PCB concentrations in intertidal sipunculan (Phylum Sipuncula) marine worms from the Pacific coast of Costa Rica

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Abstract: Specimens of the sipunculans *Phascolosoma perlucens, Antillesoma antillarum*, and *Sipunculus nudus*, were collected for PCB analysis in three coastal embayments on the Pacific coast of Costa Rica in October 2005. After collection the worms were kept in cool sea water and transferred to the laboratory for freeze drying. Half of the specimens of *S. nudus* were depurated in filtered sea water for 72 h before freeze drying. The whole worm sample was ground, using a mortar and pestle prior to being extracted with dichloromethane solvent. The extract was cleaned using a florisil column and analyzed using a 6890 Hewlett-Packard gas chromatograph equipped with a mass selective detector. Recovery efficiencies averaged between 72 and 80 %. Data are not corrected for blank or recovery efficiencies. Concentration data were calculated based on comparison with standards for Arochlor 1221, 1242, 1248 and 1254 (Supelco, Inc. ®). No worm samples were identified with six or more chlorine atoms. However, the higher chlorinated congeners were identified in the standards. Therefore, the lack of chlorination in the worms was not an analytical artifact. The sum of identifiable PCBs ranged from a barely discernable 0.01 ng/g dry wt in an *A. antillarum* sample from Culebra Bay to 67.71 ng/g dry wt in a *P. perlucens* sample obtained near the Rincón river in the inner Golfo Dulce. *S. nudus* worm samples from the Cocorocas sand flat in the Gulf of Nicoya had relatively high concentrations of PCBs whether they were depurated or not. The samples from Costa Rica are low in PCBs, especially compared to the marine sediment samples measured previously in Costa Rica. This was the first attempt to identify and quantify the presence of PCBs in any marine organisms from the Pacific coast of Costa Rica. Rev. Biol. Trop. 54 (Suppl. 1): 27-33. Epub 2006 Sept. 30.

Key words: PCB, sipunculans, Costa Rica, Gulf of Nicoya, *Phascolosoma perlucens, Antillesoma antillarum, Sipunculus nudus*, marine pollution, estuaries.

Samples of several species of marine worms were collected for PCB analysis in Culebra Bay (upwelling region), Golfo de Nicoya (estuary) and Golfo Dulce (fjord-like embayment), Pacific coast of Costa Rica (Fig. 1A), in October 2005. Background information on these ecosytems is found in Bednarski and Morales-Ramírez (2001), Maurer and Vargas (1984), and Dalsgaard *et al.* (2003), respectively. Representatives of the Phylum Sipuncula were chosen for this study due to their ubiquitous appearance in the coastal waters of Costa Rica, and around the world. These worms are commonly referred to as 'peanut worms' due to the appearance of some species, with a body shape which is divisible into a retractible introvert and a trunk. They are entirely marine and estuarine and can be found from the intertidal zone to abyssal depths at all latitudes. Most sipunculans are deposit feeders, although a few are filter feeders. They all consume detritus and fecal material as well as bacteria, algae, and small invertebrates. They are in turn eaten by fish, mollusks, crabs, and other predators (Cutler 1994).

Previous research on PCB concentrations within the coastal sediments of Costa Rica have been conducted from 1996 to 2003 (Spongberg

2004a). The highest concentrations were found in the Golfo Dulce embayment, with concentrations of total PCBs averaging around 5 000 ng/g dry sediment sample for the entire gulf, and one sample in the port of Golfito, with values greater than 15 600 ng/g dw (Spongberg 2004b). Samples from the Gulf of Nicoya estuary had a lower overall average of around 2 300 ng/g dw, and a highest value of 6 949 ng/g dw (Spongberg 2004c). Culebra Bay contained the cleanest samples with respect to PCBs, with an average total concentration of 1 417 ng/g dw (Spongberg 2004a). The fauna of coastal sipunculans of Costa Rica is relatively well known, and several species are found in high densities and are relatively easy to identify (Cutler *et al.* 1990, Dean 2001).

The current study was conducted to determine whether any PCBs in the area are capable of moving into and up the food chain. Since the sipunculans are detritivores, they are the first step between the contaminated sediments and higher trophic levels.

