5	24.61	15.44	121.25	256.3	15.52	2.24	2.46	33.13	31.9	ND	0.46	0.06	1	4	5
A10	0.57	0.21	11.31	5.29	13.93	5.87	13.43	34	33.87	ND	2.9	0.01	0	1	1
A11	18.88	9.19	19.89	274.1	11.77	39.05	23.19	39.4	32.07	ND	ND	0.13	1	0	1
B17	11.55	3.2	35.85	181.5	12.43	22.21	15.55	34.73	38.11	ND	ND	0.01	1	2	3
B18	3.93	3.13	75.61	253	13.88	23.91	12.71	32.94	29.9	ND	ND	ND	0	8	9
B19	0.57	0.25	9.12	5.31	8.77	25.19	36.36	78.03	35.62	ND	0.06	0.01	1	4	5
B20	2.75	0.41	6.68	166.1	10.4	17.6	5.03	70.52	28.85	ND	ND	0.02	0	0	0
C38	41.33	0.87	111.88	546	15.24	22.56	4.74	79.29	34.75	ND	ND	ND	0	0	0
D40	3.34	37.06	7.35	133.2	17.02	13.29	5.29	80.11	29.74	ND	0.95	0.12	0	13	13
D44	5.14	0.61	4.85	143.8	19.31	18.17	6.79	73.51	30.66	ND	0.04	ND	0	0	0
D45	1.94	0.45	8.43	123.6	16	11.27	13.02	32.88	36.55	ND	ND	0.01	0	0	0
D47	39.18	31.4	259.46	416.3	24.52	21.54	4.9	76.6	35.94	ND	ND	ND	1	40	41
D49	14.94	7.23	34.6	180.2	20.84	35.12	10.85	74.97	33.18	ND	0.1	0.01	0	0	0
D76	110.23	15.3	953.41	602.3	14.13	74.91	33.05	47.91	34.2	ND	0.07	0.01	0	0	0
D84	12.86	12.37	133.9	209.5	10.77	109.5	29.5	17.88	32.42	ND	1.24	ND	1	16	17
D89	12.87	7.47	164.5	226.4	16.03	492.4	149	74.73	39.39	ND	ND	ND	1	4	5
D90	43.86	33.81	396.06	377.7	13.09	61.64	13.31	33.31	30.13	ND	ND	ND	1	14	15
F98	12.94	4.79	14.99	262.1	13.17	7.5	19.67	33.43	29.7	ND	0.11	0.01	0	7	7
F101	41.69	23.67	214.2	234.6	10.6	11.39	16.56	38.66	30.42	ND	4.06	0.01	0	0	15
F104	0.46	0.27	6.29	6.08	19.33	26.11	6.19	77.28	35.76	ND	11.4	ND	1	0	1
G117	4.5	1.42	36.35	271.7	23.1	16.1	6.15	79.71	34.82	ND	ND	0.02	1	9	10
G122	6.63	2.84	29.88	197.7	16.5	21.69	4.96	75.68	33.17	ND	8.56	0.03	0	0	0
G123	9.42	5.69	76.28	206.3	47.66	31.7	16.25	214	34.88	ND	ND	0.03	1	0	1
G128	6.63	3.55	48.31	233.7	14.12	9.42	12.8	33.15	37.33	ND	0.22	ND	3	0	3
H157	5.59	4.05	72.19	113.2	13.46	21.31	39.33	70.63	35.5	ND	1.01	0.04	0	0	0
H182	18.65	11.64	143.32	246.9	11.75	47.59	64.83	126	42.26	ND	0.12	ND	0	42	42
J185	4.4	2.83	87.19	111.2	15.27	49.28	26.12	152.9	30.12	ND	0.06	ND	2	0	7
K194	1.85	1.1	49.97	62.42	13.66	16.99	10.28	72.33	26.43	ND	1.77	ND	0	0	0
L206	5.11	2.42	27.5	141.9	18.93	39.39	12.63	6.03	30.48	ND	1.5	ND	0	0	0
L230	12.89	4.99	23.58	193.6	36.49	111.2	50.09	214.7	30.7	ND	ND	0.02	0	0	0
M243	0.97	0.48	24.16	18.29	16.62	32.34	10.31	71.36	31.77	ND	0.29	ND	0	0	0
N244	1.78	1.09	39.13	31.81	6.12	122	36.56	58.76	29.78	ND	0.45	ND	3	1	6
N158	24.26	16.64	219.78	242.3	10.63	24.55	14.23	31.55	28.8	ND	30.2	0.18	0	0	0
R256	16.54	7.7	153.11	174.7	9.65	10.82	13.95	33.57	30.41	ND	2.71	ND	1	0	1
R255	14.75	8.43	122.22	261	11.41	42.57	24.54	53.23	34.98	ND	ND	0.12	1	2	3
R257	16.93	12.27	173.67	417.5	8.25	25.6	27.91	62.31	32.42	0.02	ND	ND	1	2	3
R258	104.93	63.47	33.41	21.81	11.81	18.31	26.9	55.69	32.31	ND	ND	ND	1	0	2
R259	59.47	45.56	538.38	162.6	14	26.67	53.48	66.14	34.9	0.22	ND	ND	2	0	2
S261	7.29	4.34	82.11	115.3	ND	79.07	46.58	90.9	31.05	ND	0.32	0.04	5	2	8

