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**Mudskippers: human use, ecotoxicology and biomonitoring of mangrove and other soft bottom intertidal ecosystems.**

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**Abstract (269)**

Mudskippers (Gobiidae: Oxudercinae) are air-breathing gobies, which are widely distributed throughout the West African coast and the Indo-Pacific region. They are closely linked to mangrove and adjacent soft bottom peri-tidal ecosystems. Some species are amongst the best adapted fishes to an amphibious lifestyle. All mudskippers are benthic burrowers in anoxic sediments, and since tidal mudflats are efficient sediment traps, and sinks for nutrients and other chemical compounds, they are constantly in contact with several types of pollutants produced by industrial, agricultural and domestic activities. Due to their natural abundance, considerable resistance to highly polluted conditions, and their benthic habits, mudskippers are frequently used in aquatic ecotoxicological studies. For the same reasons, mudskippers also frequently occur in urbanised or semi-natural coastal areas. Since several species are widely consumed throughout their whole geographical range, these same characteristics also facilitate their aquaculture in several countries, such as Bangladesh, Thailand, Philippines, China, Taiwan and Japan. Even when not directly used, mudskippers are often abundant and are important prey items for many intertidal transient species (marine visitors), and several species of shorebirds. Therefore, there is potential for bioaccumulation of toxicants wherever mudskippers and pollution co-occur. This chapter reviews the ecotoxicology of mudskippers, and their potential for use as biomonitors to better manage coastal swamp ecosystems. The diverse sympatric assemblages of mudskipper species allow for spatially differentiated ecotoxicological investigations along the whole intertidal zone, since adults are often territorial and/or sedentary, and show species-specific patterns of habitat differentiation. A case study is also proposed where this approach could be adopted to address potential health-risk issues in a local population who are regular consumers of mudskippers.

## Introduction

One third of the human population lives in coastal areas, which constitutes only 4% of the Earth's land area (Barbier *et al.* 2008). This concentration of human activities heavily impacts on coastal ecosystems. In particular, large estuaries host the largest soft bottom intertidal communities on Earth and are frequently sites of major urban settlements, and are heavily impacted by human activities. Along tropical and subtropical coasts, intertidal soft bottom systems, such as tidal mudflats and mangrove forests, characterize the great majority of shorelines. Habitat destruction, fragmentation and activities impairing the hydrology of the catchment area (Doody 2005; Lacerda *et al.* 2001; Farnsworth & Ellison 1997) are probably the most severe forms of anthropogenic impacts affecting these ecosystems. Only 40-50 years ago, mangrove forests fringed 70-75% of low-energy tropical shorelines around the world (Por & Dor 1984). Over the last 60 years, 30-40% of the global coverage has been lost (Ellison 2008; Blaber 2007; Wilkie and Fortuna 2003; Alongi 2002), with declining rates probably faster than that of coral reefs and tropical rainforests (Duke *et al.* 2007). Another source of anthropogenic impact on such systems is the increasingly frequent introduction of alien species through ballast water of ships (Moyle 1998). Sustainable ecosystem-based management and conservation efforts aimed at the maintenance of such systems for future generations, as they provide considerable direct and indirect services to human settlements (e.g. fishery production; sediment stabilization; shoreline protection from marine and climatic energetic events; degradation of organic nutrients; habitat for endemic, evolutionarily unique, or migratory species; and as recreational areas e.g. Polgar & Sasekumar 2010).

Water and sediment pollution, mainly resulting from the use of aquatic systems as convenient repositories for human biological and industrial wastes (e.g. sewage, industrial, agricultural and transportation effluents, oil spills), can also exert toxic effects on soft bottom intertidal systems (Hogarth 2007; PEMSEA 1999; Chaw *et al.* 1993; Sasekumar 1980). As a result, coastal communities can suffer from health issues induced either by the direct action of toxic substances in the water and sediments, or by trophic transfer, caused by the spreading of many contaminants through food webs (e.g. Di Giulio & Hinton 2008; Luoma & Rainbow 2005). Massive kills caused by the release of toxic substances in the environment are obvious detrimental effects. Although highly destructive, acute events allow for the immediate identification of causal agents, and rapid ecosystem remediation and rehabilitative actions can be attempted.

Nonetheless, sublethal effects can be equally destructive in the long term, acting in a more subtle and gradual way, often hampering the identification of the causal factors, and delaying appropriate management decisions. When contaminants can bioaccumulate, trophic transfers occur: if species at lower trophic levels become tolerant to sublethal toxic effects (e.g. Andreasen 1985), contaminants can spread through the food webs and concentrate at higher trophic levels. In several documented cases of trophic chains including fish and man, decades had passed before lethal concentrations at top trophic levels were reached, and interactions with other factors such as overfishing eventually disrupted ecosystem functioning, leading to the extinction of predator populations (Moyle and Cech 2000; Miller *et al.* 1989).

Soft bottom intertidal communities are generally tolerant to environmental change and pollution. Intertidal communities are also particularly resilient. Extremely variable environmental conditions select for strongly convergent traits in intertidal resident species, such as relatively high reproductive potential and highly dispersive early life stages (Hogarth 2007; Hartnoll 1987). Such adaptations generally enable these species to recolonise rehabilitated systems (e.g. Townsend & Tibbetts 1995) even after mass extinctions at the community level have locally occurred, such as those caused by massive releases of toxic contaminants (e.g. major oil spills; Chaw *et al.* 1993); fires of industrial plants (Otitoloju *et al.* 2007); or to the combined action of habitat destruction, pollution and introduction of alien species (e.g. Bennett & Moyle 1996). Of course, recolonisation would necessarily imply the presence of unimpacted ecosystems within the species' range of dispersal. Furthermore, local extinctions or degradation of intertidal communities can also affect adjacent terrestrial and aquatic ecosystems, by disrupting the trophic links connecting their food webs (Unsworth *et al.* 2009; Nagelkerken *et al.* 2008). For instance, top predators of soft bottom intertidal communities such as mangrove forests and tidal mudflats generally are transient marine and terrestrial visitors (e.g. predatory fishes and shorebirds; Clayton 1993).

Ecotoxicological studies also require insights to the dispersal and fate of bioavailable toxic chemicals in the environment (i.e. nutrients, organic, inorganic and organo-metallic contaminants (Di Giulio & Hinton 2008; Braunbeck *et al.* 1998). Since the 1960s, ecotoxicological studies on the effects of contaminants on fishes have provided insights to anthropogenic environmental impacts on aquatic systems. In fact, environmental contamination can be assessed either by chemically characterising water and sediments for known contaminants (e.g. Tam & Wong 1995), or observing rapid fish responses (mortality, behavioural changes) to suspect contaminated water in the laboratory (e.g. Heath 1987). However, rapid sub-lethal and lethal bioassays frequently do not provide information on past conditions (Helfman *et al.* 1997). In this respect, *body burdens* of bioaccumulated contaminants provide more information on the history of contamination; in particular, sedentary and territorial fishes reasonably make better sentinel models to indicate contamination in a particular habitat or location. The *biomarker* approach (Huggett *et al.* 1992; McCarthy & Shugart 1990) provides more sensitive and more immediate responses at the cellular and suborganismal level such as physiological, histological, cytological, genetic and biochemical endpoints. Other measures include structural and functional parameters (e.g. deformities, energetics, metabolism, growth rate, reproductive activity, immune competence). These can be correlated to the environmental conditions. At the community level, combined measures of fish populations and trophic structure, health and behaviour can be compared with reference communities, providing health bioindicators [e.g. Indices of Biotic Integrity (IBI); Roset *et al.* 2007].

