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Potential interactions between metazoan parasites of the Mayan catfish *Ariopsis assimilis* and chemical pollution in Chetumal Bay, Mexico

V.M. Vidal-Martínez[1,2]*, M.L. Aguirre-Macedo[1], E. Noreña-Barroso[1], G. Gold-Bouchot[1] and P.I. Caballero-Pinzón[3]

[1]Laboratories of Parasitology and Marine Geochemistry, Center for Research and Advanced Studies, National Polytechnic Institute (CINVESTAV-IPN), Carretera Antigua a Progreso Km. 6, C.P. 97310 Mérida, Yucatan, Mexico: [2]Laboratory of Parasitology, Faculty of Natural Sciences, Autonomous University of Queretaro, A.P. 184 Cerro de las Campanas, 76010 Santiago de Querétaro, Querétaro, Mexico: [3]Centro de Estudios Tecnológicos del Mar No.10 (CETMAR Chetumal) Boulevard Bahía s/n, 77010 Chetumal, Quintana Roo, Mexico

Abstract

The effect of pollutants on the intensity of infection of metazoan parasites in the Mayan catfish, *Ariopsis assimilis* was investigated. Data were collected on pollutants and metazoan parasites from 76 catfish from five localities in Chetumal Bay in October, 1996. Nineteen pollutants (pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)) were found in the catfish livers. Heavy metal content was not determined. Nineteen metazoan parasite species were recovered. After controlling for fish length and sampling station, there was a significant negative linear relationship between the intensity of the larval digenean *Mesostephanus appendiculatoides* and 1,1,1,-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) concentrations. This negative relationship may be explained either by the effect of the pesticide on the mortality of (i) free-living larval forms, (ii) metacercariae in the fish, (iii) infected fish or (iv) intermediate host snails. There were significant differences between fish parasitized and not parasitized with *M. appendiculatoides* with respect to their DDT concentrations. There were also significant differences between the variances of the mean Clark's coefficient of condition values between catfish parasitized and not parasitized by *M. appendiculatoides*, with the variance of non-parasitized catfish being significantly larger. The results provided statistical evidence that DDT has a detrimental effect on *M. appendiculatoides* infection intensity. Furthermore, the significantly larger variance value of Clark's coefficient for non-parasitized fish suggested that DDT affects both the parasite and general host condition.

*Fax: ++52 (999) 9812334
E-mail: vvidal@mda.cinvestar.mx

Introduction

In recent years, a major interest of ecological parasitologists has become determining whether metazoan parasites of fish (helminths and crustaceans) are good indicators of anthropogenic environmental impact (MacKenzie *et al.*, 1995; Lafferty, 1997; Soucek & Noblet, 1998). This interest stems from the fact that these parasites may be straightforward and relatively cheap bioindicators. Current theory in this field is based on information from three scenarios: (i) field evidence without experimental support (D'Amelio & Gerasi, 1997; Dušek *et al.*, 1998; Gelnar *et al.*, 1997; Valtonen *et al.*, 1997); (ii) experimental or laboratory evidence without field data (Siddall & des Clers, 1995; Marcogliese *et al.*, 1998; Morley *et al.*, 2001); and (iii) metazoan parasites as heavy metal sinks (Sures & Taraschevski, 1995, Sures *et al.*, 1997, 1998; Qian & Pin, 2000).

There are problems associated with these indicators, because in field studies without experimental support, different metazoan parasite species can be positively or negatively affected by pollutants (Lafferty, 1997). Furthermore, without experimental evidence researchers are reluctant to accept that pollutants really affect the intensity of metazoan parasites in a fish population (e.g. Kennedy, 1997). The core of the problem is that correlation between pollutant concentrations and increases or decreases in the number of metazoan parasites per fish does not necessarily mean causality. For experimental studies with no field component, the main problem is that the number of parasites and pollutant concentrations used in laboratory experiments are not necessarily those to which fish are exposed in field conditions (Iwama & Greer, 1980; Moles, 1980).

That intestinal adult acanthocephalans and cestodes, as well as monogeneans, can act as sinks for heavy metals such as lead, cadmium and zinc has been demonstrated by a number of authors (Sures & Taraschevski, 1995; Sures *et al.*, 1997, 1998; Qian & Pin, 2000). This approach is very robust as these authors collected fish and parasites from the field and examined them for heavy metals in the laboratory.

A challenge in studying metazoans and pollutants in many places in southeast Mexico is that there are few intestinal parasites or monogeneans in the fish, but a very high digenean larval component in the metazoan parasite communities (Salgado-Maldonado *et al.*, 1997; Salgado-Maldonado & Kennedy, 1997; Vidal-Martínez *et al.*, 1998). In this case, a strategy needs to be used to determine which metazoan species may be good environmental impact indicators. The best candidates could be those metazoan parasites with a pollutant sensitivity higher than that of their hosts, as is the case for *Posthodiplostomum minimum* in bluegill sunfish *Lepomis macrochirus* (Soucek & Noblet, 1998). To determine if such sensitivity exists, however, data for both metazoan parasites and pollutants from the same individual host would be necessary. With this data it would then be possible to answer whether or not fish with high pollutant concentrations also have low numbers of sensitive metazoan parasites.