I_r = clustering index value; *P* value = significance level. Only parasites and symbionts infecting more than four hosts were included, that is, *Prochristianella penaei* (Apro) and symbionts (Asymb). All = sum of all metazoan parasite species individuals, including *Hysterothylacium* sp., *Opecoeloides fimbriatus*, and an unidentified intestinal cestode. Capital letters in the first column are trawls from which shrimp pools were made; ND, not detected.
BAPir, Benzo (a) pyrene; Pir, pyrene; Naph, Naphthalene; Phena, Phenantrene; Cu (range 0.0–7.8 $\mu g\, g^{-1}$; $I_r = 0.96$; *P* value = 0.50); Pesti = pesticides, the sum of hexachlorocyclohexanes isomers (alpha-HCH [0.0–2.77; $I_r = 0.99$; *P* value = 0.46]; delta-HCH [0.0–1.09; $I_r = 0.95$; *P* value = 0.49, gamma-HCH [0.0–0.48; $I_r = 1.13$; *P* value = 0.22]); Hepta = heptachlor, metoxychlor [0.0–36.02; $I_r = 0.94$; *P* value = 0.66] and DDTs [0.0–30.16; $I_r = 0.80$; *P* value = 0.74], the sum of concentrations of op-DDD [0.0–0.06; $I_r = 0.75$; *P* value = 0.84], op-DDE [0.0–2.79; $I_r = 0.70$; *P* value = 0.68], op-DDT [0.0–8.56; $I_r = 0.77$; *P* value = 0.75], pp-DDD [0.0–0.95; $I_r = 0.79$; *P* value = 0.89], pp-DDE [0.0–8.64; $I_r = 0.85$; *P* value = 0.72] and pp-DDT [0.0–26.91; $I_r = 0.77$; *P* value = 0.88]; PCBs [0.0–018; $I_r = 0.84$; *P* value = 0.67], the sum of concentrations of PCB 1016 [0.0–0.12; $I_r = 0.74$; *P* value = 0.90], 1221 [0.0–0.07; $I_r = 0.96$; *P* value = 0.46], 1232 [0.0–0.05; $I_r = 0.51$; *P* value = 0.98], 1242 [0.0–0.18; $I_r = 1.17$; *P* value = 0.25], 1248 [0.0–0.04; $I_r = 1.28$; *P* value = 0.14], 1254 [0.0–0.08; $I_r = 0.69$; *P* value = 0.94] and 1260 [0.0–0.01; $I_r = 0.72$; *P* value = 0.80].

Due to the geographical proximity of Campeche Sound to the US Gulf Coast and the sharing of shrimp species between these areas, the symbionts and metazoan parasites of Campeche Sound can be expected to be similar to those reported in US waters (Overstreet, 1973, 1985; Couch, 1978). Indeed, *F. duorarum* species composition in the study zone (table 1) was similar to that described by Overstreet (1973) and Vidal-Martínez *et al.* (2002).

We are aware of the limitations of histological slides for detecting total number of parasites and symbionts in an individual host, and for this reason traditional infection descriptors such as prevalence or mean abundance were not used. Use of histology was justified because we were interested in describing possible associations between the parasites/symbionts identified in the slides and pollutants from the same individual host rather than describing infection parameters of each parasite species. Though this method can produce an underestimate of the number of symbionts and metazoan parasites, it is the only way to guarantee detection of both parasites and pollutants from the same sample or pool. Given the positive results, histological slides appear to be useful in determining associations between symbionts and organic chemical pollutants in mobile hosts like the shrimp. This histological approach has also been successfully applied in determining the possible association of environmental impact on fish parasites (Overstreet, 1997; Marcogliese & Cone, 2001; Khan, 2004).

Sediment, water and shrimp pollutant concentrations (tables 2 and 3) suggest that shrimps were exposed to and acquired different pollutants depending on the environment in which they lived. When pink shrimp juveniles inhabit the Terminos Lagoon, they acquire pesticides, 2–3 ring PAHs (from petroleum) and probably heavy metals (like Zn). En route to, and once in their mating grounds, the shrimps are in the vicinity of offshore platforms (see García-Cuéllar *et al.*, 2004), where they acquire 4–5 ring PAHs or heavy metals such as vanadium or copper.

The results suggest natural PAH spillovers throughout the study area (table 2), which is further supported by data from García-Cuellar *et al.* (2004) and Soto *et al.* (2004) on natural PAH spillovers in Campeche Sound. The UCM in sediments were significantly clustered in the platform zone (table 2; fig. 2C), which is probably associated with oil drilling taking place on the platforms. However, low concentrations of UCM were present in both water and sediments throughout the study area (table 2), probably due to distribution by ocean currents. Available data are insufficient to unequivocally determine if these concentrations are associated with petroleum extraction or natural seeps.

Overall the levels of PAHs, PCBs, heavy metals and pesticides recorded here were within permitted levels, with some exceptions (UCM; DDTs), and could not be clearly linked to the presence of oil drilling platforms. This does not mean, however, that they pose no potential risk to humans and shrimps. The PAH concentrations in water (0.12 to 177.29 $\mu g\, l^{-1}$) were below the EPA criterion (4,600,000 $\mu g\, l^{-1}$) (EPA, 1999), but UCM exceeded the

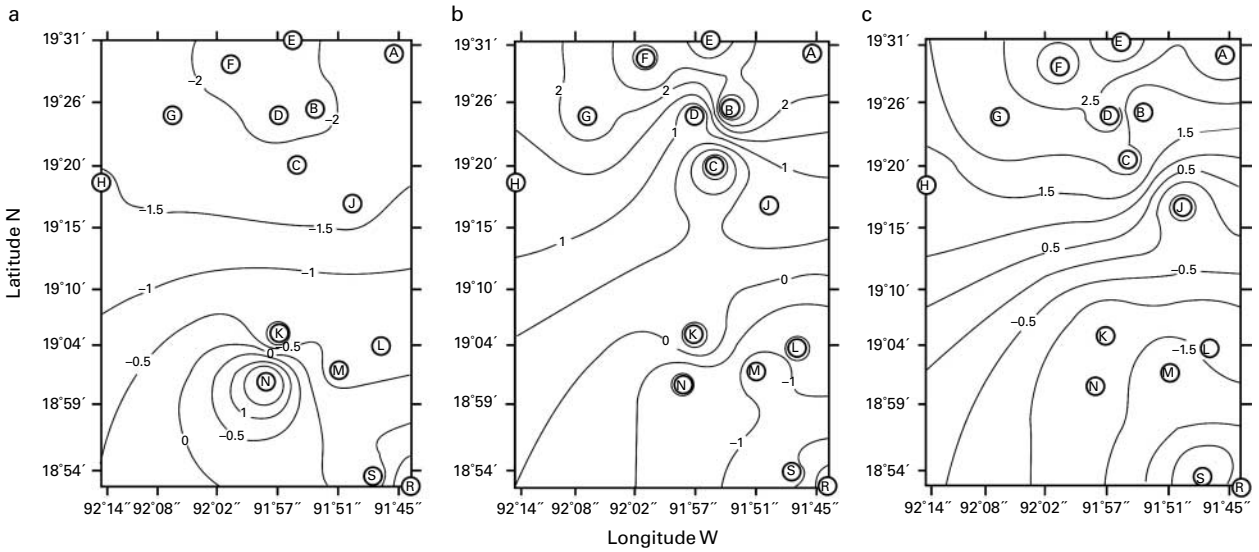

Fig. 2. Cluster indices of selected pollutants in shrimp pools, sediments and water in the study area. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A. Heptachlor in shrimp; B, copper in shrimp; C, unresolved complex mixture (UCM) in sediments. The letters in circles refer to the sampling points shown in fig. 1.