The bioaccumulation of organic and inorganic contaminants in fish tissues can occur through either aqueous uptake or ingestion. In the latter case, bioaccumulation mediates the distribution and concentration of contaminants through food webs (Braunbeck *et al.* 1998). Chemicals can bioconcentrate or biomagnify in the biota depending on their properties such as their persistence

and octanol-water partition coefficients (K<sub>ow</sub>). Both bioconcentration and biomagnification in fishes depend on the bioavailability of waterborne or ingested contaminants, which in turn is determined by the dynamic relationship between the water column and sediments, acting as source and sink compartments, respectively. In particular, the bioavailability of organic contaminants in different aquatic environments is often determined by their hydrophobicity (Di Giulio & Hinton 2008).

Due to the lower environmental energy conditions and high sedimentation rate, soft bottom intertidal systems are efficient sediment traps (Duke & Wolanski 2001). On mudflats, very fine sediments accumulate large quantities of organic carbon and nitrogen through adsorption onto mineral surfaces (Logan & Longmore 2003). In particular, organic and anoxic muds are known to extract trace metals from the water column by reaction with sulfides (Chapman *et al.* 1998), and provide binding surfaces for hydrophobic organic pollutants, rapidly concentrating them (Di Giulio & Hinton 2008; Di Pinto *et al.* 1993). Not surprisingly, soft bottom intertidal and subtidal benthic communities, characterised by detritus-based food webs (e.g. Odum & Heald 1975) are particularly impacted by such contaminants.

Hydrophobic contaminants are well known for resisting chemical and biological degradation in the environment (viz. persistent organic pollutants, or POPs; e.g. Bhatt *et al.* 2009; Rand 2003). Furthermore, their hydrophobic nature promotes increasing bioaccumulation in lipid tissues in higher trophic organisms. Apart from fat content, the age of contaminated organisms (duration of exposure) is an important factor for biological uptake, since time is required to achieve equilibrium between tissue and contaminants (Larsson *et al.* 1991). Also for this reason, fish are often more useful than invertebrates in biomonitoring hydrophobic contaminants, since the lifespans of many invertebrates are frequently too short to record steady-state concentrations in their tissues (e.g. Nakata *et al.* 2002). However, bioaccumulation results from a balance between uptake and elimination rates. In particular, species-specific detoxifying metabolic mechanisms (Blanchard *et al.* 1997) and spawning (Guiney *et al.* 1979) can play an important role in decreasing hydrophobic contaminant levels in the organism.

In the aquatic environment, metal and metalloid contaminants are also particularly important, being present in a variety of anthropogenic effluents. Nonetheless, the evaluation of ecological risk from metal contamination is particularly complex, involving metal-specific, geochemical, exposure route and species-specific biological factors (Luoma & Rainbow 2005; Wang 2002). In addition, other abiotic factors can affect the bioavailability and hence toxicity to exposed organisms such as pH, redox potential, hardness and organic ligands. The same relationship between bioaccumulation and toxicity is not simple, and biomonitors must be carefully tested for their capacity to drive ecosystem changes in response to metal contamination, relative to other components of the investigated community. Assuming steady state conditions in the organism, a simple biodynamic model (DYMBAM: Schlegel *et al.* 2002), takes into account metal uptake (both aqueous and dietary) and loss rates by measuring simple standardised parameters in the laboratory. Highly significant 1:1 correlations between predicted and field observations in several case studies are encouraging (Luoma & Rainbow 2005). Based on such relationships,

considerable variability in the above mentioned factors requires consideration of peculiar characteristics of the ecosystem, community and selected biomonitor(s), whenever risk assessments for these contaminants are conducted. Biodynamic models offer the opportunity to compare and explain the variability of bioaccumulation in different species within a study community, when investigating the ecological effects of contamination.

This review of the biology and ecotoxicology of mudskippers aims at demonstrating the potential use of these species as biomonitors in environmental assessments of tropical and subtropical soft bottom intertidal systems. Such potential would greatly benefit from future research effort to fill the gaps in the biological and ecological knowledge of this group. A possible case study where such an approach could be adopted (lower Fly River and Delta, Papua New Guinea) is also presented (Polgar *et al.* 2010).

### **Mudskippers: ecology, physiology and interactions with humans**

The following overview outlines some biological traits that make mudskippers excellent biomonitors and biomarkers for ecotoxicological studies. Mudskippers (Gobiidae: Oxudercinae: Periophthalmi; Murdy 1989) are amphibious gobies which are closely linked to tropical and subtropical soft bottom inter- and peritidal ecosystems. This group presently includes 34 species in seven genera: *Periophthalmus* Bloch & Schneider, 1801; *Periophthalmodon* Bleeker, 1874, *Boleophthalmus* Valenciennes, 1834; *Scartelaos* Swainson 1839; *Pseudapocryptes* Bleeker, 1874; *Zappa* Murdy, 1989; and *Apocryptes* Valenciennes, 1837. The highest species richness occurs in South-East Asia, Australia and New Guinea. Nonetheless, the group's distribution spans a wide biogeographical range, from the west coast of Africa to the whole Indo-west-Pacific region (Murdy 1989). At the habitat level, adults are differentially distributed from the upper sub-tidal to the high intertidal zone, including tidal reaches of rivers and supratidal ecotones to freshwater swamps (Polgar *et al.* 2010). In some regions, diverse sympatric assemblages of up to 11 species can be found (Polgar & Bartolino 2010; Polgar & Crosa 2009; Md Ali & Norma-Rashid 2005; Takita *et al.* 1999).

Mudskippers are relatively small fish species (maximum size: 4-25 cm total length), which are either primary (Yang *et al.* 2003) or secondary consumers (Kruitwagen *et al.* 2007a; Colombini *et al.* 1996), or omnivores (Bucholtz *et al.* 2009; Milward 1974). In particular, primary consumers among mudskippers (e.g. *Boleophthalmus* spp.) are benthic phytoplanktivores, and feed by scraping microbial biofilms on exposed mud surfaces. It is known that such biofilms are composed of a matrix of extracellular polymeric secretions (EPS), and are able to effectively chelate metals and other contaminants (Decho 2000), mediating their trophic transfer into benthic food webs.

These species can be very abundant locally (Polgar & Bartolino 2010), being predated by many intertidal transient fishes (marine visitors: Gibson 1999), including species of commercial importance, reptiles and shorebirds (Clayton 1993; Jayne *et al.* 1988; G. Polgar, pers. obs.). All known species are burrowers, and are, therefore, reasonably sedentary. In several species of the