MATERIALS AND METHODS

The sipunculan species used in this study were collected from three intertidal marine environments on the Pacific coast of Costa Rica (Fig. 1A). The species include *Phascolosoma perlucens*, which is a elongate, endolithic, white worm (Fig. 1B) found within crevices in intertidal rocks. Extraction of the worms from the rocks often required the use of a hammer to break apart the rock (sandstone) since the worm can extend into very small, tight spaces between rock layers. The plumper brown peanut worm, *Antillesoma antillarum* (Fig. 1C) was usually found in the same vicinity as the

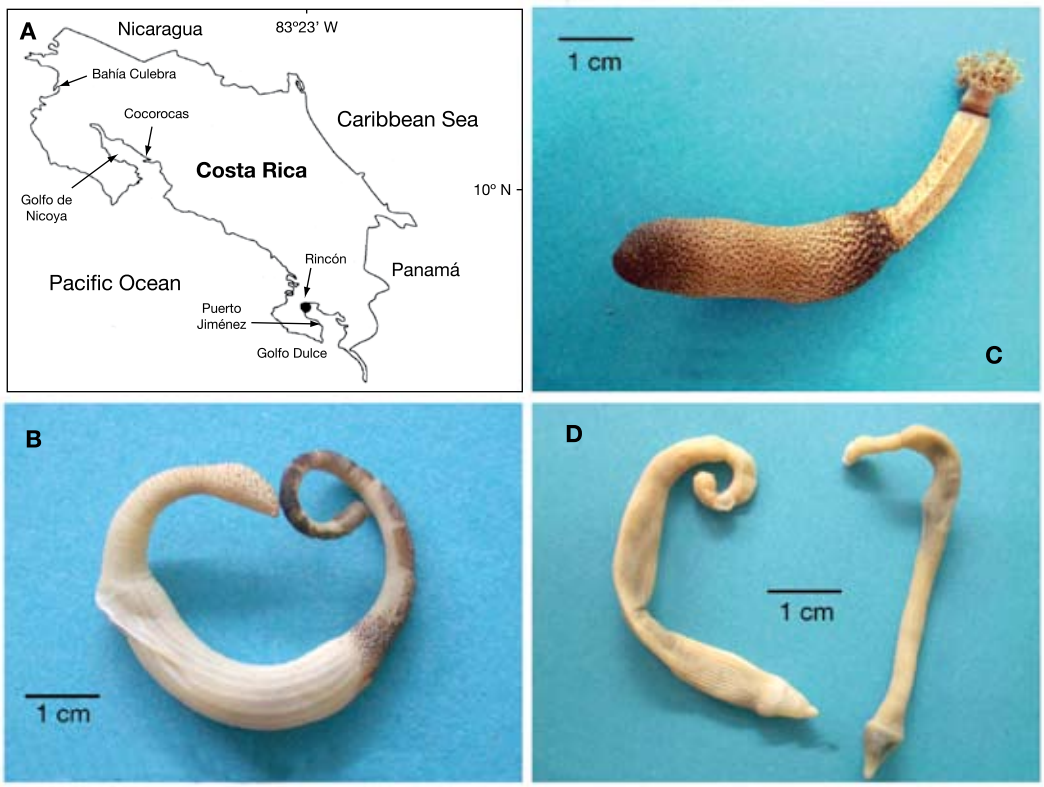

Fig. 1. A. Location of the three sampling stations (Culebra Bay, Gulf of Nicoya, Golfo Dulce) on the Pacific coast of Costa Rica, October 2005. B,C,D. Preserved specimens of the three species of sipunculan worms collected. B. *Phascolosoma perlucens*. C. *Antillesoma antillarum*. D. *Sipunculus nudus.*

Phascolosoma species, however, they lived either underneath larger rocks or in larger or looser crevices that were more easily parted. *Sipunculus nudus* (Fig. 1D) was only found in the Cocorocas sand flat near Punta Morales in the mid upper Gulf of Nicoya (Dittmann and Vargas 2001). Identification of sipunculan species was checked using Cutler (1994), and confirmed by José A. Vargas of the Center for Marine and Limnological Research (CIMAR) at the University of Costa Rica. A list of the species collected and locations is included in Table 1.

Specimens were collected and stored in cool sea water until they could be processed in the laboratory. Samples were frozen and freeze-dried prior to shipment to the University of Toledo. Half of the *S. nudus* samples were depurated for 72 h in filtered sea water from the collection site, to remove sediments from their guts prior to processing.