National Oceanic and Atmospheric Administration (NOAA) limit (70 $\mu g\, g^{-1}$). However, shrimps from all trawls possessed PAHs (table 3), though at levels deemed non-toxic for humans (0.11 to 253.97 $\mu g\, g^{-1}$). Nevertheless, the ubiquitous presence of these compounds deserves attention since many (e.g. benzo (a) pyrene) are known carcinogens in humans (ATSDR, 1999). For heavy metals, Cu, Fe, Ni, V and Zn concentrations were all below international regulations for sediments or water (e.g. O'Connor, 1990; ATSDR, 2004), though again they were present throughout the study area. Pesticide levels were low in the coastal and platform areas, only heptachlor was significantly clustered in the study area and DDT was clearly present in the shrimp. The occurrence of the latter two is cause for some concern as both are known to be toxic to shrimps and humans. The α and δ hexachlorocyclohexanes and heptachlor are considered carcinogenic in humans, while the γ isomer produces liver and kidney toxicity in humans (http://cfpub.epa.gov/iris). While the recorded concentrations may not exceed limits for humans

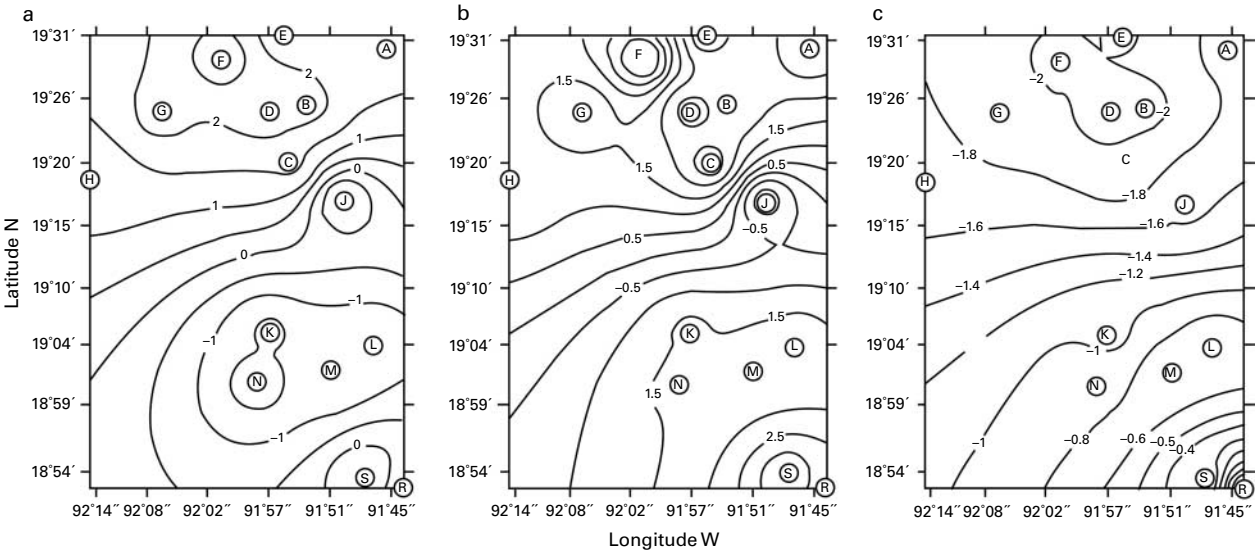

Fig. 3. Cluster indices of selected pollutants in shrimp pools, sediments and water in the study area. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A, nickel in sediments; B, vanadium in sediments; C, polycyclic aromatic hydrocarbons (PAHs) in water. The letters in circles refer to the sampling points shown in fig. 1.

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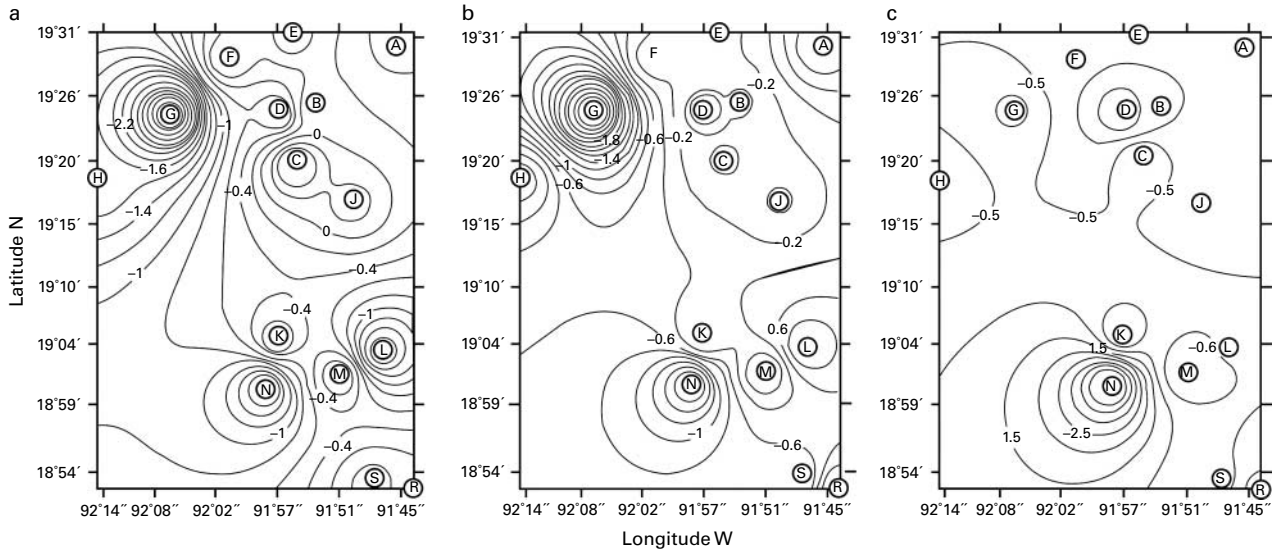

Fig. 4. Local values of correlation coefficients (X_k) of clustering indices of symbionts with selected pollutants from trawl data, after controlling for shrimp size and autocorrelation. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A, alpha-HCH ($X = -0.69$; $n = 15$; $P = 0.99$); B, HCH ($X = -0.52$; $n = 15$; $P = 0.98$); C, heptachlor ($X = 0.53$; $n = 15$; $P = 0.03$). The letters in circles refer to the sampling points shown in fig. 1.