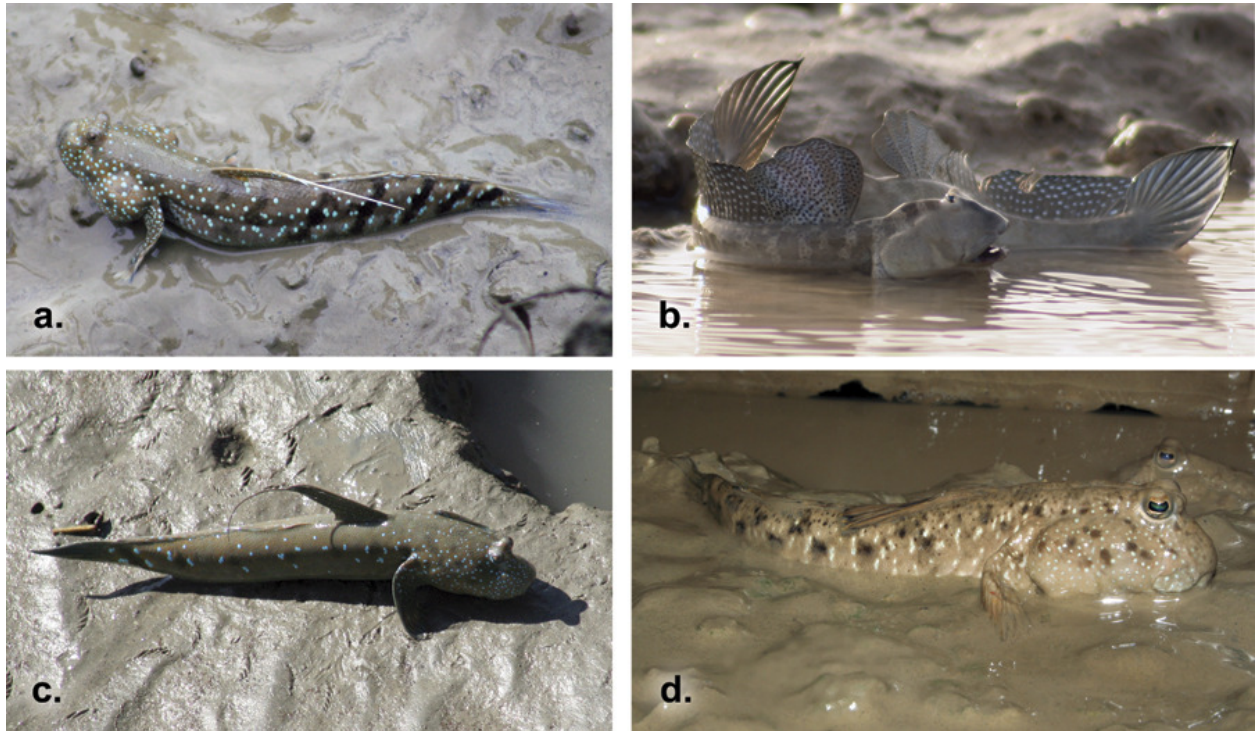
genera *Boleophthalmus*, *Periophthalmodon* and *Periophthalmus*, territorial behaviour has been extensively described (e.g. Clayton & Vaughan 1988; Brillet 1975).

The reproductive biology and life history of oxudercine species is little known, and a generalised model can only be assumed on the basis of observations made on a few genera and species (Mazlan & Rohaya 2008; Dinh *et al.* 2007; Hong *et al.* 2007; Etim *et al.* 2002; King & Udo 1997; Hagiwara *et al.* 1993; Saitoh 1993; Hoda 1986; Matoba & Dotsu 1977; Brillet 1976; Kobayashi *et al.* 1971). Based on this model, the male attracts the female into a reproductive burrow in the mud substrate, and eggs are attached to the ceiling of a specialised chamber filled with air (Ishimatsu *et al.* 2000; 1998). As is usual in mudskippers, eggs are guarded by the male (Ishimatsu *et al.* 2007). Larvae are planktonic, and after 30-50 days of pelagic life (Kon & Yoshino 2001), they settle in the intertidal zone (Chen *et al.* 2008; Dinh *et al.* 2007). Young apparently perform exploratory behaviours (errants: Clayton & Vaughan 1988, G. Polgar, pers. obs.) and eventually establish stable territories around burrows (e.g. Clayton & Vaughan 1988; Brillet 1976).

The extremely dynamic conditions of tropical soft bottom intertidal habitats have selected for extreme physiological adaptations to environmental changes over the evolutionary history of mudskipper species. In particular, the anoxic and hypercarbic sediments where mudskippers dig their burrows are characterised by low redox potential values and high ammonia, soluble phosphate and acid sulphide concentrations all of which can affect the bioavailability of some chemicals such as metals, ammonia, etc. (Hogarth 2007; Ishimatsu *et al.* 2000). All mudskippers are bimodal breathers (Graham 1997): they breathe in air both by gulping air into the buccopharynx and expanded opercular chambers, and by cutaneous respiration (Graham 1997). The same gas-exchange surfaces are utilised for respiration in both water and air environments. The degree of anatomical and physiological adaptations to aerial respiration varies amongst different species (Kok *et al.* 1998; Low *et al.* 1990), with more amphibious ones even incapable of repaying an oxygen debt while under water, and have a bradycardic diving syndrome which is probably unique amongst fishes (Takeda *et al.* 1999; Kok *et al.* 1998; Garey 1962). In fact, mudskippers are generally poorly adapted to respire aquatically in hypoxic conditions, and use aerial respiration to avoid hypoxic stress (Lee *et al.* 2005; Ishimatsu *et al.* 2000; 1998; Aguilar, 2000). Even almost completely aquatic mudskipper species can live in waters with high organic loads [e.g. *Pseudapocryptes elongatus* (Cuvier, 1816); Bucholtz *et al.* 2009; Takita *et al.* 1999; G. Polgar, pers. obs.].

Mudskippers are also highly tolerant of environmental ammonia concentrations. Not unlike most teleosts, they are essentially ammoniotelic, with excretion mainly through the gills (Evans *et al.* 1999). *Periophthalmodon schlosseri* (Pallas, 1770) can tolerate concentrations of 450  $\mu\text{M}$   $\text{NH}_3$  for at least 7 days, a condition that according to Peng *et al.* (1998) is only comparable to *Oreochromis alcalicus grahami* (Boulenger, 1912) [= *Alcolapia grahami* (Boulenger, 1912), a cichlid living in alkaline African lakes], but at much lower pH values. The relatively more aquatic *Boleophthalmus boddaerti* (Pallas, 1770) [= *B. boddarti* (Pallas, 1770) sensu Murdy 1989; Fig. 1] can tolerate about 40  $\mu\text{M}$   $\text{NH}_3$  (Peng *et al.* 1998), a performance comparable to

*Periophthalmus cantonensis* (Osbeck, 1765) (= *P. modestus* Cantor, 1842 sensu Murdy 1989; Iwata 1988). These latter values are comparable to ammonia concentrations that are lethal for a range of freshwater, estuarine and tide pool gobioid aquatic fish species (about 45  $\mu\text{M}$   $\text{NH}_3$  over 24 hours: Iwata 1988).



**Figure 1.** Three mudskipper species frequently utilised in ecotoxicological studies. a. *Boleophthalmus boddarti* (Pallas, 1770), distributed from west India (Mumbai) eastward to southern Viet Nam and Indonesia (Murdy 1989); b. *Boleophthalmus dussumieri* Valenciennes, 1837, distributed in the Persian Gulf, Gulf of Oman, Pakistan, eastward to west India, up to Mumbai (Murdy 1989); c. *Boleophthalmus pectinirostris* (Linnaeus, 1758), distributed from southern Japan and southern Korea southward to mainland China, Indonesia and South-East Asia (Murdy 1989; Polgar & Crosa 2009); d. *Periophthalmus waltoni* Koumans, 1955, distributed in the Persian Gulf, Gulf of Oman and Pakistan. The former three congeneric species, whose geographic distribution partially overlaps, were probably frequently misidentified in ecotoxicological studies.

