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Organochlorine compound residues in the eggs of broad-snouted caimans (*Caiman latirostris*) and correlation with measures of reproductive performance

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ABSTRACT

Organochlorine compounds (OCCs), like pesticides (OCPs) and polychlorinated biphenyls (PCBs), are persistent lipophilic chemicals classified as endocrine-disruptors. *Caiman latirostris* inhabits wetlands throughout north-eastern Argentina and may accumulate OCCs. The aims of this study were to determine OCC residues in the eggs of *C. latirostris* and to correlate OCC burden with clutch size, hatching success and hatchling survival as measures of reproductive performance. Fourteen caiman clutches were harvested from sites with different degrees of anthropogenic intervention on wetlands surrounding Paraná River tributaries. Two to four eggs by clutch were used to quantify OCCs. OCP residues were found in all clutches. The principal contributors to the OCPs burden were the DDT family (range BDL-153.0 ng g^{-1} lipid) and oxychlordane (range BDL-34.3 ng g^{-1} lipid). PCBs were present in 92.9% of the clutches (range BDL-136.6 ng g^{-1} lipid). Both higher concentrations and higher diversity of pesticides, including endosulfan sulfate, were found in the nests harvested close to croplands. A negative correlation was found between clutch size and ∑OCCs ($p = 0.02$, Pearson $r = -0.53$, $r^2 = 0.28$), mainly due to the ∑OCPs ($p = 0.04$, Pearson $r = -0.54$, $r^2 = 0.30$). Since egg OCCs concentrations predict maternal burden, present findings suggest that higher OCCs exposure could lead to smaller clutches. Although, other factors like mother age could influence clutch size. Additionally, as caimans are a long-lived and non-migratory species, the maternal OCCs burden reflects the environmental status throughout their home range; thus, caiman eggs could be useful as a biomonitor of local contamination.

1. Introduction

Organochlorine compounds (OCCs), such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), are persistent lipophilic chemicals identified as endocrine disruptor compounds (EDCs). These EDCs may have a broad range of adverse effects on wildlife (Matter et al., 1998; IPCS, 2002). Expansion of agricultural frontiers, intensive deforestation, increased use of agrochemicals, growing industrial development and frequent non-adherence to environmental protection rules are major threats to the ecohealth in Argentina. Despite the steady increase of the above mentioned activities (INTA – www.inta.gov.ar), little is known about the concentrations of OCCs in Argentinean wetlands. In Argentina, ANMAT and SENASA are responsible for the regulation of OCCs (ANMAT – www.anmat.gov.ar; SENASA – www.senasa.gov.ar). Most OCPs were banned in 1968 (Ley Nacional N° 17.751/68). Despite these restrictions, some of them remained in use as household insecticides until 1998 (ANMAT 7292/98). Uses, handling and safe disposal of PCBs were firstly regulated in 1991 (Ley Nacional N° 24051/91). PCBs were completely banned in 2002 (Ley Nacional 25.670/02) but the replacement and disposal of PCB transformers is still in process (Decreto N° 657/07 Entre Ríos; Resolución N° 043/03 Santa Fe).

Recently, we reported the high frequency and the elevated concentrations of OCC residues in breast adipose tissue of women from an urban area adjacent to the Parana fluvial system. In addition, our findings showed a positive association between breast OCC concentrations and freshwater fish consumption (Muñoz-de-Toro et al., 2006). Furthermore, PCBs and OCPs are known to increase in concentration with increasing trophic level (Gross et al., 2003).

Caiman latirostris (broad-snouted caiman) is a species of aquatic habits that is widely distributed across South America's wetlands. Caimans have ecological and physiological features that make them particularly vulnerable to exposure to OCCs. Due to their longevity, carnivorous diet and position at the top of the food net, caimans have the potential to accumulate high concentrations of OCCs. In Argentina, the *C. latirostris* population has

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been re-established due to successful ranching programs and protective legislations (CITES – www.cites.org; Larriera and Imhof, 2006).