($3\,\mu g\,g^{-1}$ [300 ppb] for heptachlor, ATSDR, 1993; 15–17 mg kg^{-1} body weight for HCH, WHO, 1991), little is known about what effect the concentrations found in the shrimp may have (LD_{50} for *F. duorarum* is $0.17\,\mu g\,l^{-1}$; Schimmel *et al.*, 1977). Most DDT concentrations recorded in the shrimp ($1.85–1110.88\,\mu g\,g^{-1}$) (table 3) exceeded levels known to cause adverse responses in benthic organisms (WHO, 1991). Finally, PCB concentrations in the shrimp were below NOAA limit for benthic organisms ($200\,\mu g\,g^{-1}$) (WHO, 1993) and the limit for human consumption (0.2–3 ppm) (ATSDR, 2000), though levels as low as 0.9–4.0 ppb are known to kill *F. aztecus* (Nimmo *et al.*, 1975). Even though most compounds here were within permitted concentrations, caution must be taken when interpreting these data since extensive research is still needed to definitively determine damaging levels in

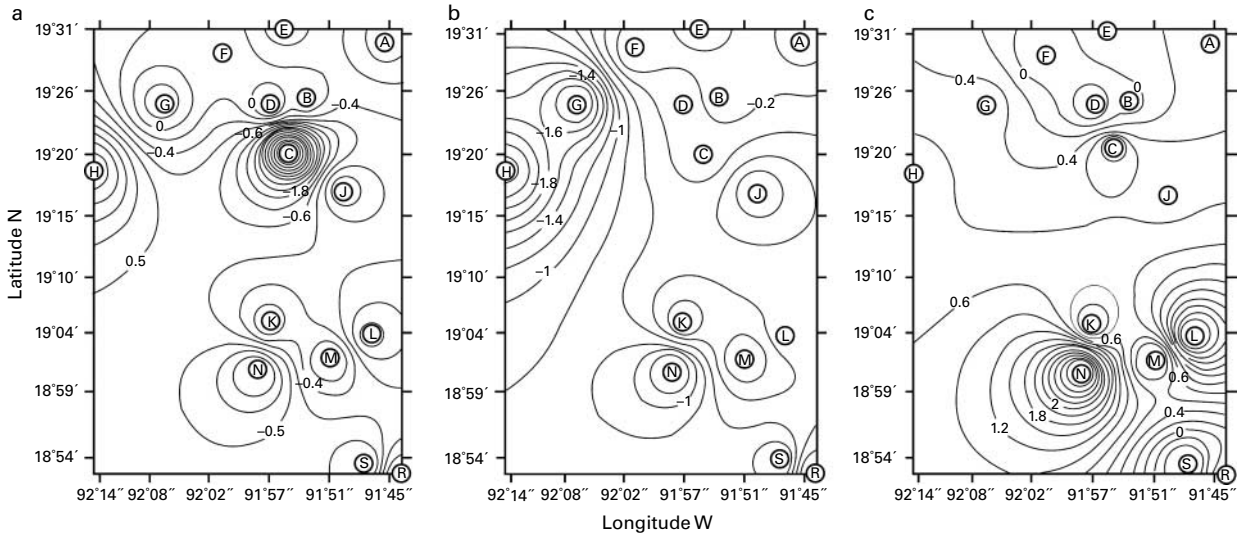

Fig. 5. Local values of correlation coefficients (X_k) of clustering indices of symbionts with selected pollutants from trawl data, after controlling for shrimp size and autocorrelation. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A, unresolved complex mixture (UCM) in sediments ($X = -0.54$; $n = 15$; $P = 0.98$); B, UCM in water ($X = -0.59$; $n = 15$; $P = 0.99$); C, nickel in water ($X = 0.51$; $n = 15$; $P = 0.03$). The letters in circles refer to the sampling points of fig. 1.

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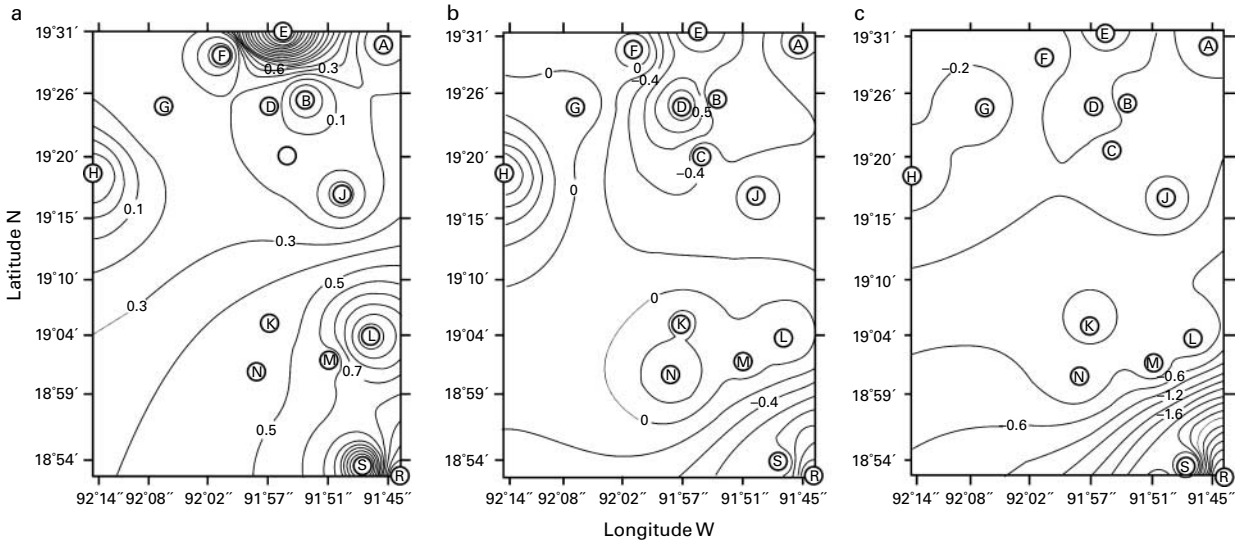

Fig. 6. Local values of correlation coefficients (X_k) of the clustering indices of symbionts with selected pollutants from 39 shrimp pools, after controlling for shrimp size and autocorrelation. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A, Phenanthrene ($X = 0.38$; $n = 39$; $P = 0.008$); B, alpha-HCH ($X = -0.39$; $n = 39$; $P = 0.9918$); C, heptachlor ($X = -0.76$; $n = 39$; $P = 0.9999$). The letters in circles refer to the sampling points of fig. 1.

shrimps or humans, and some of them (e.g. PCBs) bioaccumulate (WHO, 1993), meaning exposure to them for any length of time holds potential risks to human health.