The details of sample preparation and PCB analysis can be found in Spongberg (2004a). The whole worm sample was ground, using a mortar and pestle prior to being extracted with dichloromethane solvent. The extract was cleaned using a florisil column and analyzed using a 6890 Hewlett-Packard gas chromatograph equipped with a mass selective detector. Solvent and GC parameters are identical to those used for the sediments (Spongberg 2004a). The detector was run in both scanning and selected ion monitoring modes to detect minute quantities of PCB congeners within the sample. Blanks, spiked samples, recovery and quantitation standards were also run. Most samples were run in triplicate or more. Recovery efficiencies averaged between 72 and 80 %. Data are not corrected for blank or recovery efficiencies.

Concentration data are calculated based on comparison with standards for Arochlor 1221, 1242, 1248 and 1254 (Supelco, Inc. ®). In only one sample were congeners with 5 chlorines identified in quantifiable amounts. Therefore, eight peaks with 1, 2, 3 or 4 Cl were identified that were consistently present in the samples and standards. Standard concentration curves were created for these congeners for use in quantifying the Sum of PCB values reported below.

RESULTS

The data for PCB concentrations in the sipunculan worm samples from Costa Rica are included in Table 1. Also included in this table are the percentage of the total PCB burden with 2, 3, 4, or 5 chlorine atoms. No worm samples were identified with 6 or more chlorine atoms, however, the higher chlorinated congeners were identified in the standards. Therefore, the lack of chlorination in the worms was not an analytical artifact.

Figure 2 shows two gas chromatograms. The first is from the Arochlor 1248 standard. The second shows the peaks present in one of the Golfo de Nicoya samples that had one of the highest overall PCB concentrations of all samples. This figure shows the shift towards the lesser chlorinated congeners that is present in all samples. This phenomenon is apparent in almost all biological samples since the lesser chlorinated congeners are more water soluble and more easier absorbed through the skin, while the more chlorinated compounds generally must be ingested (Jager *et al.* 2003).

DISCUSSION

The sum of identifiable PCBs ranged from a barely discernable 0.01 ng/g dry wt in an *A. antillarum* sample from Culebra Bay to 67.71 ng/g dry wt in a *P. perlucens* sample obtained near the Rincón river in the inner Golfo Dulce (Fig. 1). However, overall, the majority of samples with detectable levels of PCBs were from the Gulf of Nicoya estuary. Of the seven samples from Culebra Bay (all run in triplicate) only two had identifiable PCB peaks. All Culebra Bay samples were obtained from the intertidal areas to the east and west of Panama Beach, where construction of new housing and resorts is rapidly changing the landscape. Erosion is apparent in several areas. Nevertheless, the PCB load on the marine worms is not evident. Golfo

TABLE 1
Sample indentification and extract data for Phylum Sipuncula marine worm collection, October 2005, Pacific coast of Costa Rica

Location	Species	x if depurated	Sum PCB ng/g dry wt.	(S.D.)	2 Cl %	3 Cl %	4 Cl %	5 Cl %
Gulf of Nicoya (Golfo de Nicoya)								
Cocorocas sand flats	*Sipunculus nudus*	x	17.75	12.22	37.50	62.50	0.00	0.00
	S. nudus	x	ND	-	-	-	-	-
	S. nudus	x	ND	-	-	-	-	-
	S. nudus	x	23.18	7.42	0.00	100.00	0.00	0.00
	S. nudus	x	41.38	16.34	7.30	92.70	0.00	0.00
	Duplicate	x	23.71	20.33	7.70	90.30	1.90	0.00
	S. nudus	x	ND					
	S. nudus	-	17.75	9.03	12.60	87.40	0.00	0.00
	S. nudus	-	19.99	6.33	0.70	25.30	74.00	0.00
Cocorocas sandstone	*Phascolosoma perlucens*	-	6.99	3.24	16.90	83.10	0.00	0.00
	P. perlucens	-	ND	-	-	-	-	-
	P. perlucens	-	ND	-	-	-	-	-
	P. perlucens	-	30.64	20.01	23.80	76.20	0.00	0.00
	Duplicate	-	21.14	13.23	7.60	92.40	0.00	0.00
Culebra Bay (Bahía Culebra)								
Panama Beach east	*Antillesoma antillarum*	-	0.01	0.01	0.00	100.00	0.00	0.00
	P. perlucens	-	ND	-	-	-	-	-
	A. antillarum	-	ND	-	-	-	-	-
	A. antillarum	-	ND	-	-	-	-	-
	A. antillarum	-	ND	-	-	-	-	-
	A. antillarum	-	ND	-	-	-	-	-
	P. perlucens	-	1.13	0.87	0.00	100.00	0.00	0.00
Golfo Dulce								
Puerto Jiménez	*P. perlucens*	-	0.60	0.28	0.00	100.00	0.00	0.00
	P. perlucens	-	ND	-	-	-	-	-
East of Rincón	*P. perlucens*	-	ND	-	-	-	-	-
	P. perlucens	-	67.71	19.96	13.40	71.80	24.20	4.20