The exposure to EDCs, however, could interfere with caiman population stability by affecting their reproductive performance and immune system (Beldoménico et al., 2007; Rey et al., 2009; Stoker et al., 2003, 2008). Caimans may be exposed to contaminants at embryonic stages by maternal transfer or exposure through the eggshell. After hatching, the remaining yolk sac, diet and aquatic environment are the main sources of exposure. Diet is an important source of exposure to persistent lipophilic compounds, which, are absorbed through the digestive tract after ingestion. Subsequently, the contaminants are transported to other tissues and deposited into lipid stores (Bargar et al., 1999). Reptile eggs have a high lipid content (Ashpole et al., 2004; Rauschenberger et al., 2007) and lipophilic compounds are depurated into the eggs by maternal transfer (Rauschenberger et al., 2007; Russell et al., 1999). Therefore, the egg burden reflects the biological availability of persistent lipophilic compounds in their maternal local environment (de Solla and Fernie, 2004). Clutch size and hatching success are measures of reproductive performance in oviparous species. Field and laboratory studies suggested that parental exposure to OCCs may contribute to low clutch viability (de Solla et al., 2008; Lundholm, 1997; Rauschenberger et al., 2004, 2007; Sepúlveda et al., 2006). In this background, we conducted a study to estimate the OCC residues present in the eggs of *C. latirostris* from north-eastern Argentina and to determine the relationship between OCC concentrations and measures of reproductive performance.

2. Materials and methods

2.1. Study sample

Fourteen *C. latirostris* clutches were harvested from wetlands on Paraná River tributaries in north-eastern Argentina. Sites with different degrees of anthropogenic intervention were selected for the study. Nests came from a wildlife refuge in a remote subtropical area of the Chaco Province (CH), from a region in Entre Rios Province (ER) where agriculture activities increased substantially in the last decades, and from an area in Santa Fe Province (SF) with low anthropogenic intervention (Fig. 1). To minimize OCC transfer from nest material or any post-laying environmental factors, eggs were harvested soon after they were laid and were transported to the lab (nesting was monitored by local residents). One or two eggs by clutch were opened in the field to stage embryos (Stoker et al., 2003). Only the nests with embryos in stages lower than 12 were transported to the lab for this study.

Two to four eggs from each clutch ($n = 46$ eggs) were selected randomly to determine the OCC egg burden (Rauschenberger et al., 2007; Wu et al., 2000a,b). The embryo was removed and the whole egg content was stored at −70 °C until OCC analysis, in glass vials pre-cleaned with acetone/hexane. The remaining eggs from each clutch were incubated in controlled conditions at 33 °C or 30 °C (temperatures at which 100% of males or females are produced, respectively) (Stoker et al., 2003). Upon hatching, neonates were identified using tags (style 1005-1, National Band and Tag Co., USA) and housed in controlled conditions.

2.2. Detection of OCC residues

Forty-six egg samples were analyzed for the presence of the following compounds: hexachlorobenzene (HCB), hexachlorocyclohexane isomers (α-HCH, β-HCH, and lindane), aldrin (ALD), oxychlordane (OCLD), α-chlordane (α-CLD), γ-chlordane (γ-CLD), heptachlor (HPT), heptachlor epoxide (HTX), dieldrin (DLD), endrin, mirex (MRX), methoxychlor (MTX), dichlorodiphenyldichloroethylene (p,p′-DDE and o,p′-DDE), tetrachlorodiphenylethane (p,p′-TDE, o,p′-TDE), dichlorodiphenyltrichloroethane (p,p′-DDT, o,p′-DDT), α-endosulfan (α-END), β-endosulfan (β-END), endosulfan sulfate (ENDSULF) and 14 polychlorinated biphenyl congeners (PCBs) (International Union of Pure and Applied Chemistry numbers 1, 5, 25, 28, 50, 52, 86, 101, 138, 153, 154, 180, 200 and 209).