That metazoan parasites exert sublethal intensity dependent effects (e.g. energy balance) is well known. This in turn depends on the ability of the host to compensate for such effects, and this depends on body size and general condition (Holmes & Zohar, 1990; Goater & Holmes, 1997; Lafferty & Kuris, 1999; Sagerup *et al.*, 2000). A fish in a polluted environment is clearly more prone to become sick and die as a result of parasites normally acquired in its habitat, as is the case with fish exposed to cadmium, zinc or hydrocarbons (Sarot & Perlmutter, 1976; Pascoe & Cram, 1977; Moles, 1980). It is also reasonable to expect that highly parasitized fish exposed to pollutants would be more prone to exhibit a demonstrable shift from a normal condition. Holmes & Zohar (1990) suggested that the net effect of this sublethal intensity dependent effect would be to increase condition variation in an entire population. Thus selection pressures such as predation, mate choice or inclement weather would cause infected fish in polluted environments to either die or exhibit a decrease in general condition.

In field work it is extremely difficult to link the effect of pollutants in sediments or water directly to fish. In view of this difficulty, a different approach was taken in the present study in which the effects of pollutants and parasites on individual fish were simultaneously analysed to determine if these two variables have an additive effect on fish body condition. In October 1996, 76 catfish *Ariopsis assimilis* were collected in Chetumal Bay, in the state of Quintana Roo, southeast Mexico. Data on the number of metazoan parasite species and individuals, and pollutants (pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)) were collected from each individual fish. As pesticide use is common practice in the large sugarcane, cotton and rice fields of the region (INEGI, 1998), and due to the bottom-feeding habits of the catfish, there were good reasons to suspect that these fish had been chronically exposed to pollutants. Therefore, the aims of the present study were two-fold: (i) to determine whether or not catfish with high concentrations of pollutants have a low number of metazoan parasites; and (ii) to determine if both infection intensity and pollutants (in a host) influence the condition of *A. assimilis* in Chetumal Bay.

Materials and methods

Chetumal Bay is located in the southeast portion of the Yucatan Peninsula (18°21′ and 18°52′ N, and 87°54′ and 88°23′ W), on the border between Mexico and Belize. The bay is shallow, with a mean water depth of 3 m, and is fed by the Hondo River, which empties into its southwest corner.

Individual fish were used as the unit of replication, comparing the tissue concentration of pollutants in the liver of a single fish with the number of individuals of a particular metazoan parasite species in that single infected fish (= infection intensity *sensu* Bush *et al.*, 1997). To obtain a wide range of metazoan parasites and pollutants, and because these fish are mobile and pollutants are dispersed across the bay, a total of 76 catfish from five localities distributed throughout the bay were sampled (Noreña-Barroso *et al.*, 1998).

Catfish were located by snorkelling in mangrove formations known locally as 'mogotes'. Once a catfish

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was detected, a gill net was placed around the 'mogote', and the catfish were induced into the net. Captured fish were transported live to the laboratory at the Marine Technological Studies Center (Centro de Estudios Tecnológicos del Mar; CETMAR), Chetumal, and kept alive until necropsy. Clearly, the sampling strategy was oriented toward collecting the largest possible number of fish, and though snorkelling is not a strictly random sample collection method, it was the only way of collecting samples given the challenging circumstances.

External and internal parasitological examinations were undertaken following the procedures recommended by MAFF/ADAS (1988). All individuals of the metazoan parasite species present in each host were collected. Metazoan parasites were given preliminary identifications, counted *in situ* and stored for definitive identification. Catfish livers were divided into two portions, one for determining pollutants, and one for parasitology. The part set aside for parasitology was used to estimate the infection intensity in the entire liver. While this method could produce an underestimation of the number of metazoan parasites in the liver, it was the only way to guarantee the detection of both parasites and pollutants in the same organ.

Hydrocarbon and chlorinated compound (pesticides and PCBs) levels were determined according to methods described by Sericano *et al.* (1990) and Wade *et al.* (1993). Liver 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, then further 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. Sediment concentrations for these pollutants, and their distributions in Chetumal Bay, were taken from Noreña-Barroso *et al.* (1998). Financial constraints did not permit analysis of heavy metals or other pollutants.

For metazoan parasites, the prevalence and mean intensity of infection were used as recommended by Bush *et al.* (1997). Exposure frequency was determined as the proportion of individual catfish with a particular pollutant from a sample of catfish, and the mean value (± SD) of concentrations of each pollutant in the liver of each individual fish calculated.

It has been argued that univariate measures such as diversity can be insensitive indicators of pollution because different parasite species can be negatively or positively affected by pollutants (Lafferty, 1997). For this reason, a more sensitive analysis was conducted that allowed detection of idiosyncratic responses of each metazoan parasite species. Multivariate statistical methods were used to determine a possible relationship between pollutant concentrations and the infection intensity of each metazoan parasite species present in each individual fish. Principal component (PCA) and redundancy (RDA) analyses were applied to the intensities of the parasites using CANOCO software (ter Braak & Šmilauer, 1998). Redundancy analysis, the constrained form of PCA, was used because the lengths of the ordination axes were less than 2 standard deviations (1.6, in fact). In this case it is assumed that most of the response curves of the number of individuals of the metazoan parasite species with respect to the environmental variables (pollutants) will be linear. If the length of the ordination axes is longer than 2 standard deviations, then unimodal models, such as canonical correspondence analysis (CCA), should be used (Jongman *et al.*, 1995).