Both *Pn. schlosseri* and *B. boddarti* can decrease the rate of proteolysis and amino acid catabolism under ammonia loading conditions, thus slowing down the accumulation of ammonia (Lim *et al.* 2001). *Pn. schlosseri* is capable of actively excreting  $\text{NH}_4^+$ , even at pH 9.0 (Randall *et al.* 2004; Chew *et al.* 2003), and of decreasing skin permeability to  $\text{NH}_4^+$  ions at increasing environmental ammonia concentrations. *B. boddarti* is not capable of actively eliminating  $\text{NH}_4^+$ , but can concentrate ammonia in its muscle, liver and plasma, thus preventing further  $\text{NH}_4^+$  passive uptake. *Pn. schlosseri* can even excrete  $\text{H}^+$  and lower the pH of small water volumes (e.g. inside burrows), thus reducing the  $\text{NH}_3$  concentration around the body and preventing a

back flux of NH<sub>3</sub> through the gills (Ip *et al.* 2004a). Adaptations to cope with high levels of endogenous ammonia, probably highly adaptive for intertidal mud burrowers, were possibly crucial pre-adaptations to an amphibious lifestyle. In fact, gills drastically decrease their excretory function in air, and ammonia tends to rapidly accumulate in body fluids when out of water. During terrestrial activities, *Pn. schlosseri* detoxifies ammonia through partial amino acid catabolism (alanine synthesis): this metabolic pathway also produces energy and allows this species to perform intense terrestrial exercise whilst not consuming glycogen reserves (Ip *et al.* 2004b). A similar strategy is probably adopted by *P. modestus* (Iwata 1988).

All the investigated oxudercine species are euryhaline and can withstand rapid and drastic changes in salinity. *Pseudapocryptes elongatus* shows no apparent behavioural reaction to instantaneous changes from 25 ppt to 0–50 ppt, after 96 hours of exposure (Bucholtz *et al.* 2009). In northern Australia, *Periophthalmus minutus* Eggert, 1935 can live for several days inside burrows with 40–70 ppt salinities during the dry season (Tatsusuke Takeda, unpub. mat.). Osmoregulation in hypersaline conditions is accommodated through the accumulation of free amino acids (FAA) and ammonia in muscles (Iwata *et al.* 1981), and through the rapid activation of the gills' *chloride cells* (Evans *et al.* 1999), which in some amphibious species are also found in skin patches behind the pectoral fins (Sakamoto & Ando 2002). The resistance to dehydration upon emergence, which is related to hyperosmotic osmoregulatory capabilities (Evans *et al.* 1999), was also measured in a few amphibious mudskippers, which were found to have an evaporative water permeability capacity comparable to that of frogs (Gordon *et al.* 1978; 1969). The structure of the skin and some particular mucous secretions can partly account for such capabilities (Zhang *et al.* 2003; 2000). In several mudskipper species regulation in hyposmotic conditions is at least partially behavioural (cutaneous evapotranspiration: Clayton 1993).

Relative to aquatic fishes, mudskippers also cope with extreme environmental temperature changes. Out of water, daily temperatures on the substrate surface can range between 10–15°C (Tytler & Vaughan 1983), while in tide pools water can reach temperatures of about 40°C (Taylor *et al.* 2005). Thermoregulation in mudskippers is mainly behavioural (microhabitat selection; evaporative cooling; orientation with respect to solar radiation; changes in colouration: Taylor *et al.* 2005; Clayton and Vaughan 1988; Tytler & Vaughan 1983; Stebbins & Kalk 1961). In particular, the use of burrows is of crucial importance in amphibious species (Lee & Graham 2002; Aguilar 2000; Tytler & Vaughan 1983). Mudskippers living in temperate zones remain in their burrows during the winter months (Takegaki *et al.* 2006; Townsend & Tibbetts 1995; Kobayashi *et al.* 1971), while some tropical species are able to aestivate in deep burrows closed with a plug of mud when supra-tidal tide pools dry up during the dry season (Swennen *et al.* 1995; Hora 1935; 1933).

Interactions between humans and mudskippers are both frequent and intense. Due to the tolerance of these species to organic pollution, available habitats frequently host large mudskipper populations close to coastal urban settlements. Mudskippers are either consumed or used in traditional medicine and as baits throughout their geographic range. In Bangladesh, China, Japan, Korea, Philippines, Taiwan, Thailand and Viet Nam several species are considered



a delicacy, thus are extensively farmed, as witnessed by the relatively rich tropical aquaculture literature on these species (e.g. Bucholtz *et al.* 2009; Kizhakudan & Shoba 2005; Zhang & Hong 2003; Shusen & Jiazhong 1995; Chung *et al.* 1991; Chen 1990; Hong & Wang 1989; Fineman-kalio & Alfred-Ockiya 1989; Koga *et al.* 1989; Zhang *et al.* 1989; Chen & Ting 1986; Chen 1982). Traditional techniques are employed for fishing several species, viz. pitfall traps (Sierra Leone: Turay *et al.* 2006; Japan: Dotsu 1974), valved traps (Nigeria: Mariogahe 1990), traps to extract mudskippers from their burrows (Viet Nam: Bucholtz *et al.* 2009); and long rods with special hooks (Japan: Dotsu 1974). Mudskipper flesh can have high nutritive value (Banerjee *et al.* 1997; Misra *et al.* 1983) and their consumption is only limited by local traditions and beliefs (G. Polgar, pers. obs.). In a preliminary test evaluating the palatability of some mudskippers species (Swennen *et al.* 1995), their taste scores were higher than that of the widely consumed species (mulletts).

Not unlike many air-breathing fishes (Graham 1997), mudskippers are cultured because of their considerable tolerance to environmental stressors, and organic and inorganic toxicants. Therefore, it is highly recommended that when they are used as a food source in polluted areas they should be carefully and periodically monitored for contaminants to assess the health risk to consumers. Where necessary, prompt environmental remediation and ecosystem rehabilitation should be implemented to maintain the sustainability of this resource (McLeod *et al.* 2005). For these purposes, basic research at sub- and supra-organismal levels is needed to further our understanding of the ecotoxicology and biology of these fishes, in particular the toxicodynamics in different species and their effects at the community level.

### **The ecotoxicology of mudskippers (4,538)**

A bibliographic online search (Google Scholar, PubMed) of the scientific literature on the ecotoxicology of mudskippers, resulted in 45 papers and 3 book chapters, spanning 40 years: 1970s (n = 2), 1980s (n = 6), 1990s (n = 17), and 2000s (n = 23), with the most recent paper and chapter published in 2009 and 2010, respectively. Twenty two papers were published in journals with a national or regional scope, i.e. not included in the database of Thomson Reuters (2011) making up about 50 % of the whole sample. Nonetheless, these references were frequently cited in recent research papers, suggesting that scientific interest on this topic is at the regional level. Over the last decade, more than 80 % of the papers were published on ISI journals.

The history of the ecotoxicology of mudskippers follows the historical trends in scientific methodology developed in ecotoxicology (Luoma & Rainbow 2005; Rand 2003). Early studies consisted of dose- and duration-dependent experiments at the organismal and sub-organismal levels, and on the ecotoxicology of heavy metals. More recent studies were on stress responses and propagation through food webs at the supra-organismal level, with a focus on the ecotoxicology of hydrophobic organic compounds.

### *Heavy metals and metalloids*

Heavy metals (HM), metals and metalloids include toxic elements of great concern, such as Cu, Zn, Cd, Hg, Pb, Al, Cr, Se, Ag, As, and Sb, released into the environment by industrial and domestic activities. Water quality greatly affects their speciation and toxicity, which also varies widely with the biotic system affected (e.g. Rand 2003).