Concentrations of OCCs were measured following the method described in Muñoz-de-Toro et al. (2006), with minor modifications. Briefly, for lipid extraction, defrosted egg homogenates were dehydrated with anhydrous sodium sulfate (Merck, Germany) and extracted twice with *n*-hexane/acetone (4:1). The clear organic phase was separated and evaporated to dryness. The percent of extractable lipids were gravimetrically determined in the residue (Mettler Toledo AB 204 scale). 100 mg of lipids were dissolved in 0.3 mL of *n*-hexane. Eight milliliters of acetonitrile (1% acetic acid) saturated in *n*-hexane were added, mixed by vortex and centrifuged. The organic phase was discarded. Then, 4 mL of *n*-hexane, 0.5 mL of a NaCl-saturated solution and 25 mL of water were added to the acetonitrile phase and mixed. The organic phase was kept apart. The extraction step was repeated. The aqueous phase was discarded, and the new organic phase was added to the former. The resulting organic phase was evaporated to dryness and redissolved in 1 mL of *n*-hexane. The extract was transferred into a Solid Phase Extraction (SPE) cartridge containing Alumina (0.5 g), Florisil (1 g) and anhydrous Na_2SO_4 for the cleanup. Solid-phase extraction cleanup was performed using Supelclean LC-Alumina-N SPE tubes (Supelco, Bellefonte, PA, USA). The SPE cartridge was eluted with 10 mL of *n*-hexane and 5 mL of *n*-hexane/diethyl ether (85:15). The eluent was concentrated to 0.5 mL, and a second cleanup was performed using Supelclean Envi-Florisil SPE tubes (Supelco). The solvent was reduced to dryness with a nitrogen stream and redissolved in 1 mL of iso-octane.

Gas Chromatography (GC) analysis was performed with GLC (Hewlett Packard Model 5890) 63Ni ECD. Pas 5 (25 m, 0.32 mm ID, film th. 0.52 mm) and Pas 1701 (25 m, 0.32 mm ID, film th. 0.25 mm) columns were used. Organochlorine pesticide standards Pestanal (Honeywell Riedel–de Haen Fine Chemicals, Seelze, Germany) and PCB congener standards (Ultra Scientific, North Kingstown, RI, USA) were used. All the solvents used were of pesticide-grade quality (Merck, Darmstadt, Germany). The presence and concentrations of OCC were confirmed using a GC–MS system (VG Trio 2; VG Analytical, Manchester, UK) in randomly-selected samples. Surrogate samples of chicken eggs (*Gallus gallus*) were prepared using known concentrations of OCPs and PCBs. The average percentage recoveries were ⩾80%. The Relative Standard Deviation (RSD) was lower than 20% for all residues. The Detection Limit (DL), based on three times the average SD of the blank replicates, was 10 ng g^{-1} lipids for β-HCH, CLD, p,p′-DDT and ENDSULF; 2 ng g^{-1} for BZ 1, 5, 25, 86, 101, 138, 154, 180, 200 and 209 and 5 ng g^{-1} for the remaining OCPs and PCB congeners assessed. The quantitation limit (QL) was calculated as 6 times the average SD of blank for compounds with DLs between 5 and 10 ng g^{-1} and 7.5 times for those with DLs of 2 ng g^{-1}.

2.3. Reproductive parameters

Parameters evaluated to assess caiman reproductive performance included clutch size (number of eggs by nest), hatching success (number of eggs that hatch successfully by nest) and hatchling survival at 10 d (% of survival at 10 d of age by nest). The total number of eggs in the nest was established in the field during the collection process. The hatchability of eggs was calculated as the number of hatchlings divided by the total number of eggs during the entire incubation period. To determine whether the OCC egg

