Title: Maternal transfer of organic pollutants in sea turtles undergoing extensive oceanic migrations

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Note: *Due to the COVID-19 Pandemic and travel restrictions between Japan and The Netherlands, I (Cynthia C. Muñoz) could not travel to Ehime University to conduct laboratory work. As a solution, the originally submitted plan for Joint Usage/Research Center, LaMer in Fiscal Year 2021 was adapted in consultation with Prof. Kunisue. This report reflects the activities conducted under the revised plan.*

1. Aim:

The project aimed to quantify the exposure of early life stages of loggerhead sea turtles nesting in Kochi prefecture, Japan, to persistent organic pollutants, (POPs). Specifically, we intended to answer the following research questions:

1. Do individual adult females differ in their diet and habitat preferences, thereby potentially affecting POP levels observed in eggs from different mothers?
2. Do POP levels vary among different internal tissues?
3. Which pollutants are maternally transferred from mother towards egg albumen and yolk, and how does the chemical structure of individual compounds relate to the transfer rate?

2. Procedure:

2.1. Research question 1, I conducted an analysis of ontogenetic shifts in diet and habitat resource use of loggerhead sea turtles occurring in coastal waters near Kochi Prefecture. Therefore, I applied stable isotope analysis to time series consisting of consecutive layers of turtle carapace scute, an inert tissue in which chemical composition is preserved over time.

2.2. Research question 2, I conducted a systematic literature search for publications reporting POPs in multiple sea turtle tissues. A subsequent meta-analysis of data extracted from these publications, then, quantified the tissue distribution of POPs across all sea turtle species.

2.3 Research question 3, in collaboration with the laboratory team of Prof. Kunisue, I analyzed samples of loggerhead sea turtle eggs (4 albumen and 4 yolk samples), collected at Iburi, Kochi prefecture. Samples were stored at -20°C until transfer into the es-Bank, where they were stored until analysis. These samples, complement analyses of the mother´s blood (1 sample) and eggs (4 albumen and 4 yolk samples) were conducted during the 2020 'Joint usage of equipment' collaboration between Radboud University and Ehime University[1]. All samples were collected

[1] http://lamer-cmes.jp/performance-en/2766

under the appropriate permit and ethics approvals in collaboration with Prof. Saito (Usa Marine Biological Institute, Kochi University).

2.4. Chemical analysis: Chemical analysis targeted PCBs and OCPs in the blood plasma of 1 female loggerhead turtle, and PCBs, OCPs, and PBDEs in 8 of her eggs (yolk and albumen, Table 1). The analysis of eggs followed the protocol reported by Coelho et al. (2016). Yolk (approximately 20 g) and albumen (6–10 g) samples were freeze-dried and then extracted with hexane/acetone (1:1 v/v) on a SE-100 High-Speed Solvent Extractor (Mitsubishi Chemical Analytech, Japan). The extract was cleaned in gel permeation chromatography for lipid removal (GPC) and activated silica-gel, and then concentrated. PCBs and PBDEs were analysed using a gas chromatograph (GC: Agilent 7980 A) coupled with a quadrupole mass spectrometer (qMS: Agilent 5975C), and OCPs were analysed using a GC (Agilent 7890 A) coupled with a triple quadrupole mass spectrometer (MS/MS: Agilent 7000). The blood plasma sample (2.8 g) was liquid-liquid extracted using hexane/methyl tert-butyl ether (1:1 v/v) according to the protocol by Eguchi et al. (2015), subjected to the same clean-up method described above, and then PCBs and OCPs were determined using a GC (Agilent 6890A) coupled with a magnetic sector high-resolution mass spectrometer (HRMS: JEOL JMS-800D) using instrumental settings reported by Goto et al. (2020).

2.5. Lipid content determination: To allow comparison with other studies, and to assess uptake relative to lipid-based partitioning capacities of different tissues, we analyzed the lipid content (i.e. total extractable solids) of the blood, yolk, and albumen samples gravimetrically using aliquots (10%–20%) of the respective sample extracts for chemical analysis.