One-dimensional PCA and RDA (i.e. with a single ordination axis) are between simple regression and multiple regression. In simple linear regression, the number of individuals of each species is separately regressed on a single explanatory variable x (e.g. a known variable such as pH). The model can be written as $y_{ik} = a_{k+}b_k x_i +$ error, where y_{ik} is the intensity of species k in sample i, x_i is the known value of the explanatory variable in sample i, and a_k and b_k are unknown regression coefficients to be estimated. If the values x_i are unknown, theoretical explanatory variables can be found by providing the best fitting straight lines with data from m species. PCA finds the optimal value x_i for which the model, $y_{ik} = a_{k+}b_k x_i +$ error, fits best. These values need not be related to any measured environmental variable. The task of RDA is to constrain the values by requiring that x_i be a linear combination of measured environmental variables (a weighted aggregate). RDA is thus a simultaneous multiple regression for all species (i.e. a multivariate regression) with linear constraints on the regression coefficients. Due to these constraints, RDA uses fewer parameters than a multivariate multiple regression. In summary, RDA is both a constrained form of PCA and a constrained form of multivariate multiple regression (see Jongman *et al.*, 1995 and ter Braak & Šmilauer, 1998). The non-parametric Spearman's correlation coefficient was used to determine any possible association, and parametric linear regression (using $\ln + 1$ transformed data) was used to determine any possible relationships between the infection intensity of each metazoan parasite species and pollutant concentrations in the liver of each fish.

Covariance analysis was used to control confounding variables such as standard length and weight, and to determine the actual influence of the pollutant concentrations in each *A. assimilis* upon the infection intensity of each metazoan parasite species. Monte Carlo tests were used to select the variables to include in the analysis, and to determine the significance of the canonical axes for both metazoan parasites and pollutants. The pollutants were aggregated into major chemical classes (i.e. DDTs is the sum of DDT + DDD + DDE; PCBs is the sum of all congeners, etc.) to increase the power of the statistical test and to decrease the number of undetected compounds. Log-transformed values were transformed to zero, mean, and unit standard deviation. The normality of transformed variables was determined by using rankit plots (Sokal & Rohlf, 1981). The use of constrained models produced decreases in the percentage of explained variance, though the models were still statistically significant (Jongman *et al.*, 1995).

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To determine the influence of both the number of individuals of all metazoan parasite species and the concentrations of pesticides, PCBs and PAHs separately on the general condition of the catfish, Clark's condition coefficient (K) was used. This coefficient is defined as $K = W \times 100/L^3$ where W is weight (g) and L is length (cm) of the fish. As the catfish were all infected (see table 1), they were divided in two groups: slightly infected fish (between 4 and 674 individual helminths) and highly infected fish (between 1378 and 3719 individual helminths). Additionally, one way ANOVA and Bartlett's test for variance homogeneity were used to determine differences between those catfish parasitized and not parasitized with the most frequent and abundant metazoan parasite species. The significance of all statistical analyses was established at $\alpha = 0.05$ unless otherwise stated.

Results

Of 19 metazoan parasite species found in the catfish from Chetumal Bay, 12 (63%) ocurred in the larval stage. *Contracaecum* sp. and tetraphyllidean larval cestodes were the most prevalent and abundant species, followed by *Ascarophis ayalai* and *Mesostephanus appendiculatoides* (table 1). These four species were present at all five localities (table 1). There were no differences in the mean number of parasite species per host between localities (one-way ANOVA; $F_{4,74} = 1.34$; $P = 0.25$) (table 1).

Three different kinds of organic pollutants were found in the catfish livers: organochlorine pesticides, PCBs, and PAHs (table 2). For exposure frequency (percentage of catfish exposed to one pollutant), based on the 100 entries in table 2, only 8% were below 40%. In fact, all the pesticides were present in catfish from all five localities, and 48% of 50 entries for pesticides had exposure frequency values between 80 to 100% of fish contaminated. The DDTs were present in 100% of fish examined at four of the localities. The mean pesticide values were highly variable, with the lowest value for endosulfan II ($0.3 \pm 1\,ng\,g^{-1}$ of liver) at Punta Calera, and the highest concentration for Drins at Ramonal ($79 \pm 88\,ng\,g^{-1}$). The PCBs accounted for 63% of 35 entries above the 80% exposure frequency. The lowest mean value for PCBs was for NonaCBs from Nictechan and Punta Calera ($2 \pm 4\,ng\,g^{-1}$), while the highest value was for HeptaCBs from Punta Calera ($153 \pm 483\,ng\,g^{-1}$) (table 2). For PAHs, 90% of ten entries were above the 80% frequency. Mean values for high molecular weight PAHs were far higher than those of low molecular weight PAHs. The highest value for high molecular weight PAHs was that of Ramonal ($212 \pm 206\,\mu g\,g^{-1}$).

By using all the metazoan parasite species data in a PCA analysis, the percentage of explained variance was 64% for the first three principal axes, with eigen values of 0.35, 0.15, and 0.14 respectively. Two species, *Contracaecum* sp. and a tetraphyllidean cestode (fig. 1), were strongly associated with the first principal axis, while *M. appendiculatoides* was strongly associated with the second principal axis. A PCA for pollutants showed that both DDTs and PCBs explain 72% of the variance (fig. 2). The RDA, including only those variables considered as covariables (standard length (Ls), and sampling station (not shown)), accounted for 18.4% of the total variance, and was highly significant ($F = 3.71$; $P = 0.002$; 1999 permutations). Therefore, as standard length increased there was an increase in the number of individual parasite species, such as the tetraphyllidean cestodes and the nematode *Contracaecum* sp. (fig. 3). However, after controlling for the effect of standard length and sampling station, DDTs were shown to have had a significant effect on the infection intensity of *M. appendiculatoides*, whereas PCBs had a significant effect on the infection intensity of tetraphyllidean cestodes ($F = 2.35$; $P = 0.006$; 1999 permutations) (fig. 4). As fish size increased, the infection intensity of tetraphyllidean cestodes also increased, and this species was consequently eliminated from the analysis. This analysis explained an additional 6.8% of the variance, for a total of 23.6%. The DDTs had a significant effect upon the infection intensity of *M. appendiculatoides* ($r = -0.49$; $P < 0.01$; $n = 74$), with a negative linear relationship between DDT concentrations and infection intensity for this species ($Y = -0.23(X) + 3.76$; $r^2\,adj = 0.14$, $F_{1,73} = 13.22$; $P = 0.0005$).