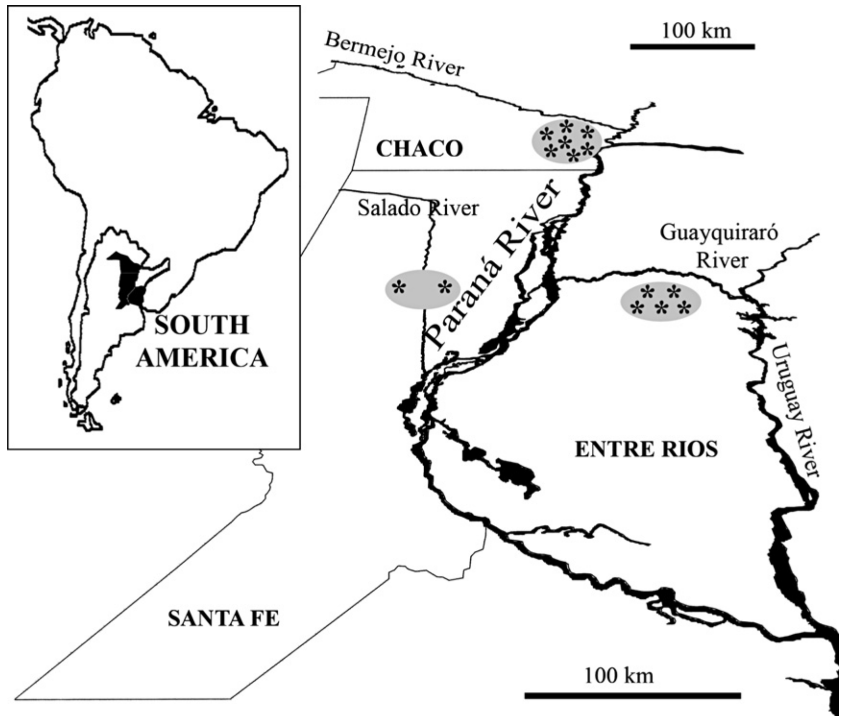

Fig. 1. Geographical distribution of the sampling area in the north-eastern Argentina. *Caiman latirostris* nests were harvested in wetlands on Paraná River tributaries. Sampling areas are represented by gray ellipses and nests by asterisks. 100 km scale bars differ because of perspective.

burden affects temperature sex determination (TSD), the gonad sex was assessed in all the hatchlings of the study which were assigned to other experiments (Stoker et al., 2003).

2.4. Statistical analysis

The distributions of OCC data were not normal in their original form, thus they were log transformed (ln) for statistical analyses. All clutch values were expressed as the mean ± SEM. To determine statistical differences among clutches, OCC concentrations and lipid contents, were analyzed with *t* test. Clutches from Santa Fe were not included in the analyses because of the small sample size. For statistical analysis, OCCs concentrations below detection limit (BDL) were replaced by values representing the half of the detection limit for that compound, while the values below quantitation limit (BQL) were considered to be half of the assay quantitation limit. Correlations between OCC concentrations and reproductive parameters were performed using Pearson analysis. A value of $p < 0.05$ was accepted as significant.

3. Results and discussion

3.1. OCC residues in C. latirostris eggs

Detectable concentrations of OCCs were found in all *C. latirostris* clutches ($n = 14$). The contaminant burden varied among the sites, both in the absolute values and the relative proportions. The geographical distribution of the nests and the concentrations of OCCs ($\sum$PCBs and $\sum$OCPs) are presented in Fig. 1 and Table 1, respectively. No differences in egg lipid content were observed among nests (21.6% ± 0.6% with a range from 17.9% to 25.3%, $p = 0.14$). Recently, Guirlet et al. (2010) reported that OCC concentrations and the percentage of lipid in leatherback turtle eggs declined in successive clutches in the same season, highlighting a process of off-loading from females to their eggs and a decreasing investment of lipid from females into their clutches. *C. latirostris* lay only one clutch each season or every two years. Because of their position in the upper trophic levels, prey preferences, lack of migratory habits and longevity, they have the potential to accumulate OCCs. Thus, it is a species that provide a representative scenario of exposure.

We did not find differences in egg lipid content, either within a clutch or between clutches, from different mothers. Moreover, as described for wild alligators (Rauschenberger et al., 2004, 2007), variations in egg lipid content were not associated with OCC concentrations ($p = 0.74$, Pearson $r = 0.09$, $r^2 = 0.01$).