Dulce worm samples were also generally clean of PCBs (below detection limits). The sample of *P. perlucens* from Puerto Jiménez were extracted from concrete structures near a boat docking area where water and road travel were relatively high. Levels of PCBs were expected to be high at this location. The Golfo Dulce sample with the highest overall PCB concentration, however, was obtained near the head of the gulf on a relatively deserted shoreline. Both Culebra Bay and Golfo Dulce samples had few PCB peaks. However, the selected ion monitoring mode used on the mass selective detector made their identification positive.

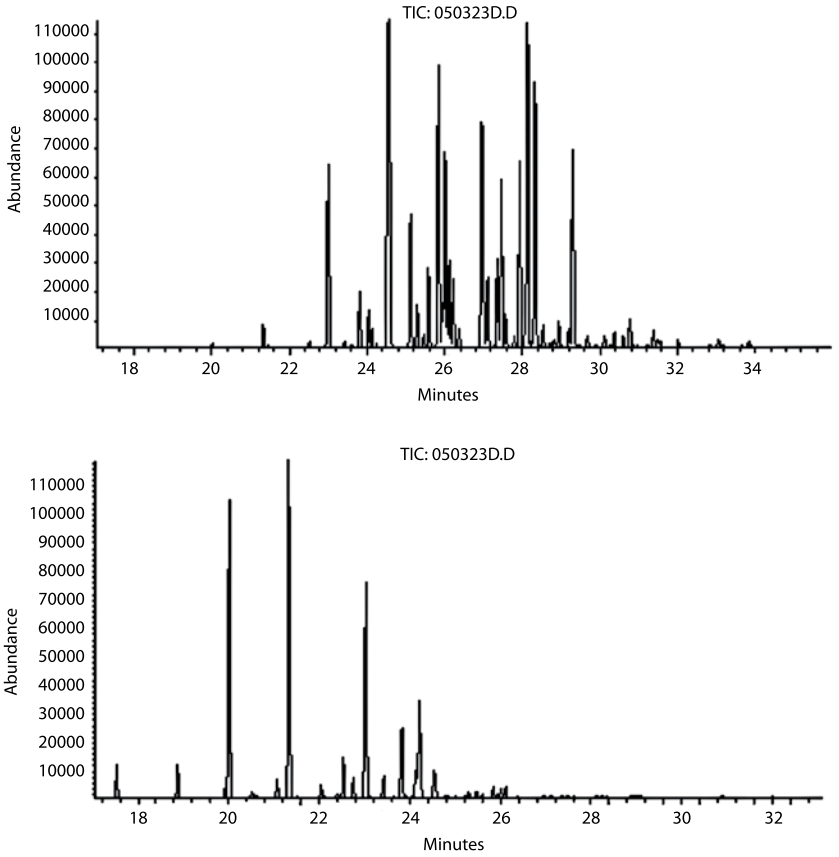

Fig. 2. Gas chromatograms of the 1248 PCB standard and one worm sample from G. Nicoya. Worm PCB concentrations skew towards the lower chlorinated congeners. Data were obtained using a mass selective detector in Selective Ion Monitoring mode to enhance the detection of PCB compounds.