3. Results and discussion

3.1. Research question 1: ontogenetic shifts in diet and habitat

Our research identified that loggerhead sea turtles undergo ontogenetic shifts from oceanic subadults with a pelagic diet toward neritic adults with a benthic diet. Within the population occurring near Kochi Prefecture, those ontogenetic shifts have occurred at different times, hence suggesting a mixed-age population structure. Moreover, during the adult stage, individuals have high individual specialization in their resource use. Combined, these results suggest that adults might be exposed to higher pollution burdens than subadults, due to their higher trophic level and proximity to the coast. Additionally, different individuals likely are exposed to different levels, depending on their individual-based resource use patterns. *(see publication 1 for details)*

3.2. Research question 2: internal pollution distribution

Our meta-analysis identified that the concentrations of POPs in several tissues can be predicted by the partitioning capacities determined by the lipid content of the tissues. However, egg tissues deviate from this expected equilibrium. This has implications for the toxicity of pollutants in eggs, and subsequently, to embryos. To explain deviations from equilibrium partitioning, accurate measurements of lipid contents of different tissues are needed. Nonetheless, our literature review identified that lipid contents for many sea turtle tissues are poorly known. Therefore, we developed a lipid content database to support research in this area, which includes a limited number of records for lipid contents in egg compartments (Table 1), relevant for research question 3. *(see publication 2 for details)*

3.3. Research question 3: Maternal transfer of POPs in loggerhead turtles

3.3.1. Lipid contents

Lipid contents decreased in the order of yolk > blood plasma > albumen (Table 1). These results complement the limited data available on lipid contents in sea turtles with information for loggerhead turtles and details on yolk and albumen as individual compartments. Our results fall within the range of previously reported values. Lipid contents in egg albumen were low (Table 1). Measurement of lipid contents at such low quantities is challenging, which is reflected in the variability across the 8 samples. Nonetheless, the median value corresponds with the known lipid content of 0.020 % in the albumen of snakes. Consequently, we lipid-normalized all concentrations in egg albumen samples against the median lipid content of 0.017 %. Lipid contents in egg yolk were much higher than in albumen (Table 1), as was expected given the role of egg yolk in providing energy reserves to developing embryos. Lipid contents varied little between yolks, therefore, we lipid normalized measured POP concentrations against the sample-specific yolk lipid content.

Table 1 Known data on lipid percentages (%) reported as median; mean; and range (min. - max.) in compartments of female blood and her eggs for 3 species of sea turtle (Sp.: *C.c.*: loggerhead, *C.m.*; green, *D.c.*: leatherback turtle).

Study	Sp.	Whole blood	Plasma*	Whole egg	Egg yolk	Egg albumen
This report	*C.c*	-	0.62	-	8.75; 8.34; 6.40 - 9.70	0.017; 0.151; 0.008 - 0.560
van de Merwe et al., 2010	*C.m*	Na; 1.5 (0.1SE); 1.1-2.1	-	Na; 8.9; 6.8 - 10.9	-	-
Guirlet et al., 2010	*D.c*	Na	-	Na; 12.9; 3.8 - 25.5	-	-
Stewart et al., 2010	*D.c*	0.48; 0.56; 0.22-1.00	-	5.02; 5.13; 4.67 - 5.69	-	-

*1 Blood plasma from 1 turtle

3.3.2. Concentrations of OCPs, PCBs and PBDEs

Individual POP compounds reached the highest concentration in wet mass in yolk followed by the plasma and the albumen. By contrast, median lipid normalized concentrations of POP compounds recorded in individual albumen samples were one to two orders of magnitude greater than in yolk and plasma samples (Fig. 1). The results for OCPs only show measurements for eggs T1-T4, with the measurement for eggs T5-T8 currently under analysis (OCPs will therefore not be further discussed in this report). PCBs and PBDEs were analysed across the 8 eggs. PBDEs were only reported in albumen and yolk, with only PBDE-28, -100, -153, -154, and -155 measured at detectable levels. Meanwhile, nearly all major tri- to deca-chlorinated PCB congeners were detected in multiple egg yolk samples.