OCC residues were found in 100% of clutches. Residues most frequently detected at measurable concentrations included: OCLD in 92.9% of the clutches; p,p′-DDE in 85.7%; HCB and HPT in 35.7%; o,p′-TDE, MTX and HTX in 21.4%; o,p′-DDT and ENDSULF in 14.3%; and o,p′-DDE in 7.0% of clutches. Concentrations of HCH isomers, ALD, CLD isomers, DLD, endrin, MRX, p,p′-TDE, p,p′-DDT and END isomers, if present, were below the detection limits in all nests sampled. PCBs were present in 13 of the 14 clutches. Of the 14 targeted PCBs congeners, only BZ 138 (78.6%), BZ 28 (57.1%), BZ 153 (42.9%), and 180 (28.6%) were detected.

It is not surprising that DDE and oxychlordane were the compounds more frequently found, because they are the most persistent metabolites from DDT and chlordane, respectively. Chlordane was used extensively on agricultural crops and livestock worldwide. As it was already mentioned, in Argentina, the use of DDT and chlordane on livestock was banned a long time ago but they remained in use as household insecticides until 1998 (Ley Nacional N° 17.751/68; ANMAT 7292/98). Restrictions and recommendations on uses and disposal of PCBs were considered later (Ley Nacional N° 24051/91; Ley Nacional 25.670/02) but replacement of PCB transformers is still in process (Decreto N° 657/07 Entre Ríos; Resolución N° 043/03 Santa Fe).

OCP residues have been detected in eggs and/or somatic tissues of several crocodilian species, such as the American alligator

Table 1
Reproductive parameters and concentrations of organchlorine compounds in *C. latirostris* nests from Santa Fe (SF), Entre Ríos (ER) and Chaco (CH) provinces, Argentina.

Nest identification	Clutch size	Hatching success	Hatchling survival	$\sum$PCBs (ng g^{-1} lipid)	$\sum$OCPs (ng g^{-1} lipid)	Lipid Content
SF1	39	91.9%	91.9%	11.6	61.9	19.5%
SF2	35	85.2%	85.2%	BDL	63.0	25.3%
Mean ± SEM	37 ± 2	88.5 ± 3.3	88.5 ± 3.3	7.1 ± 4.6	62.5 ± 0.6	22.4 ± 1.6
Range	35–39	85.2–91.9	85.2–91.9	BDL-11.6	61.9–63.0	19.5–25.3
ER1	30	92.6%	92.6%	35.5	159.5	22.0%
ER2	34	96.6%	93.1%	7.7	37.2	19.9%
ER3	33	82.7%	79.3%	BQL	28.9	23.3%
ER4	28	76%	44%	32.0	75.8	22.4%
ER5	18	0%	0%	44.5	189.0	24.3%
Mean ± SEM	28.6 ± 2.9	69.6 ± 17.8	61.8 ± 17.9	24.4 ± 8.2	98.1 ± 32.4	22.4 ± 0.7
Range	18–34	0–96.6	0–93.1	BQL-44.5	28.9–189.0	19.9–24.3
CH1	37	5.8%	0.7%	BQL	33.5	21.9%
CH2	33	100%	100%	136.6	166.4	19.5%
CH3	39	94.6%	94.6%	BQL	36.3	22.3%
CH4	36	91.2%	91.2%	21.0	46.0	19.9%
CH5	31	82.1%	78.6%	13.5	29.3	17.9%
CH6	30	82.1%	82.1%	13.0	31.0	20.7%
CH7	40	86.5%	86.5%	11.5	39.3	23.1%
Mean ± SEM	35.1 ± 1.5	78.7 ± 10.6	76.2 ± 12.9	30.1 ± 17.8	54.5 ± 18.8	21.6 ± 0.6
Range	30–40	5.8–100	0.7–100	BQL-136.6	29.3–166.4	17.9–23.1

PCBs: polychlorinated biphenyls. OCPs: organochlorine pesticides. BDL: below detection limit. BQL: below quantitation limit.

(*Alligator mississippiensis*) (Cobb et al., 1997, 2002; Heinz et al., 1991), the American crocodile (*Crocodylus acutus*) (Hall et al., 1979; Wu et al., 2000a), Morelet's crocodile (*C. moreletti*) (Wu et al., 2000b, 2006) and the Nile crocodile (*C. niloticus*) (Skaare et al., 1991). In agreement with the above-mentioned reports, DDT family members were the most frequent OCCs found in *C. latirostris* eggs.

