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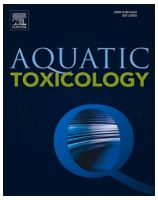

The Irrawaddy dolphin, *Orcaella brevirostris* from the Mekong river Cambodia: Preliminary health and toxicological investigations

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ABSTRACT

The subpopulation of the Irrawaddy dolphin (*Orcaella brevirostris*) living in the Mekong River, Cambodia, is considered to be critically endangered. The aim of the investigation was to gain information about the genetic variation, health status and exposure to toxic compounds of these dolphins. Tissue samples from 27 Irrawaddy river dolphins found dead along the Mekong River between 2004 and 2009 were analysed with regards to genetics, pathology and ecotoxicology. Genetic maternal lineage detection, based on polymorphisms of the mitochondrial d-loop sequences, was performed. Data indicate a genetic separation of the Mekong dolphins from both the coastal population and the Mahakam dolphins. Pathological investigations revealed acute moderate multifocal suppurative bronchopneumonia, moderate periportal hepatic lipidosis, moderate diffuse hepatic atrophy and acute severe diffuse suppurative leptomeningitis. Residue levels of organochlorines and polybrominated diphenyl ethers in Irrawaddy dolphins from the Mekong River were lower than the concentrations reported for other cetaceans in the coastal and riverine waters of Asia, except for Dichlorodiphenyltrichloroethane. A high percentage of organic mercury compared to the immuno-toxic methylmercury was observed. Due to numerous confounding factors, it is not possible to relate levels of pollutants to observed morphological lesions. However, it is likely that chemical contaminants do adversely impact on the health of the Irrawaddy dolphins at present, and have also affected previous generations.

The subpopulation of the Irrawaddy dolphin (*Orcaella brevirostris*) living in the Mekong River is considered to be critically endangered, based on the criteria of the International Union for Conservation of Nature (IUCN) (Smith & Beasley, 2004; Reeves et al., 2008). The population size has been estimated to be 80 individuals in 2015 in the Mekong River (Phan et al., 2015; Limsong et al., 2017).

Irrawaddy dolphins of the Mekong River face numerous anthropogenic threats, including being caught in fishing nets, fragmentation of their habitat by dam construction, and exposure to the environmental pollution (Reeves et al., 2008; Smith, 2009; Phan et al., 2015). The dolphin's range in the river is now restricted to an area spanning from the main stem from Khone Falls, near the Laos-Cambodia border, to about 200 km downstream in Cambodia (Brownell et al., 2017). The net effect is a reduction in the ecological niches available for freshwater biodiversity, including large mobile predators such as the Mekong's dolphin population (Brownell et al., 2017).

Sixty-three percent of the 94 Mekong dolphin deaths recorded since 2001 involved calves, which puts the remaining population under threat of extinction (Ryan et al., 2011). Different investigations have concluded that the deaths of young dolphins may be the result of several factors (Dove, 2009), including: (1) genetic inbreeding, (2) infectious diseases caused by *Aeromonas hydrophila* and other opportunistic bacteria; (3) chemical pollution. But reports investigating pathological and pollutant findings are rare in Irrawaddy dolphins.

We focused our investigation on additional information about the health status, exposure to toxic compounds and genetic variation. Tissue

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samples of 27 Irrawaddy river dolphins which were found dead along the Mekong River between 2004 and 2009 were analysed in several laboratories in Europe and Cambodia. Pathological investigations included a necropsy, as described previously (Kuiken & Garcia Hartmann, 1993; Siebert et al., 2001). Depending on the state of preservation (up to condition score 3: moderate decomposition), samples for histology and microbiological analyses were collected. Organ systems were examined macroscopically, and samples of lesions and different organ systems were fixed routinely in 10% formalin and embedded in paraffin wax. For histological evaluation, 5 µm sections were cut and stained with hematoxylin-eosin.

Microbiological analyses were conducted by the Institut Pasteur du Cambodge, Laboratoire de Biologie Medicale, Phnom Penh, Cambodia. Samples were taken under abacterial conditions from 14 dolphins, including the abdominal cavity, amniotic fluid, aorta, blood, blowhole, blubber, faeces, muscle, kidney, liver, lung, spleen, umibilicus, bone, urine, and uterus. The samples were frozen at –25 °C until further processing. For cultivation, samples were decontaminated superficially by heat after unfreezing, and examined according to standard procedures (Siebert et al., 2009).

Genetic maternal lineage detection, based on polymorphisms of the mitochondrial d-loop sequences, was performed. This approach has a long-standing tradition in investigations of the molecular phylogenetics of extant cetaceans (e.g., Arnason et al. 1996). However, a detailed analysis of genetic structure is still missing for Irrawaddy dolphins from the Mekong River.