To our knowledge, the first ecotoxicological studies on mudskippers were conducted in the 1970s by Uchida *et al.* (1971), who measured the concentration of heavy metals in *Boleophthalmus pectinirostris* (Fig. 1) and other organisms in the Ariake Sea (Japan).

Patel *et al.* (1985) investigated the presence of Zn, Mn, Cu, Fe, Co, Ni, Cd, Cr, Pb and Sr in water, sediments, the clam *Anadara granosa*, and the mudskipper *Boleophthalmus boddarti* (= *B. boddarti*) in the Mumbai harbour area, to biomonitor the effects of urbanisation and anthropogenic pollution. No evidence of dangerous levels and no systematic or substantial spatial or temporal fluctuations of these elements in the biotic and abiotic matrices over a period of 4 years was found in this study. However, the high percentage mortality of clams in one of the surveyed locations was correlated to the presence of anoxic conditions, which was due to organic and industrial pollution.

Mahajan & Srinivasan (1988) measured Hg concentrations in sediments, bivalves, benthic fishes (*Boleophthalmus boddarti*), crabs, prawns, gastropods and pelagic fishes. Hg levels were higher in the first two groups of organisms. Interestingly, they found that contaminant levels increased after the rainy season, suggesting an increased input through increased surface runoff.

*Boleophthalmus dentatus* (= *B. dussumieri* Valenciennes, 1837 sensu Murdy 1989; Fig. 1) was utilised in a series of ecotoxicological studies in India. Lakshmi *et al.* (1990; 1991a) studied the chronic dose and duration-dependent inhibition of HgCl<sub>2</sub> on ATPases and acid and alkaline phosphatases in the gills of this species. Similar studies (Lakshmi *et al.* 1991b,c) were conducted on different ATPases of the intestine and kidney. In all of these studies, the authors found an approximately linear inhibition of all the studied enzymes with increasing sublethal concentrations and durations of exposure of the contaminant. The severe cellular damage observed was related to the apparent blockage of transport mechanisms across cell membranes.

Kundu *et al.* (1992a,b) investigated the enzymological and histopathological effects of sublethal doses of Hg<sup>++</sup> (0.50, 1.00, 1.25, 1.50 ppm HgCl<sub>2</sub> ; LC<sub>50</sub> = 1.65 ppm) on the kidneys, gills, intestine, liver, brain and muscle of *Boleophthalmus dentatus* (= *B. dussumieri*) for up to 3 days. Their results indicated a dose and duration-dependent inhibition of ATPase enzymes in all the studied tissues, at longer durations and higher concentrations of the contaminant. Kidneys, gills and liver were the most impacted organs. Using the same mudskipper species as a model, Kundu *et al.* (1995) measured significant dose and duration dependent changes in the activity of 5 types of ATPases of the brain and muscle, induced by exposure to Cr(VI) (30-60 mg/l for 1-3 days). In several trials a significant stimulation of the enzymes' activity was observed, and interpreted as a response to the toxicant. However, a general dose dependent inhibition of the enzymes' activity was found. Neurological and behavioural effects were also observed.

Also the mudskipper *Periophthalmus dipes* (= *P. dipus* Bleeker, 1845 = *P. argentilineatus* Valenciennes, 1837 sensu Murdy 1989) was utilised in several studies on heavy metals in India.

Thaker *et al.* (1996; 1997; 1999) studied the dose and duration-dependent effects of Cr(VI), an important carcinogenic pollutant in water bodies, on the activities of five types of ATPases and alkaline phosphatases in several tissues. Based on the ATPases, the authors found evidence for a cascade of metabolic effects in different anatomical compartments: first the gills, with mainly dose-dependent effects; then the kidneys, with both dose and duration-dependent effects; and finally the intestine, with mainly duration-dependent effects. The authors speculated that this cascade phenomenon probably affected osmoregulation, acid-base balance, nutrient assimilation, and mobility of intestinal muscles (see also Kundu *et al.* 1995). Effects on phosphatases (Thaker *et al.* 1997) were more variable.

In a preliminary study conducted in Indonesia, Amin & Nurrachmi (1999) found higher levels of Ni, Pb and Cd in the liver and muscle of *Periophthalmus* sp. from an industrialized area (Dumai) relative to a nearby unimpacted area (Sumatra, Straits of Malacca).

Kapil & Ragothaman (1999) found that mercury, copper and cadmium contaminations induced changes in the total protein content of the muscles of *Boleophthalmus dussumieri*.

Ni *et al.* (2000) conducted one of the first studies on the assimilation efficiencies (AEs) of heavy metals in fishes (Cd, Cr, and Zn), to quantify the relative importance of bioconcentration and biomagnification. They used two species of zooplankters, an ambassid fish, and a mudskipper (*Periophthalmus cantonensis* = *P. modestus*). While AEs were generally lower than in carnivorous invertebrates, their results clearly demonstrated that trophic transfers occur, with species-specific differences in the AEs for different metals which follow different metabolic routes from zooplankters to fishes.

Ni *et al.* (2005) described the aqueous uptake, dietary assimilation and elimination of Cd, Se and Zn in *Periophthalmus cantonensis* (= *P. modestus*) acclimated at different salinities (10-30 ppt). The dietary assimilation efficiency based on consumption of radiolabeled polychaetes was tenfold higher for Se than for Zn and Cd, and was not influenced by salinity. In contrast, the highest concentration factor (CF: equilibrium ratio between concentration in the organism and concentration in water) was found for Zn, followed by Cd and Se. Salinities began to affect CFs only after 12 h, with final CFs significantly higher at lower salinities. This trend was similar for all contaminants studied, and could be due to physiological changes. Elimination rates were not significantly affected by salinity, but Se was more rapidly eliminated following aqueous uptake relative to dietary ingestion. Accumulated Cd was mainly found in the gut, whereas the other metals were more abundant in the muscle.

Eboh *et al.* (2006) measured the levels of copper, zinc, lead, mercury, arsenic, chromium and calcium in the muscle, gills and liver tissues of *Periophthalmus koelreuteri* [= *P. barbarus* (Linnaeus, 1766)] and other fish species from Nigeria, finding that the levels of these metals were generally lower than those found in other commercial species in the USA.

Liu *et al.* (2006) measured the activities of the enzymes xanthine oxidase (XOD), superoxide dismutase (SOD), catalase (CAT) and malonyldialdehyde (MDA) in the liver of *Boleophthalmus pectinirostris* exposed to different levels of Cd<sup>2+</sup>, concluding that only XOD and SOD are sensitive to Cd<sup>2+</sup> stress.

Liu and Zhou (2007) observed the chronic effects of sublethal doses of cadmium (1.0, 0.1 mg/l for 10 days) on the hepatic cells of *Periophthalmus modestus*, finding dose-dependent effects on intracellular organelles (mitochondria, endoplasmic reticulum, nucleolus, and, at higher concentrations, the nucleus). They inferred that damage was caused by lipidic over oxidation.