The available data do not permit attribution of the presence of the studied compounds in shrimps, sediments and water solely to petroleum extraction on the platforms. Low molecular-weight PAHs probably result from road runoff or oil leaks (WHO, 2003) into coastal zones, heavy metals and pesticides likely enter the marine environment from river runoff, and PCBs come from electrical capacitors, transformers, marine paint and rust protection for marine structures (EPA, 1999). Though banned, DDT is still used in agricultural pest control, and so continues to enter the Gulf of Mexico (Benítez & Bárcenas, 1996).

The influence of total shrimp length as a covariable is to be expected since as shrimps grow, and 'sample the

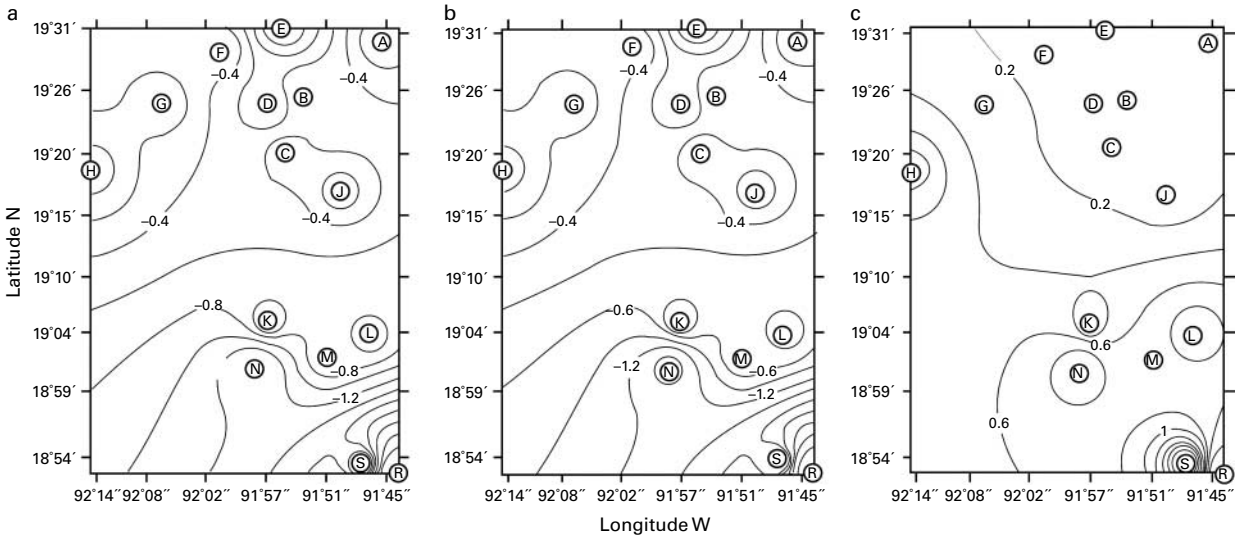

Fig. 7. Local values of correlation coefficients (X_k) of the clustering indices of symbionts with selected pollutants from 39 shrimp pools, after controlling for shrimp size and autocorrelation. The coastal zone is located on the bottom margin and the platform zone on the top margin in all figures. A, PCB1016 ($X = 0.64$; $n = 39$; $P = 0.0001$); B, PCB1242 ($X = -0.77$; $n = 39$; $P = 0.9999$); C, zinc ($X = 0.32$; $n = 39$; $P = 0.0244$). The letters in circles refer to the sampling points shown in fig. 1.

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environment' while finding food, they accumulate an increasing number of symbionts and metazoan parasites. This is supported by data for the same phenomenon in a variety of fish species and other vertebrates that accumulate larval-stage metazoan parasites as they grow (Hudson & Dobson, 1995). The influence of depth is also predictable because shrimps are migratory, moving from shallow coastal lagoon waters to mate in the deeper open sea (Ramírez-Rodríguez *et al.*, 2003). This migration occurs when they have reached adult stage, explaining the correlation between host length and depth.

Once shrimp size and autocorrelation were controlled for, useful patterns emerged between the symbiont and pollutant cluster indices values in both trawl and shrimp pool data. Negative correlations in figs 4A, B and 6B suggest that the sampling points with high cluster values for alpha-HCH and HCH had low symbiont values, especially near the coast. The apparent contradiction between overall values and correlations for heptachlor and symbionts cluster values (figs 4C and 6C) in trawls and shrimp pools near the coast does not exist. Figure 4C shows that the positive overall value is due to low concentrations of both symbionts and pesticides, while fig. 6C, with an increase in sampling size (39 shrimp pools), presents a clear pattern where high and low indices occur for heptachlor and low symbionts respectively. Low symbiont clustering values in the presence of HCH may be related to an inhibitory effect since lethality due to HCH in *Amphidinium carteri* (dinoflagellate) is attained at $2\,mg\,l^{-1}$ and in *Tetrahymena pyriformis* (ciliate) at $>10\,mg\,l^{-1}$ (Jeanne-Levain, 1974). A similar situation can be expected for heptachlor since this pesticide is reported to cause a drop in cell density in the marine dinoflagellate *Exuviella baltica* at $50\,\mu g\,l^{-1}$ (Magnani *et al.*, 1978).

The phenanthrene and symbiont correlation values (X) were higher near the coast than in the platform zone, which may be explained by organic productivity being higher in Terminos Lagoon due to the dumping of untreated sewage from Ciudad del Carmen. This in turn may cause an increase in the number of symbionts, as well as in the amount of PAHs.