There were no significant differences in the mean Clark's condition coefficient (K) values between slightly and highly infected catfish affected by pesticides (one way ANOVA; $F_{1,73} = 0.54$; $P = 0.46$), PCBs (one way ANOVA; $F_{1,73} = 0.41$; $P = 0.52$), or PAHs (one way ANOVA; $F_{1,73} = 0.21$; $P = 0.64$).

There were significant differences between fish parasitized and not parasitized with *M. appendiculatoides* with respect to their DDT concentrations (one way ANOVA; $F_{1,73} = 9.04$; $P = 0.0036$). In fact, the mean DDTs values for non-parasitized fish were twice ($43 \pm 2\,ng\,g^{-1}$ of liver) the mean values of parasitized fish ($21 \pm 3\,ng\,g^{-1}$ of liver). There were no significant differences in the mean of K values between parasitized and non-parasitized fish (one way ANOVA; $F_{1,73} = 0.25$; $P = 0.61$). There were, however, significant differences between variances in the mean Clark's condition coefficient (K) values of parasitized and non-parasitized fish (Bartlett's test for equal variances: $X^2 = 45.32$; D.F. $= 1$; $P = 0.0001$). The variance of K was significantly higher for the non-parasitized fish (Tukey (HSD) pair-wise comparison of means; critical Q value $= 2.18$; rejection level 0.05). There were no significant differences between parasitized and non-parasitized fish in length (one way ANOVA; $F_{1,73} = 2.43$; $P = 0.12$), age (one way ANOVA; $F_{1,73} = 3.02$; $P = 0.08$) or weight (one way ANOVA; $F_{1,73} = 2.31$; $P = 0.13$).

Discussion

The present results provide statistical evidence that DDTs have an effect upon the intensity of *M. appendiculatoides* infection in *Ariopsis assimilis*, once the effect of length and sampling locality have been controlled. Furthermore, significantly higher variance values in the Clark's condition coefficient for catfish not parasitized by *M. appendiculatoides* suggest that DDTs probably affect the parasite, in addition to affecting general host condition.

The fact that both pollutants and the most frequent and abundant larval metazoan parasite species (*Ascarophis*

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Table 1. Metazoan parasites in *Ariopsis assimilis* (n = 76) from five localities in Chetumal Bay, southeast Mexico in October 1996.

Locality		Ramonal		Nictechan		Bellavista		Punta Verde		Punta Calera	
Geographical position		18°29′58″N; 88°05′12″W		18°41′29″N; 88°02′41″W		8°29′47″N; 88°17′01″W		18°43′50″N; 88°09′56″W		18°42′27″N; 88°10′50″W	
Number of fish examined		12		11		25		17		11	
Standard length (cm)		30 ± 2		33 ± 3		29 ± 3		29 ± 3		30 ± 2	
Weight (g)		420 ± 80		463 ± 86		324 ± 104		363 ± 90		390 ± 95	
Age (years)		3–6		4–6		2–6		3–5		3–5	
Body condition		0.81 ± 0.25		0.83 ± 0.07		0.80 ± 0.12		0.84 ± 0.11		0.84 ± 0.06	
Species richness at infracommunity level		6 ± 2		7 ± 2		6 ± 2		6 ± 2		7 ± 2	
Species	Habitat	%	MI	%	MI	%	MI	%	MI	%	MI
Monogenea											
Neotetraonchus bravohollisae [A]	Gills	33	3 ± 2	27	4 ± 2	40	2 ± 2	47	2 ± 3	45	3 ± 1
Digenea											
Elongoparorchis sp.[A]	Swim bladder	8	1	27	1	12	3 ± 2	23	1	18	1
Diplostomidae gen. sp.[L]	Eyes	8	94	–	–	4	2	–	–	–	–
Mesostephanus appendiculatoides [L]	Muscle	17	22 ± 18	64	45 ± 31	28	27 ± 27	65	57 ± 52	45	11 ± 11
Pseudacanthostomum panamense [A]	Intestine	17	8 ± 5	54	16 ± 11	36	7 ± 6	47	4 ± 3	72	8 ± 9
Cestoda											
Otobothrium sp.[L]	Muscle	58	7 ± 8	54	19 ± 21	68	9 ± 8	76	6 ± 7	91	6 ± 4
Tetraphyllidea gen. sp.[L]	Gall bladder	83	140 ± 177	64	913 ± 895	44	123 ± 257	47	52 ± 48	72	92 ± 135
Trypanorhyncha gen. sp.[L]	Intestine	58	9 ± 7	64	10 ± 8	72	4 ± 3	88	4 ± 4	82	6 ± 4
Nematoda											
Ascarophis ayalai [L]	Stomach	33	27 ± 21	36	24 ± 14	40	12 ± 13	6	10	18	20 ± 26
Contracaecum sp.[L]	Liver, kidney, stomach and intestinal wall	100	114 ± 196	100	321 ± 354	92	224 ± 453	100	59 ± 101	100	96 ± 173
Contracaecum type 2[L]	Mesenteries, intestinal wall	–	–	9	1	–	–	–	–	–	–
Cucullanus bagre [A]	Intestine	8	1	9	1	24	2 ± 1	–	–	18	1
Eustrongylides sp.[L]	Stomach wall	–	–	–	–	–	–	6	1	–	–
Goezia sp.[L]	Stomach	–	–	–	–	4	1	–	–	–	–
Procamallanus chetumalensis [A]	Intestine	8	1	9	1	4	4	–	–	–	–
Pseudoterranova sp.[L]	Mesenteries, intestinal wall	–	–	36	436 ± 681	12	1	6	1	9	15
Acanthocephala											
Gorgorhynchus bullocki [A]	Intestine	17	3 ± 2	–	–	12	2 ± 1	18	2 ± 2	45	2 ± 1
Polymorphidae[L]	Mesenteries	17	13 ± 11	–	–	12	14 ± 18	6	3	9	6
Arthropoda											
Argulus flavescens [A]	Skin	33	2 ± 1	72	1 ± 1	–	–	35	2 ± 2	18	1 ± 1
Ergasilus sp.[A]	Skin	–	–	–	–	20	2 ± 1	–	–	–	–