Bu-Olayan & Thomas (2008) measured the acute and chronic effects (aqueous uptake) of Zn, Cu, Cd and Fe in *Periophthalmus waltoni* Koumans, 1955 (Fig. 1) under laboratory conditions. Acute toxicity tests showed that Cd was the most toxic metal (LC<sub>50</sub> values of the other metals were 2-4 times higher), followed by Fe, Cu and Zn. The bioaccumulation factors (BAF) calculated after sub-lethal chronic exposure (lowest observed effect concentration (LOEC) and LC<sub>15</sub> for 60 d exposure) showed an identical pattern, with more toxic metals having higher BAFs. For all contaminants and at both concentrations, bioaccumulation was highest in the liver, intermediate in muscles, and lowest in gills. Nonetheless, the authors did not specify whether fishes could emerge at will (they were maintained in glass tanks filled with filtered seawater and no substrate), which would probably influence both the degree of exposure of the gills to the contaminants and contaminant uptake during aquatic respiration.

#### *Organic contaminants*

Organic contaminants have been massively introduced in the environment since the 20<sup>th</sup> century; these include polychlorinated biphenyls (PCBs), polychlorinated aromatic hydrocarbons (PAHs), chlorinated dioxins and furans, aliphatic and aromatic hydrocarbons, synthetic detergent and several types of pesticides (Rand 2003). The toxic effects on the aquatic fauna are extremely variable. Several of these chemicals are particularly persistent and hydrophobic (hence lipophilic), and are consistently biomagnified through food webs.

Parmar & Patel (1993) found a significant decrease in the glycogen content in *Boleophthalmus dentatus* (= *B. dussumieri*), after lethal and sub-lethal doses of the organochlorine pesticide endosulfan. Patel & Parmar (1993) found that endosulfan also induces a significant decrease of the protein content in *B. dussumieri*, and maximally in the liver. Several PCBs are well known fish endocrine disrupters and inhibitors of antioxidants, being associated with oxidative stress, impairment of protein metabolism, and hyperglycemia through glycogenolysis (e.g. Gill *et al.* 1991). It should be noted here that hyperglycemia, induced by cortisol or catecholamines is a generalised response to environmental stressors in fishes (Thomas 2008; Wendelaar Bonga 1997).

Feng *et al.* (2001a) investigated the variation of the concentration of reduced-glutathione (GSH) in the liver and ovaries of *Boleophthalmus pectinirostris* at concentrations of 0, 0.05, 0.2 and 0.5 mg/L of benzo(a)pyrene (BaP) for up to a week. The GSH levels in the liver and ovaries of BaP-exposed fishes respectively increased and decreased significantly with dose, relative to controls. Such results indicated either adaptation of or toxic effects on these organs, which appear to be heavily affected by this toxicant. Feng *et al.* (2001b) found that BaP significantly increased the activities of the antioxidants superoxide dismutase and glutathione peroxidase at

relatively higher concentrations, while no effect was measured on the catalase, in the liver of *B. pectinirostris*.

In one of the first studies of the impact of contaminants at the community level, Nakata *et al.* (2002) studied the accumulation of PCBs in mudskippers (*Boleophthalmus pectinirostris*, *Periophthalmus modestus*) and worm-eel gobies (*Odontamblyopus rubicundus*; Gobiidae: Amblyopinae), crabs and mussels living on the tidal flats of the Ariake Sea, Japan. All fishes showed a larger percentage of heavier and more hydrophobic 5-7 chlorinated PCB congeners, with some species-specific patterns, while 2-3 chlorinated congeners were more abundant in sediments. The concentration ratios of heavier PCBs between organisms and sediments were highest in carnivorous fishes (*P. modestus* and *O. rubicundus*), intermediate in omnivores (crab), lower in herbivorous fishes (*B. pectinirostris*), and lowest in mussels, possibly due to their shorter lifespan. Such results indicated that trophic level plays a key role in PCB bioaccumulation. Interestingly, this pattern was reversed for non-ortho coplanar CBs (a minor class in this sample), suggesting different toxicokinetic mechanisms. The authors also found positive and significant relationships between PCB levels and size of *B. pectinirostris*, suggesting that growth and feeding rates (hence biomagnification) are important factors for the bioaccumulation of persistent contaminants in mudskippers, and that the accumulation rate was faster than growth rate. In particular, in larger size classes a weaker correlation suggested slower growth and feeding rates in older mudskippers. Also, the weak correlation found in younger mudskippers (< 1 yr), caused by a high variability of PCB concentrations, was apparently due to very variable feeding and growth rates, which in turn were probably determined by the intense exploratory behaviour and lower social status prior to establishment of territorial behaviour in this species ("errants": Hofmann *et al.* 1999; Clayton & Vaughan 1988). As previously found in other fish species, this study also showed that female *B. pectinirostris* can eliminate PCBs through spawning, with a transfer rate of about 10% of female body burdens during each spawning event.

Nakata *et al.* (2003) made further observations on the environmental distribution, bioaccumulation and toxic potencies of PCBs in the tidal mudflats of the Ariake Sea and compared them with PAHs. While the former contaminants accumulated in higher trophic levels (coastal and tidal omnivorous fishes), the latter ones were dominant at lower levels and in detritivorous species (clams, crabs), possibly reflecting a higher degree of PAH adsorption on sediment particulates. Accordingly, a larger proportion of TCDD (tetrachlorodibenzodioxin) total toxic equivalents (TEQs) in coastal and tide flat species vs. detritivorous species was represented by PCBs and PAHs, respectively.

Nakata *et al.* (2005) monitored the environmental levels of dichlorodiphenyltrichloroethane and its isomers (DDTs), dichlorodiphenyldichloroethylene and dichlorodiphenyldichloroethane (DDD and DDE, respectively), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), hexachlorobenzene (HCB) and PCBs in three different ecosystems in northern China (Lake Tai, Hangzhou Bay, and in the vicinity of Shanghai city). Contaminants were measured in sediments, crustaceans, fishes (including *Boleophthalmus pectinirostris*), birds and aquaculture

feed. DDT and its major metabolites (DDD and DDE) were the most abundant contaminants. In particular, trophic transfer and biomagnification were particularly conspicuous in marine food webs. They also concluded that even if these contaminants had been banned in China, fresh inputs of this pesticide were apparently still taking place in coastal environments around Hangzhou Bay at the time of the study.

Wong *et al.* (2005) conducted a study on the presence of PCBs and organochlorine insecticides in aerial deposition, seawater, sediment and biota in two sites in the Hong Kong Bay, one of the busiest and most densely populated ports in the world, which receives agricultural and industrial effluents from large fluvial systems throughout the year. They compared the Mai Po Nature Reserve system (a Ramsar site), located right in front of the Pearl River delta and dominated by mudflat, mangrove and aquaculture systems, with A Chau, a small and less impacted island on the eastern side of Hong Kong. Direct measurements of contaminants were coupled with *in vitro* bioassays, in order to assess estrogenic potency, non specific cytotoxicity, and total dioxine-like activity. Several taxa were examined, including a sample of unidentified polychaetes, three species of farmed detritivorous fishes, two gobies, two crustaceans, a clam, and a mudskipper [*Boleophthalmus boddaerti* ? = *B. pectinirostris* (Linnaeus, 1758); to our knowledge, this species was never recorded in Hong Kong]. Unfortunately, different species were analysed in the two sites, possibly reducing the value of this comparison. However, the results showed that Mai Po was more heavily contaminated than A Chau, and that o,p' and p,p' isomers of a wide range of persistent organic pollutants were present in the different environmental matrices. DDTs, HCB, cyclodienes and PAHs were dominant contaminants in Mai Po. Mudskippers were highly contaminated, containing higher concentrations of all these contaminants than both polychaetes and prawn; comparable or higher amounts of cyclodienes and comparable or lower amounts of DDTs and HCB than farmed fishes; and higher amounts of PAHs. In samples with positive bioassay responses, PAHs were dominant, although the endocrine disrupting hazard could not be clearly evaluated.