Nests harvested in Entre Ríos (ER5, 1 and 4) showed the higher concentrations of $\sum$OCPs (Fig. 2). Additionally, ENDSULF was present only in ER1 and ER5 nests (18.5 and 46.0 ng g^{-1} lipid, respectively). This fact could be explained by the expansion of agriculture practices in Entre Ríos, where an overlapping of croplands with the natural caiman habitat was observed (INTA – www.inta.gov.ar).

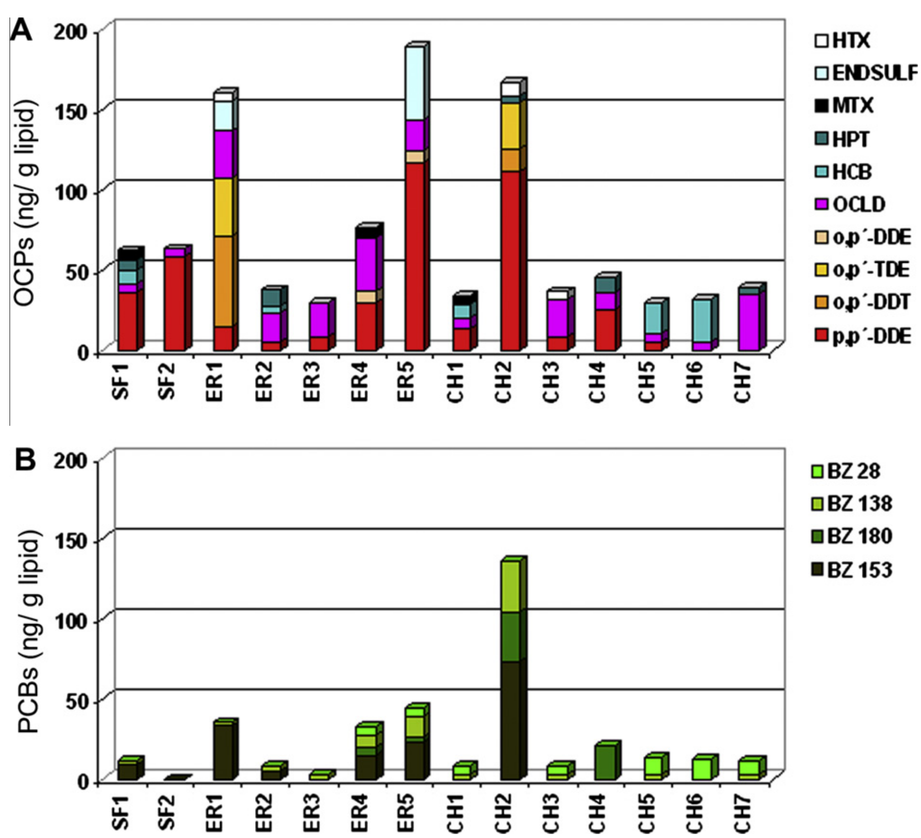

Fig. 2. (A) Proportion of organochlorine pesticides (OCPs) and (B) polychlorinated biphenyls (PCBs) (ng g^{-1} lipid) found in *C. latirostris* clutches in Santa Fe (SF), Entre Ríos (ER) and Chaco (CH) provinces, Argentina. Different colors represent mean clutch concentration for each compound. hexachlorobenzene (HCB), oxychlordane (OCLD), heptachlor (HPT), heptachlor epoxide (HTX), methoxychlor (MTX), dichlorodiphenyldichloroethylene (p,p′-DDE and o,p′-DDE), tetrachlorodiphenylethane (o,p′-TDE), dichlorodiphenyltrichloroethane (o,p′-DDT), endosulfan sulfate (ENDSULF). PCBs congeners (International Union of Pure and Applied Chemistry numbers BZ 28, BZ 138, BZ 153, BZ 180).