Infracommunities included all metazoan parasites species infecting an individual host. L, larval stage; A, adult stage; %, prevalence; MI, mean intensity of infection.

Table 2. Pollutants detected in the liver of 76 *Ariopsis assimilis* from five localities in Chetumal Bay, southeast Mexico in October 1996.

Locality	Ramonal		Nictechan		Bellavista		PuntaVerde		Punta Calera	
Number of fish examined	12		11		25		17		11	
Pollutants	F	$\bar{x} \pm$ S.D.	F	$\bar{x} \pm$ S.D.	F	$\bar{x} \pm$ S.D.	F	$\bar{x} \pm$ S.D.	F	$\bar{x} \pm$ S.D.
Pesticides (ng g^{-1})										
TCBs	67	13 ± 15	64	5 ± 7	76	7 ± 11	41	15 ± 20	64	3 ± 5
Pentachlorobenzene	50	13 ± 15	54	7 ± 7	72	5 ± 9	47	18 ± 31	37	7 ± 8
HCBs	100	24 ± 15	91	9 ± 8	100	8 ± 7	82	6 ± 5	82	3 ± 4
Pentachloroanisole	83	13 ± 21	64	5 ± 9	68	4 ± 5	53	4 ± 4	64	2 ± 2
HCHs	100	28 ± 19	91	14 ± 13	92	12 ± 11	65	10 ± 7	91	5 ± 6
Chlordanes	100	76 ± 50	100	35 ± 33	100	32 ± 23	82	26 ± 17	100	16 ± 12
Drins*	100	79 ± 88	100	22 ± 19	92	23 ± 22	76	22 ± 27	91	6 ± 7
Endosulfan II	33	5 ± 8	18	3 ± 6	16	4 ± 5	35	1 ± 3	45	0.3 ± 1
DDTs	100	48 ± 3	100	34 ± 31	100	73 ± 134	76	29 ± 24	100	76 ± 99
Mirex	50	11 ± 21	82	7 ± 8	36	3 ± 4	41	2 ± 5	45	2 ± 5
Polychlorinated biphenyls (PCBs) (ng g^{-1})										
TriCBs	100	33 ± 12	91	12 ± 12	100	14 ± 13	82	50 ± 130	100	18 ± 17
TetraCBs	83	42 ± 60	64	7 ± 8	84	36 ± 110	65	9 ± 12	100	7 ± 7
PentaCBs	84	16 ± 16	72	10 ± 10	96	13 ± 11	65	14 ± 17	91	8 ± 9
HexaCBs	92	29 ± 38	72	10 ± 9	100	18 ± 11	82	16 ± 11	100	12 ± 19
HeptaCBs	92	19 ± 19	91	27 ± 28	100	22 ± 34	65	16 ± 23	100	153 ± 483
OctaCBs	8	10 ± 15	81	4 ± 4	84	7 ± 7	65	4 ± 4	81	3 ± 9
NonaCBs	33	12 ± 13	64	2 ± 4	36	4 ± 4	35	4 ± 4	64	2 ± 4
Polycyclic aromatic hydrocarbons (PAHs) (μg g^{-1})										
Low molecular weight (2–3 rings)	100	7 ± 6	100	8 ± 11	100	6 ± 6	76	8 ± 13	91	4 ± 2
High molecular weight (4–5 rings)	100	212 ± 206	100	61 ± 40	100	116 ± 99	82	133 ± 179	100	39 ± 32
Unresolved complex mixture (UCM) (μg g^{-1})	58	427 ± 910	54	100 ± 123	72	199 ± 545	41	208 ± 438	45	102 ± 106

Exposure frequency (F) was the proportion of individual catfish with a particular pollutant from a sample of catfish. *Aldrin + Endrin + Dieldrin. TCB, trichlorobenzene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane. Based on Noreña-Barroso *et al.* (1998).