In the same sites, Wong *et al.* (2006) measured the concentrations of several organochlorine insecticides (DDTs, HCHs, HCB and 11 cyclodiene insecticides) in water, sediments, and several organisms: plankton, polychaetes, a clam, two shrimp, and several fish species, including *Boleophthalmus boddaerti* (see above considerations). Also in this study, DDTs were dominant contaminants. The concentrations of pollutants in the Mai Po sediments was higher than levels considered hazardous, while in water and organisms, concentrations were below the hazard and tolerance levels for human consumption (US EPA, FDA; ISQG). Among the organisms, fish contained higher concentrations of dominant contaminants (DDTs, Cyclodienes), and mudskippers were again amongst the most contaminated species. In Mai Po, sediment pollutant concentration along a transect from land to sea decreased within a mangrove belt of <1 km, suggesting some role played by mangrove rhizospheres in detoxifying the sediment, as reported by Lacerda *et al.* (1993) for heavy metals. The predominance of DDD and DDE relative to DDT in this study suggests that the ban of DDT after the massive releases that occurred during the 1970s and 1980s, and some low-cost management practices (periodic flushing and re-flooding of

fish ponds) reduced the presence of contaminants in cultivated and wild fish, even if the measurable levels of DDTs in the water indicated that some fresh inputs were still present.

Islam *et al.* (2006) conducted a descriptive study on the chronic effects of sub-lethal concentrations of DDT on the behaviour and liver histology of *Apocryptes bato* (Bleeker 1874), a widely farmed and consumed fish in Bangladesh [the studied species possibly was *Pseudapocryptes elongatus* (Cuvier, 1816): to our knowledge, *A. bato* is usually not consumed in this area]. At increasing concentrations, the authors observed lethargic movements, increased frequency of air gulping, irregular swimming, aggressive behaviours, flicks, thrusts and coughing. After 20 days at the maximum concentration, loss of balance and fin paralysis were observed in most fishes. These latter specimens suffered serious hepatic damage, with vacuolization, necrosis, degeneration of the reticular tissue, and disaggregation of the parenchyma, especially in heavily vascularised areas.

Nakata *et al.* (2009) documented the presence of heavy contamination of benzotriazole UV stabilizers in sediments and several marine organisms (including mollusks, mudskippers, birds and sharks) from the Ariake Sea, Japan. Contaminant concentrations were highly correlated to sediment organic carbon content, implying strong adsorption of the contaminants to organic matter. The high contaminant concentrations measured at higher trophic levels (predatory fishes and coastal birds) would suggest the presence of biomagnification. Nonetheless, environmental conditions apparently had a stronger influence than trophic levels, since organisms collected on the tidal mudflat (e.g. clams, gastropods, oysters and mudskippers) generally contained higher concentrations of contaminants than animals at higher trophic levels collected in adjacent shallow waters. This suggests that contaminant levels in the mud and interstitial water are higher than in the water column.

Takao *et al.* (2010) measured the concentrations of PAHs, alkylphenols, and organotin compounds in sediments, the mudskipper *Periophthalmus modestus* and the aquatic goby *Acanthogobius flavimanus* from nine estuaries in the Ariake Sea. Presence and distance of potential sources of these contaminants from sampling stations were also reported. The presence of contaminants in the environment was also assessed by measuring the levels of vitellogenin in males. Vitellogenin, a protein precursor of egg yolk, can be artificially induced in male fish by several chemicals, which are derived from organochlorine contaminants, and mimic the female hormone that induces its synthesis (Ohkubo *et al.* 2003). Vitellogenin levels in male mudskippers were much higher than in male aquatic gobies and several other species in literature (e.g. Soyano *et al.* 2010). A possible explanation could be related to the mudskippers' amphibious lifestyle, which may expose them more directly to organic sediments, while alkylphenols (e.g. nonylphenol) are one of many synthetic hormone disrupters in fish. Nonetheless, high levels of vitellogenin were also found in stations where alkylphenols were not detected, suggesting that other unmeasured contaminants such as natural and synthetic human female hormones (e.g. found in contraceptive pills) could be present in treated sewage effluent of these highly populated coastal areas.

### *Fluoride*

Fluoride is an important contaminant occurring in fertilizers and industrial effluents. It accumulates in fish skeletal tissues and is transferred through food webs. Toxicity to fish is both dose and duration-dependent. It acts as an enzymatic poison, interrupting glycolysis and protein synthesis. Since its toxicity decreases with water hardness ( $\text{Ca}^{++}$  and  $\text{Cl}^-$ ), its effect is usually less on marine and estuarine fishes (Camargo 2003).

Shaikh & Hiradar (1987) described the chronic effects of sub-lethal concentrations of fluoride on liver, renal and brain tissues of *Boleophthalmus dussumieri*. The most destructive effects were observed in the liver, with progressive degeneration and necrosis. They also observed that the same treatment markedly decreased the activities of acid and alkaline phosphatases and the total level of proteins in the liver and muscles (Shaikh & Hiradar 1988).

Nakata *et al.* (2006) measured the distribution of perfluorinated contaminants (PFCs) in sediments and aquatic organisms (including mudskippers) from shallow water and tidal flats of the Ariake Sea, Japan. Perfluorooctane sulfonate (PFOS) was dominant in shallow waters, and bioaccumulated at higher trophic levels (e.g. cetaceans and birds). On tidal flats, perfluorooctanoate (PFOA) was the most abundant compound. Relative to hydrophobic contaminants (e.g. organochlorines: Nakata *et al.* 2002), PFCs were significantly less abundant in sediments and tidal flat organisms, and PFOS levels in shallow water species were comparable or significantly higher than that for organochlorines. These results confirm that the aqueous phase is a major sink for polar PFCs, while organic sediments are the major sink of non-polar organic pollutants.

### *Radionuclides*

Patel *et al.* (1975) utilised *Periophthalmus schlosseri* [= *Periophthalmodon schlosseri* (Pallas 1770); to our knowledge, this species was never recorded from west Indian coasts] and other benthic species (fish, bivalves and crustaceans) to monitor the effects of radioactive effluents of an atomic research center near Bombay (= Mumbai, Gujarat, India). The authors found that  $^{137}\text{Cs}$ ,  $^{144}\text{Ce}$  and  $^{106}\text{Ru}$  rapidly accumulated in the sediments since regular discharge began. In particular,  $^{137}\text{Cs}$  was distributed throughout the whole sampled area, and bioaccumulated in muscle tissues of all the analysed organisms.

In the same area, Bangera & Patel (1984) measured the concentration of  $^{238}\text{U}$ ,  $^{226}\text{Ra}$ ,  $^{210}\text{Pb}$  and  $^{210}\text{Po}$  in the clam *Anadara granosa* and the mudskipper *Boleophthalmus boddaerti* (= *B. boddarti*). They found a higher concentration of the natural radionuclides in sediments, with a prevalence of  $^{210}\text{Po}$  and  $^{210}\text{Pb}$ , which were also bioaccumulating in the organisms' tissues.

More recently, Wang *et al.* (2003) simulated the dynamics of  $^{95}\text{Zr}$  in a controlled system including sediment, water and two biotic compartments (a gastropod, *Nassarius semiplicatus*; and a mudskipper, *Boleophthalmus pectinirostris*), and found that this radionuclide concentrated more rapidly in the gastropod than in the mudskipper.