The blubber from 10 different dolphins (up to condition score 3: moderate decomposition) was analysed for various pollutants by Galab Laboratories, Germany (accredited laboratory according to DIN/EN/ISO/17025). These included brominated flame retardants (BFR, 20 compounds), polychlorinated biphenyls (PCBs, 6 congeners), organochlorine pesticides (OCP, 37 congeners), dioxins and furans (PCDD/PCDF), organotin compounds (OTC), polyfluoroalkyl substances (PFOS/PFOA), toxaphene, methyl parathion, arsenic (As), lead (Pb), cadmium (Cd), Iron (Fe), copper (Cu), mercury (Hg), methylmercury (MeHg), selenium (Se) and zinc (Zn).

∑DDT (Dichlordiphenyltrichlorethan) were the predominant compounds found in Irrawaddy dolphin tissues (Table 1). A ∑DDT concentration greater than 180,000 ng·g^{-1} lipid weight was found in the blubber of 1 male individual dolphin. The lowest DDT concentrations were detected in a new-born individual. Amongst DDT, *p,p'*-DDE was the predominant metabolic compound in the tissues of Irrawaddy dolphins from the Mekong river and suggest exposure to aged residues from the environment, and greater metabolic transformation by the organism (Kannan et al., 2005).

PCBs were the second most abundant compounds in Irrawaddy dolphins (Table 1). Concentrations of PCBs in Irrawaddy dolphins were 1 to 2 orders of magnitude lower than the threshold PCB concentration of 8700 ng·g^{-1} lipid weight reported to generate physiologic effects in aquatic mammals (Kannan et al., 2000) and the threshold of 17 µg·g^{-1} (or 17000 ng·g^{-1} lipid weight) where PCB exposure will be biologically significant (Jepson et al., 2005).

Other pesticides, namely chlordane (cis and trans), nonachlor, hexachlorobenzene, oxychlordan, methoxychlor, mirex, camphechlor, aldrin, dieldrin, dienchlor, endisufan (alpha and beta) endosulfan sulfate, endrin, hexachlorocyclohexane (alpha, beta, delta, epsilon) and octachlorstyrene, were also analysed in selected individuals. Most of the concentrations were below the detection limit, except for trans nonachlor > hexachlorbenzene > methoxychlor > oxychlordan > mirex.

Dioxins and furans were analysed in blubber, with the most abundant congener determined to be Octachlorodibenzodioxin with values ranging from 1.5 to 56 pg·g^{-1} lipid weight. The results of this study indicate low values, with an average of only 5 out of 17 congeners detected.

Amongst brominated compounds analysed, bromodiphenyl ether BDE 47 was the most abundant congener in analysed samples (Table 1) and are 1 to 3 orders of magnitude lower than concentrations reported for harbour porpoises from the North Sea (Weijs et al., 2009), but similar to that observed in the blubber of Irrawaddy dolphins from India (Kannan et al., 2005).

Tributyltin (TBT) and the products of its degradation concentrations in Irrawaddy dolphins from the Mekong river are higher than the typically-described values from 1 to 10 µg·g^{-1} wet weight in livers of marine mammals. Accumulation of butyltin residues in Irrawaddy dolphins suggests the presence of sources in the Mekong River.

Perfluorinated compounds (PFCs), including perfluorinated acids, are today widely-distributed in the marine environment (Yamashita et al., 2005) and PFOS was the predominant compound identified in the liver of Ganges river dolphins (Yeung et al., 2009). The low PFC levels analysed in Irrawaddy dolphins from the Mekong River reflect low PFC pollution in the waterway.

Essential elements such as Fe, Cu, and Zn, analysed in the liver of Irrawaddy dolphins (Table 2), were of the same order of magnitude as compared to other marine mammal specie (Law et al., 1991; Bennet et al., 2001; Das et al., 2003). Cd, As and Pb are low in the tissues, except for 3 dolphins displaying elevated level of Cd in the kidney (9, 14 and 19 mg·kg^{-1}).

Total mercury (T-Hg) concentration in the livers of Irrawaddy dolphins from the Mekong River ranged from 0.4 to 18 mg·kg^{-1} fresh weight (Table 2). The high percentage of organic mercury is unusual, and we cannot provide any explanation for such a result. Methylmercury is immunotoxic, even at low concentrations (Wolfe et al., 1998; Das et al., 2008, 2016). The source of mercury in the Mekong River is thought to be gold mining activities (Dove, 2009).

Exposure to these persistent pollutants can compromise the health and reproductive potential of these river dolphins (Kannan et al., 2005). Chemical pollution, in addition to other reasons (e.g., habitat destruction and inbreeding), is a significant factor for the decrease of river dolphin populations (Reeves, 2002). Irrawaddy dolphins may be vulnerable to toxic effects due to their close proximity to pollution sources, and their lesser capacity to metabolise contaminants (Kannan et al., 2005).

Amongst the most severe pathological findings, acute moderate multifocal suppurative bronchopneumonia, moderate periportal hepatic lipidosis and moderate diffuse hepatic atrophy were observed in one dolphin (CID 09002), whereas another individual showed an acute severe diffuse suppurative leptomeningitis (CID 09008) (Table 3). Other findings were multifocal gastric ulcerations, acute mild multifocal tubular mineralisation in kidneys, multifocal mild ulcerative dermatitis, moderate focal to diffuse epidermal hyperplasia, and protozoal cysts morphologically consistent with *Sacrocystsis* sp. infection of the skeletal muscle.