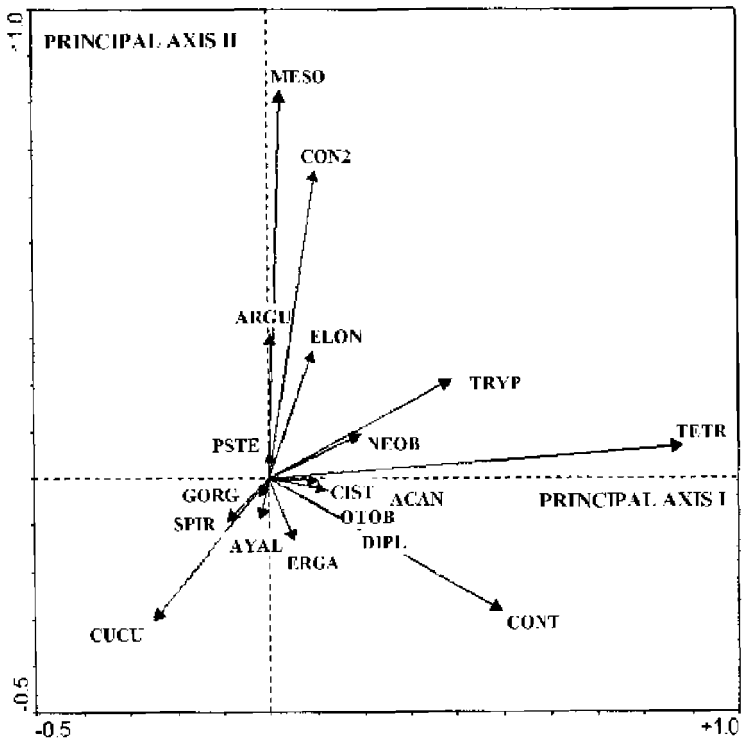

Fig. 1. Principal component analysis of the metazoan parasites of 76 *Ariopsis assimilis* represented by arrows. ACAN, *Pseudacanthostomum panamense*; ARGU, *Argulus flavescens*; AYAL, *Ascarophis ayalai*; CIST, Polymorphidae; CONT, *Contracaecum* sp.; CON2, *Contracaecum* type 2; CUCU, *Cucullanus bagre*.; DIPL, Diplostomidae gen. sp.; ELON, *Elongoparorchis* sp.; ERGA, *Ergasilus* sp.; GORG, *Gorgorhynchus bullocki*; MESO, *Mesostephanus appendiculatoides*; NEOB, *Neotetraonchus bravohollisae*; OTOB, *Otobothrium* sp.; PSTE, *Pseudoterranova* sp.; SPIR, *Procamallanus* (*Spirocamallanus*) *chetumalensis*; TETR, Tetraphyllidea gen. sp.; TRYP, Trypanorhyncha gen. sp.

ayalai, *Contracaecum* sp., *Otobothrium* sp. and *M. appendiculatoides*, table 1) were present in the 76 catfish suggests that a common factor is influencing their transport within the bay. The most likely explanation is very slow water circulation, which, via advection, moves pollutants, infective stages of metazoan parasites and/or infected intermediate hosts throughout the bay. In this case the catfish were evidently chronically exposed to all pollutants and infective stages of metazoan parasites in the five localities (tables 1 and 2).

In the PCA analysis, the relationship of the larval metazoan parasite species with a high prevalence and infection intensity to the first and second principal axes is not surprising. These species were numerically dominant (table 1), and thus they explain most of the cumulative variance, as suggested by Jongman *et al.* (1995). The RDA analysis determined that two groups of variables affected the infection intensity of metazoan parasite species in the catfish: (i) standard length (Ls) and sampling sites; and (ii) DDT and PCB concentrations. The importance of standard length as a predictor is to be expected because as a fish grows, and 'samples the environment' in its search for food, it accumulates an increasingly greater number of metazoan parasites. Further support for this suggestion comes from a variety of fish species and other vertebrates that accumulate larval stages of metazoan parasites as they grow (Hudson & Dobson, 1995). The influence of sampling site can be explained by the accumulation of both pollutants and metazoan parasite infective stages at one or several localities, which is related to the slow circulation of water in the bay.

Once the influence of standard length and sampling site is controlled for using redundancy analysis, the negative effect of DDTs on the infection intensity of *M. appendiculatoides* becomes evident. Both RDA and the significant negative linear relationship between DDT concentrations and *M. appendiculatoides* infection intensity suggest that fish with high concentrations of the pesticide may harbour fewer metacercariae of this species. The data proved inconclusive, however, in determining if DDTs truly impact upon infected fish hosts and/or host snails, miracidia, cercariae and/or metacercariae. Further experimental evidence is needed to determine which life cycle stage is affected by DDTs, though the present results do not reject the hypothesis that DDTs affect *M. appendiculatoides* infection intensity in this fish species. The results also imply that *M. appendiculatoides* could be a good indicator species of pesticide impact due to its apparent sensitivity to DDTs. In fact, there is experimental evidence that suggests that DDTs affect the eggs, miracidia and cercariae of other digeneans (Mukherjee, 1973; Varma *et al.*, 1994), and snails (Kumar *et al.*, 1992).

Even though data on heavy metals were not collected in the present investigation, it is unlikely to be an issue in Chetumal Bay. It has recently been demonstrated that as water hardness increases, the toxicity of heavy metals to

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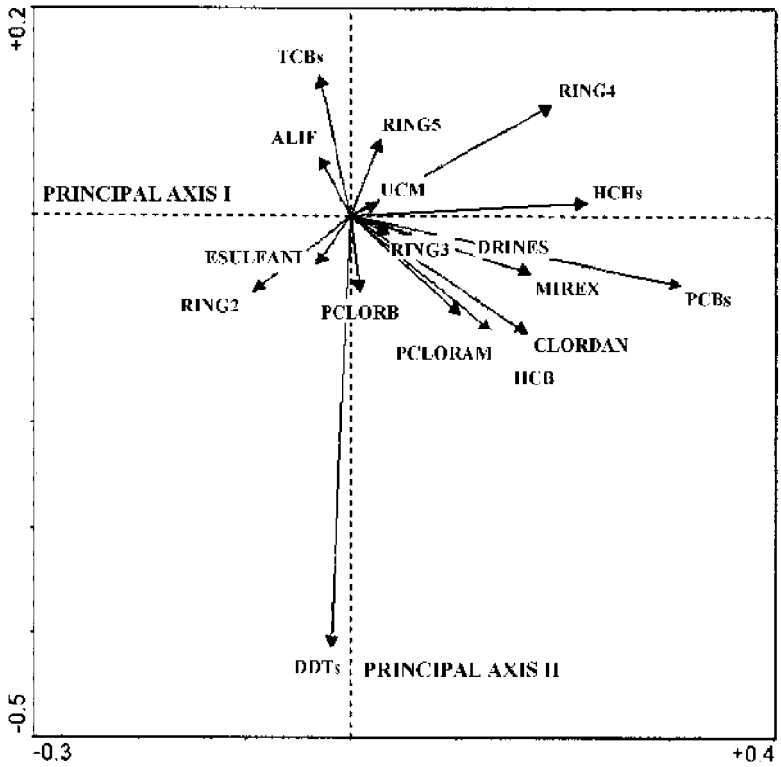