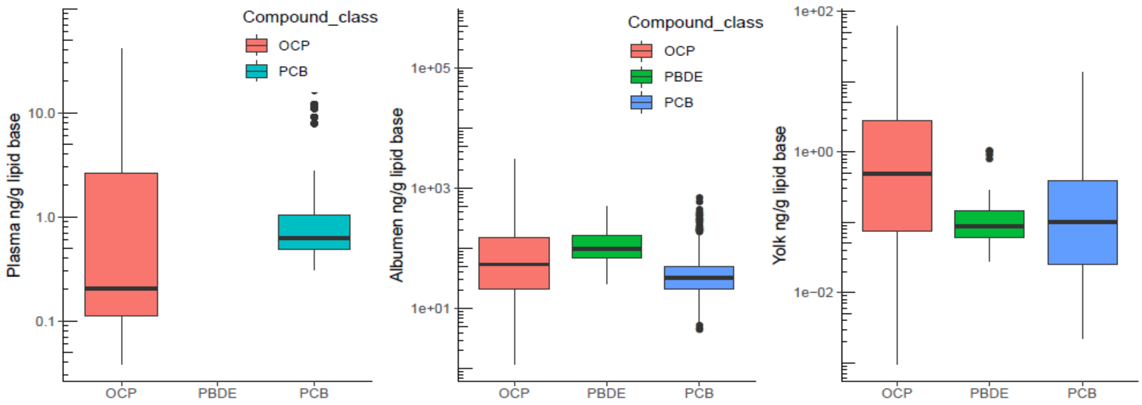

Fig. 1 Distribution of lipid normalized concentrations of PCB, OCP and PBDE compounds recorded in individual samples of blood plasma, albumen and yolk tissue. Measurements <LOD substituted by ½×LOD. Boxplots: median, 25−75th percentiles, 1.5×interquartile range, outliers as individual points.

3.3.3. Tissue distribution of POPs

Lipid normalized concentrations PCB congeners were higher in plasma than yolk (Fig. 2), suggesting the presence of a barrier or process that restricts the maternal transfer of PCB compounds into egg yolk, as I previously pointed out in a systematic review of maternal transfer in sea turtles (Muñoz and Vermeiren, 2020[2]). Individual measurements of PCBs varied between yolk samples (notice the wide spread in yolk concentrations for the same plasma concentration). Nevertheless, the trend in partitioning across the PCB congeners detected in each yolk sample showed a comparable pattern (coloured lines in Fig. 2 are roughly parallel).

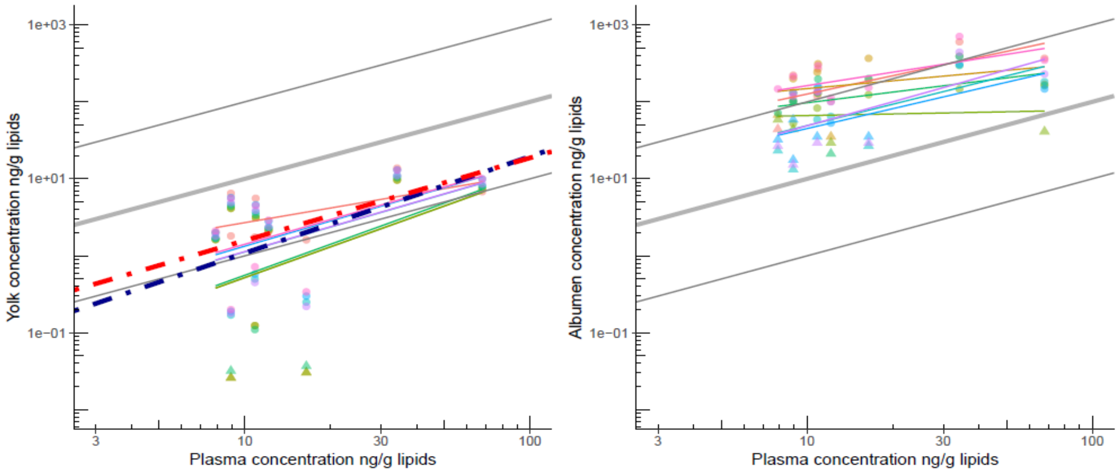

Fig. 2 Lipid-normalized concentrations of individual, detected PCBs in plasma vs. yolk (left) or albumen (right) in 1 female loggerhead turtle and 8 of her eggs. Grey line: equilibrium partitioning, Red, blue dashed lines: models for green and leatherback turtles derived from a scientific literature meta-analysis[2].