Suppurative bronchopneumonia was caused by primary or

Table 1
POP concentrations (ng·g^{-1} *lw) in blubber* of Irrawaddy dolphins, *Orcaella braevirostris,* from the Mekong River, Cambodia.
Data are presented as mean (median) ± standard deviation (min – max values).

Compound	Concentration
p,p'DDE	27543 ± 47287 (2257) <10–167256
o,p' DDE	176 ± 232 (72) <10–809
p,p'DDD	2233 ± 3030 (683) <10–9383
p,p'DDT	392 ± 589 (125) <10–2489
DDT, op'	329 ± 513 (127) <10–2212
DDD op'	340 ± 484 (145) <10–1964
ΣDDXs[3]	31014 ± 51568 (3733) 10–180086
Σ6 NDL-PCBs[1]	344 ± 256 (253) 9 – 950
ΣPBDEs[2]	120 ± 127 (120) <LOQ - 438

[1] ∑ 6 NDL-PCBs : CB 28, CB 52, CB 101, CB 138, CB 153, CB 180
[2] ∑ PBDEs : BDE 47, BDE 99
[3] ∑ DDXs : o,p'-DDT, p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p'DDE, and o, p'DDD

Table 2.
Trace element concentrations in the tissues of Irrawaddy dolphins, *Orcaella braevirostris,* from the Mekong River, Cambodia. Data are expressed as mean (median) ± standard deviation, min-max and n (number of analysed samples). All concentrations are expressed in mg·kg^{-1} fresh weight.

	Liver	Kidney
T-Hg	4.4 ± 5.6 (1.7) 0.4–18 n= 19	nd
MeHg	2.6 ± 4.3 (0.8) 0.1–15 n= 18	nd
%MeHg	52 ± 30 (51) 16–97 n= 18	nd
Se	2.1 ± 1.9 (1.4) 0.5–6.9 n=19	nd
Cd	0.29 ± 0.38 (0.04) <0.0025–1.0 n= 13	3.6 ± 6.6 (0.1) <0.002–19 n= 13
As	0.08 ± 0.07 (0.06) <0.025–0.24 n= 11	nd
Pb	0.04 ± 0.06 (0.02) <0.007–0.21 n= 12	nd
Fe	578 ± 551 (458) 60–2369 n=19	nd
Cu	25 ± 31 (7) 1.7–111 n=18	nd
Zn	51 ± 29 (44) 19–110 n=19	nd

Table 3.
Pathomorphological and histopathological findings in Irrawaddy dolphins, Orcaella braevirostris, from the Mekong River, Cambodia.

Specimen	Pathomorphological and histopathological findings
CID 06091	multifocal gastric ulcerations
CID 07009	multifocal skin ulcerations
CID 07014	multifocal mild chronic lymphocytic panniculitis
CID 09002	- acute moderate multifocal suppurative broncho-pneumonia - moderate diffuse hepatic atrophy - moderate periportal hepatic lipidosis - acute mild multifocal ulcerative dermatitis - mild multifocal renal tubular mineralization - mild anthracosis of the lymph node
CID 09008	- acute severe diffuse suppurative leptomeningitis - protozoal infection of the skeletal muscle morphologically consistent with Sarcocystis sp. - Moderate focal to diffuse epidermal hyperplasia

secondary bacterial infections. There was no morphological evidence for a viral infection (e.g., morbillivirus); however, primary damage of the respiratory tract by viral infection cannot be excluded. In addition, general immunosuppression (e.g., caused by environmental toxins) has to be considered as a predisposing factor. Bronchopneumonia was regarded as a cause of death in affected animals.

One adult male individual suffered from acute severe diffuse suppurative leptomeningitis, most likely of bacterial origin, which was considered as a possible cause of death. Unfortunately, samples for microbiological investigations were not taken from the brain, and bacterial growth in other organs and tissues was non-specific.

It can be concluded that the Mekong population shows a sufficient level of genetic variation, which currently makes inbreeding a lesser extinction risk. However, conservation programs should contribute to the preservation of the entire genetic variation of the Mekong population (Krützen et al., 2018).

We note that the adverse effect of contaminants may be insidious and could potentially combine with other stressors to have an influential effect on the incidence and severity of infectious disease. Due to numerous confounding factors, it is not possible to relate levels of pollutants to observed morphological lesions. However, it is very likely that chemical contaminants do adversely affect alone or in combination the health and the reproduction of Irrawaddy dolphins at present and should have also affected previous generations.

CRediT authorship contribution statement

Joseph G. Schnitzler: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Krishna Das:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Peter Wohlsein:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Thijs Kuiken:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Arne Ludwig:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Dietmar Lieckfeldt:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Channa Phan:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Somany Phay:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ursula Siebert:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.105812.

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