The UCM in sediments and water, and symbionts correlation values were negative at most points (fig. 5A and B). High UCM clustering values were associated with low symbiont values suggesting that this mixture of compounds seems to be toxic to symbionts. This is further supported by data showing that species that do not metabolize PAHs, like algae, oligochaetes, molluscs and protozoans, can accumulate high UCM concentrations that eventually become harmful to them (James, 1989).

The PCB 1016 and 1242 and symbiont local correlation values were negative near the coast (fig. 7A and B), suggesting that these concentrations were harmful to these protozoans. These pollutants presumably originated in Ciudad del Carmen, and are produced by the vapourization of plasticizers or incineration, industrial fluid leaks and dumping, or disposal in dumps and landfills. Accumulation of PCBs varies depending on the length of exposure and concentration in the water. For example, a diatom exposed to PCB 1242 had an accumulation factor of 1100 (Keil *et al.*, 1971), while ciliated protozoan exposed to PCB 1254 in water had an accumulation factor of 60. However, growth in certain marine diatoms is inhibited by PCB 1254 levels as low as $10–25\,\mu g\,l^{-1}$ (Cooley *et al.*, 1972).

For heavy metals, nickel in water from trawls (fig. 5C) and zinc in the shrimp pools (fig. 7C) had positive correlation values, especially near the coast. Again, a likely explanation is that high organic productivity in Terminos Lagoon from the dumping of untreated sewage may cause an increase in the number of symbionts, as well as in Ni and Zn concentrations.

The data indicate that different pollutants have different statistical associations with symbionts. A possible explanation for this is that these pollutants have different effects on symbionts since they are chronically exposed to them due to their position on the gills or cuticle of shrimps.

The *F. duorarum* catch has been declining since the late 1970s (Ramírez-Rodríguez *et al.*, 2003), and, though the present results are not shown as an explanation for all or part of this decline, they do indicate that the presence of pollutants and parasites probably has an additive effect on the Campeche Sound shrimp population's general condition. If this is true, then highly polluted and parasitized shrimps are being removed from the population through chronic exposure to a combination of deleterious agents present in the environment. It can also be expected that shrimps affected by pollutants, symbionts and metazoan parasites are unable to deal with environmental stresses (e.g. changes in temperature, salinity, oxygen concentration) that would have no effect on healthy shrimps.

Based on the present field data, both shrimps and their symbionts seem to be promising bioindicators of the environmental status of Campeche Sound (but see EPA, 2000). Furthermore, data suggest that the origin of pollutants found in the water, sediments and shrimps of Campeche Sound is not exclusively linked to PEMEX activities. Government authorities and people of coastal cities and towns also contribute by freely dumping sewage charged with PAHs (mainly petroleum products) and PCBs into Terminos Lagoon, as do farmers upstream who continue to use banned pesticides that eventually wash into the sea (Botello *et al.*, 1996). The problem is extremely complex and its mitigation will require extensive preventative measures such as sewage processing plants and natural pesticide use, among others.

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References

Agency for Toxic Substances and Disease Registry (ATSDR) (1993) Toxicological profile for heptachlor and heptachlor epoxide. Atlanta, Georgia: US Department of Health and Human Services, Public Health Service (http://www.atsdr.cdc.gov/tfacts12.html).

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) Toxicological profile for total petroleum hydrocarbons (TPH). Atlanta, Georgia: US Department of Health and Human Services, Public Health Service (http://www.atsdr.cdc.gov/tfacts123.html).

Agency for Toxic Substances and Disease Registry (ATSDR) (2000) Toxicological profile for polychlorinated biphenyls (PCBs). Atlanta, Georgia: US Department of Health and Human Services, Public Health Service (http://www.atsdr.cdc.gov/tfacts17.html).

Agency for Toxic Substances and Disease Registry (ATSDR) (2004) Toxicological profile for Copper. Atlanta, Georgia: US Department of Health and Human Services, Public Health Service (http://www.atsdr.cdc.gov/tfacts132.html).

Bénitez, J.A. & Bárcenas, C. (1996) Sistemas fluviolagunares de la Laguna de Términos: Hábitats críticos susceptibles a los efectos adversos de los plaguicidas. pp. 187–201 *in* Botello, A.V., Rojas-Galavíz, J.L., Benítez, J.A. & Zárate-Lomeli, D. (*Eds*) *Golfo de México, contaminación e impacto ambiental: diagnóstico y tendencias*. Universidad Autónoma de Campeche, México. Campeche, México, EPOMEX. Serie Científica 5.

Bengtsson, J. & Ebert, D. (1998) Distributions and impacts of microparasites on *Daphnia* in a rockpool metapopulation. *Oecologia* **115**, 213–221.

Bergey, L., Weis, J.S. & Weis, P. (2002) Mercury uptake by the estuarine species *Palaemonetes pugio* and *Fundulus heteroclitus* compared with their parasites. *Probopyrus pandalicola* and *Eustrongylides* sp. *Marine Pollution Bulletin* **44**, 1046–1050.

Botello, V.A., Rojas-Galavíz, J.L., Benítez, J.A., & Zárate-Lomeli, D. (1996) (*Eds***)** *Golfo de México, contaminación e impacto ambiental: diagnóstico y tendencias*. Universidad Autónoma de Campeche, México. Campeche, México, EPOMEX. Serie Científica 5.

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.

Castrejon, H., Pérez-Castañeda, R. & Defeo, O. (2004) Spatial structure and bathymetric patterns of penaeid shrimps in the southwestern Gulf of Mexico. *Fisheries Research* **72**, 291–300.

Cooley, N.R., Keltner, J.M. & Forester, J. (1972) Mirex and Aroclor 1254: effect on accumulation by *Tetrahymena pyriformis* strain W. *Journal of Protozoology* **19**, 636–638.

Couch, J.A. (1978) Diseases, parasites and toxic responses of commercial penaeid shrimps of Gulf of Mexico and South Atlantic coast of North America. *Fishery Bulletin* **76**, 1–44.

Dutilleul, P. (1993) Modifying the t-test for assessing the correlation between two spatial processes. *Biometrics* **49**, 305–314.

Environmental Protection Agency of the United States (EPA) (1999) *Environmental assessment of proposed effluent limitations guidelines and standards for synthetic-based drilling fluids and other non-aqueous drilling fluids in the oil and gas extraction point source category.* 248 pp. Office of Water, Office of Science and Technology, Engineering and Analysis Division, US Environmental Protection Agency (EPA-821-B-98-019), Washington, DC 20460.