Fig. 2. Principal component analysis of the pollutants recovered from the liver of 76 *Ariopsis assimilis* from Chetumal Bay. ALIF, aliphatic hydrocarbons; CLORDAN, chlordanes; DDTs, pesticide; DRINES, Aldrin + Endrin + Dieldrin; ESULFANI, Endosulfan I; HCB, hexachlorobenzene; HCHs, hexachlorocyclohexane; MIREX, pesticide; PCBs, polychlorinated biphenyls; PCLORB, pentachlorobenzene; PCLORAM, pentachloroanisole; RING2, 2-ring PCB; RING3, 3-ring PCB; RING4, 4-ring PCB; RING5, 5-ring PCB; TCB, trichlorobenzene; UCM, unresolved compounds.

free-living infective stages of digeneans, such as cercariae of *Diplostomum spathaceum*, decreases (Morley *et al.*, 2001). The waters of Chetumal Bay are very hard due to the limestone composition of the Yucatan Peninsula, and it can be reasonably assumed that heavy metal toxicity to *M. appendiculatoides* cercariae is relatively low.

The lack of significant differences for K between slightly and highly infected catfish and their respective concentrations of pesticides, PCBs and PAHs could be related to the differential response of metazoan parasite species to pollutants. Similar explanations have been suggested for other metazoan parasite species by Lafferty (1997).

Significant differences in the variance for K (Bartlett's test for equal variances: $X^2 = 45.32$; D.F. $= 1$; $P = 0.0001$), suggest that catfish exposed to high DDT concentrations are infected by all the frequently occurring and abundant larval metazoan parasite species in table 1, except *M. appendiculatoides*. This highlights the apparent high sensitivity of this parasite species to pesticides, as does the fact that DDT concentrations in fish not parasitized by *M. appendiculatoides* were twice those in parasitized fish. This suggests that the high variance for the condition coefficient may be related to harm caused the catfish from DDTs and/or the parasites. Goater & Holmes (1997) have emphasized the important role of parasites in producing sublethal intensity dependent effects, such as those on energy balance or appearance, which may influence host fitness. Holmes & Zohar (1990) suggested that the ability of the host to compensate for such effects depends largely on body size and general condition of the host. It is also well known that DDTs can impair host condition, strongly affecting the nervous system (Deustche Gesellschaft für Technische Zusammenarbeit, 1995). Consequently, DDTs and/or the metazoan parasites may be impairing the general condition of catfish. This is further supported by the suggestion of Moles (1980) that parasites can influence fish sensitivity to pollutants. He demonstrated that coho salmon fry parasitized with glochidial larvae of *Anodonta oregonensis* have an increased sensitivity to crude oil, toluene and naphthalene (Moles, 1980).

The presence of DDTs does appear to have some effect on *M. appendiculatoides* infection intensity, as shown by the fact that the parasitized fish had half the DDT concentrations of the non-parasitized fish, and a very low variance value for the Clark's condition coefficient. It is possible that the DDT concentrations in the bay were not terminal for either the fish or parasite species. The fact that the highly polluted catfish were still alive but free of *M. appendiculatoides* does suggest that this digenean species is more sensitive to DDTs than the catfish. There are no data on the effect of DDTs on *M. appendiculatoides*, but pesticides do have deleterious effects on the invasive

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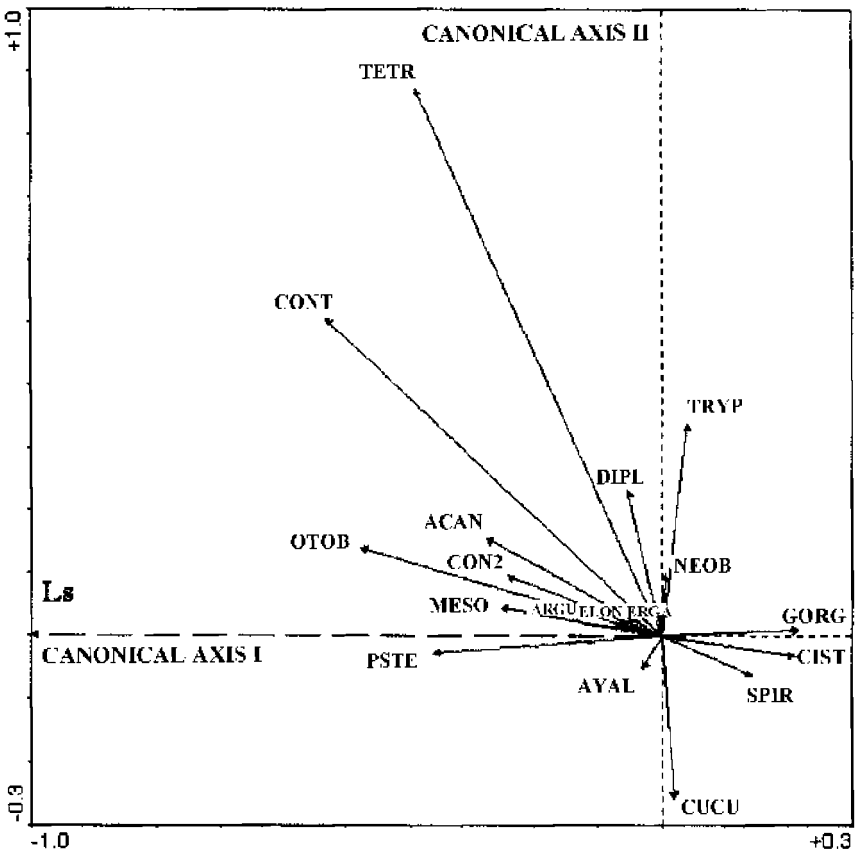