A potential hypothesis for the increased variability at lower concentrations of PCBs within yolk is the difficulty in precisely quantifying such low concentrations. Alternatively, maternal transfer rates could vary among eggs from the same female. However, turtles produce all eggs within one clutch at the same time, which is known to reduce variability in POP levels among eggs. The pattern in maternal transfer observed here for loggerhead turtles overlaps with previous data from

[2] Muñoz and Vermeiren, 2020, Environmental Toxicology & Chemistry 39:9-29 https://doi.org/10.1002/etc.4585

green and leatherback turtles that I extracted during a systematic literature review (Muñoz and Vermeiren, 2020[2], Fig. 2). These results suggest a comparable pattern (and possibly process) of maternal transfer across sea turtles, which is different from the expected equilibrium partitioning.

3.3.4. Predicting maternal transfer rates of POPs

PCBs with low lipophilicity (approximated as log K_{ow}) have lower maternal transfer rates than those with higher lipophilicity (Fig. 3). Additionally, the maternal transfer ratio levels off and decreases again for compounds with log K_{ow} > 7.5. Previous studies on maternal transfer in sea turtles only contained data on PCBs with log K_{ow} of close to 7 and higher. Potentially, those studies missed important patterns and processes operating for compounds with log K_{ow} of 6 and lower, which were measured in the current collaboration. Planned analyses of OCPs will provide a valuable contribution to these data as OCP compounds also have lower log K_{ow}.

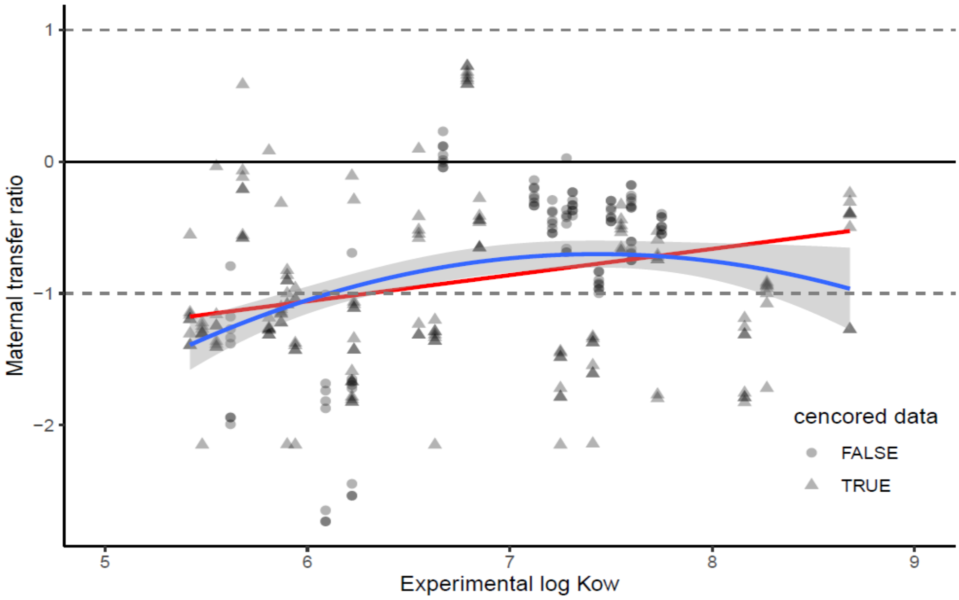

Fig. 3 Maternal transfer rates ($\log_{10}$ of lipid normalized concentrations in yolk/plasma) for PCB congeners in individual samples, expressed against the log K_{ow} of these PCBs. Triangles: data < LOD in plasma. Red line: linear regression, Blue line: quadratic regression with standard error.

4. Publication/conference presentation

1. Muñoz C.C, Saito, T., Vermeiren, P. (2021) Marine Ecology Progress Series Vol 671:175-190 https://doi.org/10.3354/meps13767

2. Muñoz C.C, Hendriks, A.J., Ragas, A.M.J, Vermeiren, P. (2021) Environmental Science & Technology 55:10012-10024. https://doi.org/10.1021/acs.est.1c02845 -> *also presented at SETAC Europe 31st Annual Meeting*

5. Perspective in future

① Our results indicated that maternal transfer rates depend on chemical properties (log K_{ow}). We will include additional chemical properties into further, advanced statistical analyses.

② We plan to analyze POPs in eggs, embryos and hatchlings of green sea turtles from Okinawa. Such analysis of POP transfer into sea turtle embryos and hatchlings is currently lacking, but highly relevant for risk assessment of early life stages. These analyses were planned for the 2021 fiscal year LAMER project, but could not be conducted due to the Covid-19 Pandemic.