Environmental Protection Agency of the United States (EPA) (2000) *Evaluation guidelines for ecological indicators*. 109 pp. Office of Research, and Development. US Environmental Protection Agency (EPA/620/R-99/005), Washington DC 20460.

García-Cuellar, J.A., Arreguín-Sánchez, F., Hernández-Vázquez, S. & Lluch-Cota, D.B. (2004) Impacto ecológico de la industria petrolera en la Sonda de Campeche, México, tras tres décadas de actividad: una revisión. *Interciencia* **29**, 311–319.

Hudson, P.J. & Dobson, A.P. (1995) Host–parasite processes and demographic consequences. pp. 155–173 *in* Clayton, D.H. & Moore, J. (*Eds*) *Host-parasite evolution, general principles and avian models*, Oxford University Press, Oxford.

James, M.O. (1989) Biotransformation and disposition of PAH in aquatic invertebrates. pp. 69–91 *in* Varanasi, U. (*Ed.*) *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*, Boca Raton, Florida, CRC Press.

Jeanne-Levain, N. (1974) Etude des effets du lindane sur la croissance et le developpement de quelques organismes unicellulaires. *Bulletin de la Societé de Zoologie Francaise* **99**, 105–109.

Karvonen, A. & Valtonen, T.E. (2004) Helminth assemblages of whitefish (*Coregonus lavaretus*) in interconnected lakes: similarity as a function of species specific parasites and geographical separation. *Journal of Parasitology* **90**, 471–476.

Khan, R.A. (2004) Parasites of fish as biomarkers of environmental degradation: a field study. *Bulletin of Environmental Contamination and Toxicology* **72**, 394–400.

Khan, R.A. & Payne, J.F. (2004) Comparative study of oil well drill cuttings and polycyclic aromatic hydrocarbons on parasitism in winter flounder: a dose-response study. *Bulletin of Environmental Contamination and Toxicology* **73**, 652–658.

Keil, J.E., Priester, L.E. & Sandifer, S.H. (1971) Polychlorinated biphenyl (Aroclor 1242): effects of uptake on growth, nucleic acids and chlorophyll of a marine diatom. *Bulletin of Environmental Contamination and Toxicology* **6**, 156–159.

Kennedy, C.R. (1997) Freshwater fish parasites and environmental quality: an overview and caution. *Parassitologia* **39**, 249–254.

Lafferty, K.D. (1997) Environmental parasitology: what can parasites tell us about human impacts on the environment? *Parasitology Today* **13**, 251–255.

Lewis, J.W., Hoole, D. & Chappell, L.H. (2003) Parasitism and environmental pollution: parasites and hosts as indicators of water quality. *Parasitology* **126**, S1–S3.

Magnani, B., Powers, C.D., Wurster, C.F. & O'Connors, H.B. (1978) Effects of chlordane and heptachlor on the

marine dinoflagellate *Exuviella baltica* Lohman. *Bulletin of Environmental Contamination and Toxicology* **20**, 1–7.

Marcogliese, D.J. & Cone, D.K. (2001) Myxozoan communities parasitizing *Notropis hudsonius* (Cyprinidae) at selected localities on the St. Lawrence River, Quebec: possible effects of urban effluents. *Journal of Parasitology* **87**, 951–956.

Marcogliese, D.J., Nagler, J.J. & Cyr, D.G. (1998) Effects of exposure to contaminated sediments on the parasite fauna of American plaice (*Hipoglossoides platessoides*). *Bulletin of Environmental Contamination and Toxicology* **61**, 88–95.

Moles, A. & Wade, T.L. (2001) Parasitism and phagocytic function among sand lance *Ammodytes hexapterus* Pallas exposed to crude oil-laden sediments. *Bulletin of Environmental Contamination and Toxicology* **66**, 528–535.

Morley, N.J., Crane, M. & Lewis, J.W. (2001) Toxicity of cadmium and zinc to *Diplostomum spathaceum* (Trematoda: Diplostomidae) cercarial survival. *International Journal for Parasitology* **31**, 1211–1217.

Nimmo, D.R., Hansen, D.J., Couch, J.A., Cooley, N.R., Parrish, P.R. & Lowe, J.I. (1975) Toxicity of Aroclor 1254 and its physiological activity in several estuarine organisms. *Archives of Environmental Contamination and Toxicology* **3**, 22–39.

O'Connor, T. (1990) *Coastal environment quality in the United States, 1990. Chemical contamination in sediment and tissues*. 34 pp. Coastal and Estuarine Assessments Division. Office of Oceanography and Marine Assessment, National Ocean Service, National Oceanic and Atmospheric Administration, Rockville, Maryland 20852.

Overstreet, R.M. (1973) Parasites of some penaeid shrimps with emphasis on reared hosts. *Aquaculture* **2**, 105–140.

Overstreet, R.M. (1985) Some parasitological aspects of shrimps culture in the United States. pp. 117–122 *in* Hargis, W.J. Jr (*Ed.*) *Parasitology and pathology of marine organisms of the world Ocean*. NOAA Technical Report, NMFS 25.

Overstreet, R.M. (1997) Parasitological data as monitors of environmental health. *Parassitologia* **39**, 169–176.

Perry, J.N. (1995) Spatial aspects of animal and plant distribution in patchy farmland habitats. pp. 221–242 *in* Glen, D.M., Greaves, M.P. & Anderson, H.M. (*Eds*) *Ecology and integrated farming systems*. Bristol, John Wiley & Sons.

Perry, J.N. & Dixon, P. (2002) A new method for measuring spatial association in ecological count data. *Ecoscience* **9**, 133–141.

Perry, J.N., Winder, L., Holland, J.M. & Alston, R.D. (1999) Red-blue plots for detecting clusters in count data. *Ecology Letters* **2**, 106–113.

Poulin, R. (1997) Species richness of parasite assemblages: evolution and patterns. *Annual Review of Ecology and Systematics* **28**, 341–358.

Poulin, R. (2003) The decay of similarity with geographical distance in parasite communities of vertebrate hosts. *Journal of Biogeography* **30**, 1609–1615.

Poulin, R. & Morand, S. (1999) Geographical distances and the similarity among parasite communities of conspecific host populations. *Parasitology* **119**, 369–374.