Fig. 3. Redundancy analysis of the number of individuals of each metazoan parasite species in *Ariopsis assimilis* controlling for standard length (Ls) and localities (not shown). Abbreviations as in fig. 1.

ability of other digeneans such as *Fasciola hepatica* (Bielecki, 1985; Babicek & Danek, 1993), and paramphistomids (Gupta & Yadav, 1993).

During May to September 1996, a selective fish kill of 33,000 Mayan catfish, *Ariopsis assimilis* occurred in Chetumal Bay (Noreña-Barroso *et al.*, 1998), and although the present results do not conclusively explain such a massive catfish mortality, the presence of pollutants and parasites is likely to have had an additive effect on the general condition of the catfish. Noreña-Barroso *et al.* (in press) found that the catfish used in the present study also had a series of lesions such as hyperaemia, tumours, granulomata, pycnosis and lymphocyte infiltration associated with the presence of pollutants in Chetumal Bay. Furthermore, the numerically dominant larval species of metazoan parasites infecting the catfish had very high mean intensity values (table 1). More than 50 cysts per fish of *Uvulifer ambloplitis* metacercariae were enough to produce parasite-induced host mortality in bluegill sunfish *Lepomis macrochirus* when temperatures were below 20–25°C (Lemly & Esch, 1984). Therefore, even though the histopathological changes induced by larval parasites in the catfish (table 1) need to be investigated further, it seems reasonable to suggest a deleterious association between these parasites and the pollutants. If this is true, then the 1996 fish kill removed highly polluted and parasitized catfish from the population through chronic exposure to a combination of deleterious agents that caused a weakening of their general condition. In turn, fish affected by pollutants and metazoan parasites would have been incapable of dealing with environmental stresses (e.g. changes in temperature, salinity, oxygen concentration) that in other circumstances would have had no affect on healthy catfish.

An experimental design that could be used to confirm this hypothesis would involve placing floating cages containing pollutant and metazoan parasite-free fish in the natural marine environment so that field-based observations could be combined with laboratory experiments to test the effect of DDTs on the parasite *M. appendiculatoides*.

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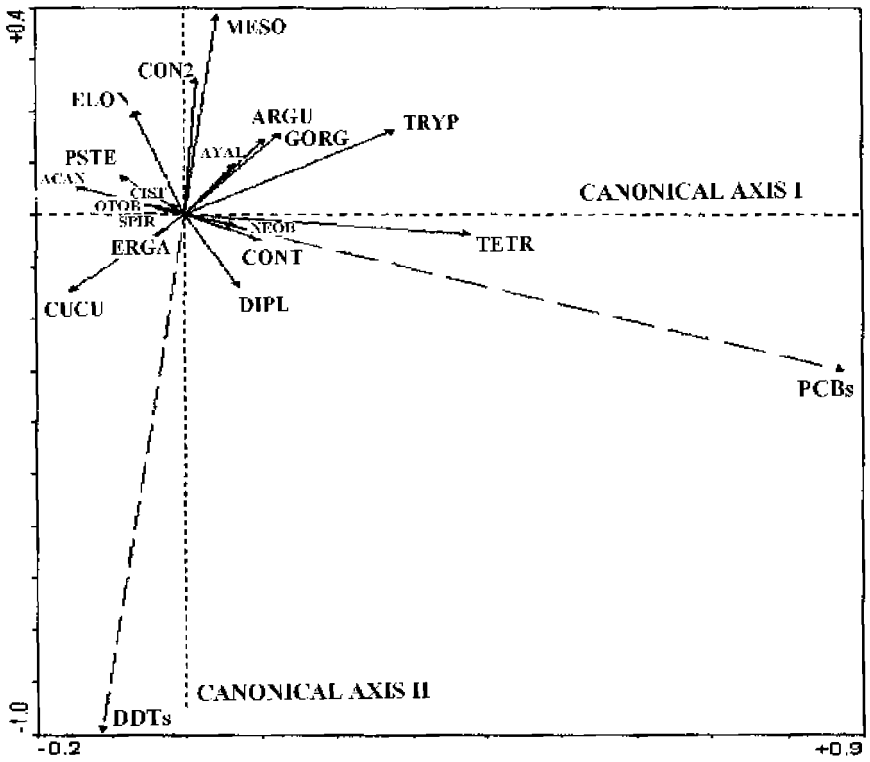

Fig. 4. Redundancy analysis showing the relationship between pollutants and the number of individuals of each metazoan parasite species in *Ariopsis assimilis* in Chetumal Bay, southeast Mexico, controlled for standard length (Ls) and localities (not shown). Abbreviations as in fig. 1.

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