S. nudus worm samples from the Cocorocas sand flat in Golfo Nicoya had relatively high concentrations of PCBs whether they were depurated or not. In most cases the Gulf of Nicoya samples had a wider range of congeners apparent on the gas chromatograms than either the Golfo Dulce or Culebra Bay samples. The GC shown at the bottom of Figure 1 is from sample 15 with a Sum of PCB of 41.38 ng/g dry wt. These chromatograms were creased in SIM mode and thus show the diversity of congeners in these samples.

S. nudus is a cosmopolitan species and many studies have been conducted on its physiology and anatomy (see Ruppert and Rice 1995). However, no studies are reported on PCB concentrations in sipunculan worms (Oberdörster and Cheek 2000). For comparison, a few studies on contaminants in various trophic levels were used in this study. Analyses by Wong *et al.* (2004) on PCBs in lipid fraction of fish from Lake Superior found 5 440 ng/g lipid (11.8 % lipid) for trout, 2 190 ng/g lipid (4.8 % lipid) for herring and 1 120 ng/g lipid (4.7 % lipid) for smelt. The associated *Mysis* and *Diporeia* crustaceans had lower PCB burdens (280 and 250 ng/g lipid (4.9 and 4.3 % lipid), respectively. Concentrations in the associated zooplankton and phytoplankton were similar to the crustaceans. VanderPol *et al.* (2004) studied several contaminants in Alaskan Murre eggs in Eastern Canada and Western Europe and included a summary of other similar studies in their paper. PCB concentrations ranged from 60 to 2 210 ng/g wet mass using several different quantitative techniques. The samples

from Costa Rica are obviously low in PCBs, especially compared to the sediment samples measured previously (Spongberg 2004a,b,c.). Of interest is the comparison of these data to the United States Environmental Protection Agency's Specific Polychlorinated Biphenyl Worm Tissue Criterion of 113 ng/g in worms found in dredge material designated for re-use as remediation materials (Fed. Register 2004). This value was chosen as the pass/fail cut off deemed safe for sediment reuse.

This was the first attempt to identify and quantify the presence of PCBs in any marine organisms from the Pacific coast of Costa Rica.

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RESUMEN

Ejemplares de los sipuncúlidos *Phascolosoma perlucens*, *Antillesoma antillarum*, y *Sipunculus nudus* de tres ambientes costeros del Pacífico de Costa Rica, fueron recolectados en octubre del 2005, para analizar bifenilos policlorados (BPC). Los ejemplares fueron colocados en agua de mar enfriada y trasladados al laboratorio para ser liofilizados. La mitad de los ejemplares de *S. nudus* fueron depurados por 72 horas en agua de mar filtrada. El total de la muestra liofilizada fue molida, utilizando un mortero y pistilo, y después sometida a la extracción con el disolvente diclorometano. El extracto fue limpiado en una columna con florisil y analizado utilizando un cromatógrafo de gases Hewlett Packard 6890, equipado con un detector selectivo de masas. La eficiencia promedio de las recuperaciones fue entre 72 y 80%. Los datos no fueron corregidos para el blanco o eficiencias de recuperación. Los datos de concentración fueron calculados por comparación con estándares para Arochlor 1221,1242, 1248 y 1254 (Supelco, Inc. ®). No hubo muestras de gusanos con congéneres de seis o más átomos de cloro. Sin embargo, los congéneres más clorados fueron identificados en los estándares. Por lo tanto, la falta de estos clorados en los gusanos no fue un artificio analítico. La suma de los bifenilos identificables tuvo un ámbito entre 0.01 ng/peso seco en una muestra de *A. antillarum* de Bahía Culebra, hasta 67.71 ng/peso seco en una muestra de *P. perlucens*, obtenida cerca del Río Rincón en el Golfo Dulce. Muestras de *S. nudus*, depuradas y sin depurar, de la planicie arenosa de Cocorocas en el Golfo de Nicoya, tuvieron concentraciones relativamente altas de BPC. Las muestras de Costa Rica son bajas en sus concentraciones de BPC, especialmente cuando se les compara con muestras de sedimentos marinos analizadas previamente en Costa Rica. Este fue el primer intento de identificar y cuantificar la presencia de BPC en organismos marinos de la costa Pacífica de Costa Rica.

Palabras clave: Bifenilos policlorados, sipuncúlidos, Costa Rica, Golfo de Nicoya, *Phascolosoma perlucens*, *Antillesoma antillarum*, *Sipunculus nudus*, contaminación marina, estuarios.

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