Qian, G. & Pin, N. (2000) Lead content in the monogenean *Ancyrocephalus mogurndae* and in different organs of its host, the mandarin fish *Siniperca chuasi*. *China Environmental Science* **20**, 233–236.

Ramírez-Rodríguez, M., Arreguín-Sánchez, F. & Lluch-Belda, D. (2003) Recruitment of the pink shrimp *Farfantepenaeus duorarum* in the southern Gulf of Mexico. *Fisheries Research* **65**, 81–88.

Schimmel, S.C., Patrick, J.M. Jr & Forester, J. (1977) Toxicity and bioconcentration of BHC and lindane in selected animals. *Archives of Environmental Contamination and Toxicology* **6**, 355–363.

Schmidt, V., Zander, S., Körting, W., Broeg, K., von Westernhagen, Dizer, H., Hansen, P.D., Skouras, A. & Steinhagen, D. (2003) Parasites of flounder (*Platichthys flesus* L.) from the German Bight, North Sea, and their potential use in biological effects monitoring. *Helgoland Marine Research* **57**, 262–271.

Schuwerack, P.M.M. & Lewis, J.W. (2003a) The mode of action of acute and chronic concentrations of waterborne Cd in the digestive gland of the acclimated infested freshwater crab (*Potamonautes warreni*). *Cell and Tissue Research* **312**, 249–263.

Schuwerack, P.M.M. & Lewis, J.W. (2003b) Cellular responses to increasing Cd concentrations in the freshwater crab. *Potamonautes warreni*, harbouring microbial gill infestations. *Cell and Tissue Research* **313**, 335–346.

Schuwerack, P.M.M., Lewis, J.W. & Jones, J.W. (2001a) Pathological and physiological changes in the South African freshwater crab *Potamonautes warreni* Calman induced by microbial gill infestations. *Journal of Invertebrate Pathology* **77**, 269–279.

Schuwerack, P.M.M., Lewis, J.W. & Jones, J.W. (2001b) The potential use of the South African freshwater crab *Potamonautes warreni*, as bioindicator species for heavy metal contamination. *Ecotoxicology* **10**, 159–166.

Sericano, J.L., Atlas, E.L., Wade, T.L. & Brooks, J.M. (1990) NOAA's status and trends mussel watch program: chlorinated pesticides and PCBs in oysters (*Crassostrea virginica*) and sediments from the Gulf of Mexico, 1986–1987. *Marine and Environmental Research* **29**, 161–203.

Siddall, R. & Des Clers, S. (1995) Effect of sewage sludge on the miracidium and cercaria of *Zoogonoides viviparus* (Trematoda: Digenea). *Helminthologia* **31**, 143–153.

Soto, L.A., Sánchez-García, S. & López-Veroni, D. (2004) Ambientes influídos por emanaciones naturales de hidrocarburos y gas en el suroeste del golfo de México. *Universidad y Ciencia* (Special number) **1**, 51–58.

Sures, B. (2003) Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. *Parasitology* **126**, S53–S60.

Sures, B. (2004a) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* **20**, 170–177.

Sures, B. (2004b) Fish acanthocephalans of the genus *Pomphorhynchus* sp. as globally applicable bioindicators for metal pollution in the aquatic environment? *Wiener Klinische Wochenschrift* **116**, 19–23.

Sures, B., Thielen, F., Baska, F., Messerschmidt, J. & von Bohlen, A. (2005) The intestinal parasite *Pomphorhynchus laevis* as a sensitive accumulation indicator for the platinum group metals Pt, Pd, and Rh. *Environmental Research* **98**, 83–88.

Vidal-Martínez, V.M. & Poulin, R. (2003) Spatial and temporal repeatability in parasite community structure of tropical fish hosts. *Parasitology* **127**, 387–398.

Vidal-Martinez, V.M., Jiménez-Cueto, A.M. & Sima-Álvarez, R. (2002) Parasites and symbionts of native and cultured shrimps from Yucatan, Mexico. *Journal of Aquatic Animal Health* **14**, 57–64.

Vidal-Martínez, V.M., Aguirre-Macedo, M.L., Noreña-Barroso, E., Gold-Bouchot, G. & Caballero-Pinzón, P.I. (2003) Potential interactions between the metazoan parasites of the Mayan catfish *Ariopsis assimilis* and chemical pollution in Chetumal Bay, Mexico. *Journal of Helminthology* **77**, 173–184.

Yeomans, W., Chubb, J.C. & Sweeting, R.A. (1997) Use of protozoan communities for pollution monitoring. *Parassitologia* **39**, 201–212.

Wade, T.L., Brooks, J.L., Kennicutt, M.C. II, McDonald, T.J., Sericano, J.L. & Jackson, T.J. (1993) GERG trace organic contaminant analytical techniques. *In* Sampling and analytical methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch projects 1984–1992, Vol. IV. *NOAA Technical Memorandum NOS ORCA* **71**, 121–139.

Williams, H.H. & MacKenzie, K. (2003) Marine parasites as pollution indicators: an update. *Parasitology* **126**, S27–S41.

World Health Organization (WHO) (1991) Lindane. Environmental Health Criteria 124. International Programme on Chemical Safety. Geneva, http://www.inchem.org/documents/ehc/ehc/ehc124.htm.

World Health Organization (WHO) (1993) Polychlorinated biphenyls and terphenyls. Environmental health criteria. International Programme on Chemical Safety, World Health Organization, Geneva, http://www.inchem.org/documents/ehc/ehc/ehc140.htm.

World Health Organization (WHO) (2003) Selected nitro- and nitro-oxy-polycyclic aromatic hydrocarbons. Environmental health criteria 229. International Programme on Chemical Safety. World Health Organization. Geneva, http://www.inchem.org/documents/ehc/ehc/ehc229.htm.

Zimmermann, S., Sures, B. & Tarachewski, H. (1999) Experimental studies on lead accumulation in the eel-specific endoparasite *Anguillicola crassus* (Nematoda) and *Paratenuisentis ambiguus* (Acanthocephala) as compared with their host, *Anguilla anguilla*. *Archives of Environmental Contamination and Toxicology* **37**, 190–195.

Zimmermann, S., von Bohlen, A., Messerchmidt, J. & Sures, B. (2005) Accumulation of precious metals platinum, palladium, and rhodium from automobile catalytic converters in *Paratenuisentis ambiguus* as compared with its fish host, *Anguilla anguilla*. *Journal of Helminthology* **79**, 85–89.

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