Despite of the diversity found in the OCPs, the principal contribution to pesticide burden in the most contaminated nests was from the sum of components from the DDT family (p,p′-DDE + o,p′-TDE + o,p′-DDT + o,p′-DDE) (Fig. 2). According to Yogui et al. (2003) and Cipro et al. (2010), p,p′-DDE/∑DDTs ratios above 0.6 indicate old DDT contamination. As might be expected, the ratio was higher than 0.6 in all but one nest. ER1 exhibited a p,p′-DDE/∑DDTs = 0.16, showing a recent exposure to the banned DDT.

As expected, all nests collected in the Wildlife Refuge (Chaco), except for one (CH2), exhibited a low OCC burden. CH2 significantly differs from its neighbor's burden. Additionally, CH2 was the nest with the greatest diversity of OCCs and was the only nest in which HTX was measurable (HTX: 12.3 ng g^{-1} lipid, Fig. 2). Regarding PCBs, the CH2 nest showed the highest concentrations of ∑PCBs, exceeding two or even three times the value of the neighbor clutches. This observation was surprising because CH2 was harvested from the site with the lowest anthropogenic intervention and no PCB sources were identified upstream. Since the nests were harvested immediately after egg laying, post-laying environmental factors could not affect egg OCC burden. An aged mother or infrequent mother migration could account for the observed differences (Ferguson, 1985; Rauschenberger et al., 2007). There is evidence for long-range transport of PCBs to regions where they have never been used or produced (Scheringer, 2009); as a consequence, these pollutants are now distributed worldwide and are even found in remote locations, such as the Polar Regions (Braune et al., 2007; Cipro et al., 2010; Schiavone et al., 2009). BZ-138 and BZ-153, both of which are hexachloro-biphenyl congeners, were frequently found in *C. latirostris* eggs. The same pattern was observed in penguin eggs from the Antarctica (Schiavone et al., 2009), a region no directly exposed to PCBs. Although, no comparison could be done between the three provinces because of the small sample size from Santa Fe, significant differences were found between Chaco (CH2 nest was not included in the analysis) and Entre Rios when ∑OCCs were compared. These differences were mainly due to differences in ∑OCPs. Consistent with other studies (Sepúlveda et al., 2006; Wu et al., 2006), we found that concentrations and class patterns of OCCs were similar between eggs from the same nest and differed among nests collected in different regions (Fig. 2). To establish the number of clutches needed to characterize a site, is beyond the scope of the present study, however, as all but one of the neighbor clutches exhibited similar OCC patterns. Caiman eggs could be sufficiently sensitive to biomonitor local contamination.

The concentrations of OCCs reported here agree with those observed in Morelet's crocodile eggs from Belize (Wu et al., 2000a,b, 2006) and in the alligator eggs from reference sites in USA (Cobb et al., 2002; Rauschenberger et al., 2004, 2007). OCC residues were detected in sediment and different trophic levels, including humans from coastal environments in Argentina (González Sagrario et al., 2002; Lajmanovich et al., 2002; Muñoz-de-Toro et al., 2006; Cid et al., 2007; Arias et al., 2010). We have reported not only a high frequency and elevated concentration of OCC residues in human breast adipose tissue but also a positive association between OCC concentrations and freshwater fish consumption (Muñoz-de-Toro et al., 2006).

3.2. Reproductive parameters

The OCC egg burden did not affect sex determination at the studied temperatures, thus all the eggs incubated at 30 °C resulted in female hatchlings and those incubated at 33 °C resulted in male hatchlings. In species with temperature-dependent sex determination, such as turtles and alligators, OCCs were able to act as xenoestrogens, overriding the effect of temperature, thus, phenotypical females were born at male producing temperatures (Bergeron et al., 1994; Matter et al., 1998). In *C. latirostris*, both 17β-estradiol (E2) and the xenoestrogen bisphenol A induced sex reversion (Stoker et al., 2003); however, neither END nor atrazine were able to induce sex reversion (Beldoménico et al., 2007; Rey et al., 2009). In American alligators, E2- and p,p'-DDE-induced sex reversion have been found (Milnes et al., 2005). In the present study, we used only incubation temperatures, which are known to produce all female or all male caiman hatchlings (Stoker et al., 2003). Developing caimans, however, could be more sensitive to OCCs at intermediate incubation temperatures that produce both sexes (Milnes et al., 2005). Studies concerning the ability of environmental contaminants to alter sex determination in TSD species should take into account not only pollutant concentration but also incubation temperature. In addition, as evidenced by nest OCC class pattern, caimans are exposed to complex pollutant mixtures. In turtles, it has been demonstrated that temperature sex determination was affected by a synergistic effect of aroclor 1242, chlordane, cis- and trans-Nonachlor and p,p′-DDE (Willingham and Crews, 1999).

A significant negative correlation was found between *C. latirostris* clutch size and mean ∑OCC concentrations ($p = 0.02$, Pearson $r = -0.53$, $r^2 = 0.28$), ∑OCPs clearly contributed to this association ($p = 0.04$, Pearson $r = -0.54$, $r^2 = 0.30$) (Fig. 3). No correlations were found between OCCs and hatching success or hatchling survival ($p > 0.05$).

For alligators, it has been demonstrated that OCP concentrations in maternal tissues and eggs were strongly correlated, allowing the mean egg burden to be used as a predictor of the OCP burden in maternal tissues (Rauschenberger et al., 2004). In the present study, a significant negative correlation was found between the clutch size and the mean sum of OCC concentrations in *C. latirostris* eggs, suggesting that the reproductive performance of the mother is affected by exposure to contaminants. It is well known that OCCs behave as endocrine-disrupting compounds. A plethora of evidence suggests that exposure to EDCs affects reproduction in several species (Colborn and Clements, 1992; Guillette and Edwards, 2008; IPCS, 2002). For alligators and turtles it has been shown that OCCs maternally transferred to the developing egg are associated with reduced clutch success and increased embryonic mortality (de Solla et al., 2008; Rauschenberger et al., 2007). Previously, we have demonstrated that *in ovo* exposure to EDCs during caiman gonadal organogenesis alters follicular dynamics, steroid concentrations and increases multioocyte follicle incidence in females (Stoker et al., 2003, 2008) and testicular histoarchitecture and testosterone concentrations in males (Rey et al., 2009). Since OCCs are EDCs, we hypothesize that similar gonadal disruptions could be caused by OCC exposure in polluted habitats, leading to impaired caiman fertility that could be evidenced at clutch size level. Although, other factors such as mother age or size

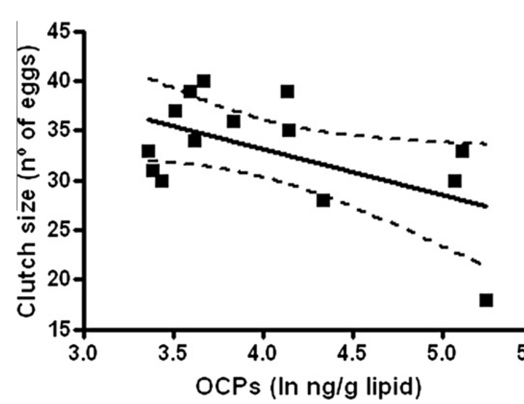

Fig. 3. Correlation between organochlorine pesticides (OCPs) burden in *C. latirostris* eggs and clutch size (Pearson $r = -0.54$, $p = 0.04$, $r^2 = 0.30$).

need to be considered to explain the observed relationship between clutch size and OCCs (Ferguson, 1985; Rauschenberger et al., 2007). Sampling a larger number of nests for OCC quantification and/or recording maternal size should help to better characterize each site.

4. Conclusions

The presence of OCPs and PCBs in caiman eggs from the Paraná River tributary regions reveals their past and recent use. Egg burden can be used as predictor of OCC burden in oviparous maternal tissues. Caimans are long-lived and non-migratory with a small home range; therefore, the maternal burden of OCCs reflects the environmental status throughout their home range. Consequently, caiman eggs could be sufficiently sensitive to biomonitor local contamination as reported for turtles and alligators (de Solla et al., 2007; Rauschenberger et al., 2007). Biomonitoring is a valuable tool for identifying exposures to chemicals that pose potential harm to the ecosystem health.

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