### *Studies on different combinations of pollutants or complex effluents*



In some cases, the effects of effluents with an heterogeneous chemical composition or the distribution of different types of pollutants were investigated.

Chhaya *et al.* (1997a,b,c) studied the effects of effluent from dyeing and printing industries on *Periophthalmus dipes* (= *P. dipus* = *P. argentilineatus*). In the study on the sub-lethal effects on Na<sup>+</sup>, K<sup>+</sup> ATPase, Mg<sup>++</sup> ATPase, Ca<sup>++</sup> ATPase and total ATPases of the liver, brain and muscle, Chhaya *et al.* (1997a) found significant changes amongst ATPase activity levels for different exposure durations (2, 4, 6 days) within doses in all the investigated ATPases and tissues, except for Mg<sup>++</sup> ATPase in the brain. Dose effects of the effluents [0.1, 0.5, 1.0%; LC<sub>50</sub> (96 hr) = 1.72%] were significantly different only for the Na<sup>+</sup>, K<sup>+</sup> ATPase in the liver and muscle. In several cases, increases in ATPase activity at lower levels and longer exposure periods suggest adaptation to the toxicants present in the effluents (e.g. Cr; Lakshmi *et al.* 1990). In all other cases, significant inhibition in the activity levels was observed. Following the same experimental design and utilising the same contaminants and model species, Chhaya *et al.* (1997b) investigated the effects on the same ATPases in the gills and intestine, while Chhaya *et al.* (1997c) studied the effects on the acid and alkaline phosphatases in gills, intestine, liver, brain and muscle. In the former case, they found strong duration-dependent effects; in the latter, they found significant dose and duration-dependent effects in the gills, and only duration-dependent effects in other tissues.

In an attempt to establish ecotoxicological methods and find suitable biomarker fish models in Bangladesh, Al-Arabi & Goksøyr (1998) studied the responses of *Apocryptes bato* to several toxicants. The authors conducted a preliminary investigation on the effects of intraperitoneal injections of β-Naphthoflavone (BNF, 50 mg/kg), a polychlorinated biphenyl (PCB) mixture (Clophen A50, 20 mg/kg) and cadmium chloride (CdCl<sub>2</sub>, 2 mg/kg) on the activity and protein level of cytochrome P4501A (CYP1A), and found a persistent induction of this protein in the liver.

Lam & Lam (2004) reviewed the results of an investigation made by the CCPC (Centre for Coastal Pollution and Conservation in Hong Kong) on the concentration of pollutants in a sample of intertidal animals living on the tidal flats of the Mai Po Nature Reserve in Hong Kong, an important migratory stop-over site for shorebirds. The organisms sampled included a mudskipper (*Boleophthalmus pectinirostris*), two aquatic teleost species, three crustacean species and a number of polychaete species. Relative to the other organisms, *B. pectinirostris* had higher levels of Pb and Hg, the highest levels of total HCHs, heptachlor, DDE, DDT and total PCBs, and lower levels of other heavy metals, PAHs, and petroleum hydrocarbons (PHCs). The evaluated risk quotients (RQs) for the analysed organisms were at a level of concern (RQ ≥ 1) for most contaminant types, either on mudflats, or in mangrove forests, or both. In some cases, RQs were exceedingly high (e.g. for Cu and heptachlor, RQs > 10). In particular, PCBs, dieldrine, DDE and DDT were considered potentially harmful to waterbirds via ingestion of contaminated prey, especially mudskippers (*B. pectinirostris*). In fact, egrets, bitterns and herons (Ardeidae) are dominant consumers of mudskippers (Clayton 1993; G. Polgar, pers. obs.). More recently, Lam *et al.* (2008) assessed the risks of organohalogenated compounds on the eggs of the populations

of two ardeid bird species from three coastal areas in South China, predicting that concentrations of dioxin-like (coplanar) PCBs and  $\Sigma$ DDEs in the eggs would probably affect bird populations.  $\Sigma$ DDTs were the predominant and most abundant residues, while  $\Sigma$ PBDEs were more abundant in areas where rapid industrialization had occurred.

Kruitwagen *et al.* (2006) described the effects of pollution on natural populations of *Periophthalmus argentilineatus* living in differently polluted coastal areas in Tanzania. They reported the occurrence in natural populations of eye malformations (anophthalmia) which imply damage of earlier embryonic processes, an unprecedented case in natural fish populations.

### *Genotoxicology*

This branch of toxicology studies the effects of toxicants on the genome: i.e. contaminants that can disrupt function and structure at both organismal and ecosystemic level (e.g. Anderson *et al.* 1994).

Krishnaja & Rege (1982) conducted a baseline study on the mutagenic effects of mitomycin C (MMC) and heavy metals (Hg, Se, Cr) on the mitotic chromosomes of the gill cells of *Boleophthalmus dussumieri* (Cuv. and Val.) (= *B. dussumieri* Valenciennes, 1837 sensu Murdy 1989). These mudskippers, which are also extensively consumed in the Mumbai area, were selected amongst 20 other fish species for their favourable karyotype and cytological traits in different tissues, low incidence of spontaneous aberrations, easy and low cost maintenance in laboratory, relatively small size, tolerance to experimental conditions, and availability in large numbers throughout the year. Thus *B. dussumieri* was proposed as a suitable model for *in vivo* studies on the direct and indirect effects of mutagenic pollutants in the environment. The authors found that chromosomal aberrations and increased mitotic activity were induced by all the studied contaminants, with MMC inducing breaks near the centromere regions, and Se being the most toxic HM in direct exposure experiments (intramuscular injections). In indirect exposure assays (toxicants released in the water) Hg induced the largest number of aberrations. The authors concluded that considerable baseline knowledge was needed to bring this model to a standardised testing protocol for the study of genotoxicity of specific contaminants. In particular, baseline cell kinetic studies of this species was needed to better understand the occurrence of spontaneous and induced chromosomal aberrations; and further investigations of the chronic effects of indirect exposure. Nonetheless, apparently no significant progress in this direction has been made to date.

Feng *et al.* (2003) measured DNA breaks as a biomarker of exposure to benzo[a]pyrene [BaP, a polycyclic aromatic hydrocarbon (PAH) strongly suspected to be carcinogenic both in aquatic species and humans] in the liver of *Boleophthalmus pectinirostris*. The authors found that at 0.5 mg/l BaP, DNA breaks were significantly more frequent than that in controls after 12 hr of exposure, being increasingly more frequent until 7 days of exposure. Dose-response positive correlations were also found both at exposures of 3 and 7 days, even though the amount of damage at 0.2 mg/l was similar than at 0.5 mg/l, suggesting saturation of the effect at concentrations >0.2 mg/l. DNA breaks were not significantly repaired after 3 days at 0.5 mg/l

exposure plus 4 days in unpolluted water, indicating that *B. pectinirostris* might be an appropriate species for this type of biomarker assay.

Gadhia *et al.* (2008) recently extended the observations of Krishnaja & Rege (1982) to the effects on the gills of *Boleophthalmus dussumieri* through direct exposure to the antineoplastic antibiotics Bleomycin and Doxorubicin. The authors confirmed the suitability of this species as a cytogenetic model for *in vivo* detection of potential mutagens, finding dose and time dependent increases in chromosomal aberrations after treatment with all the antibiotics.

## **The lower Fly River and delta: A possible case study**

### *Introduction*

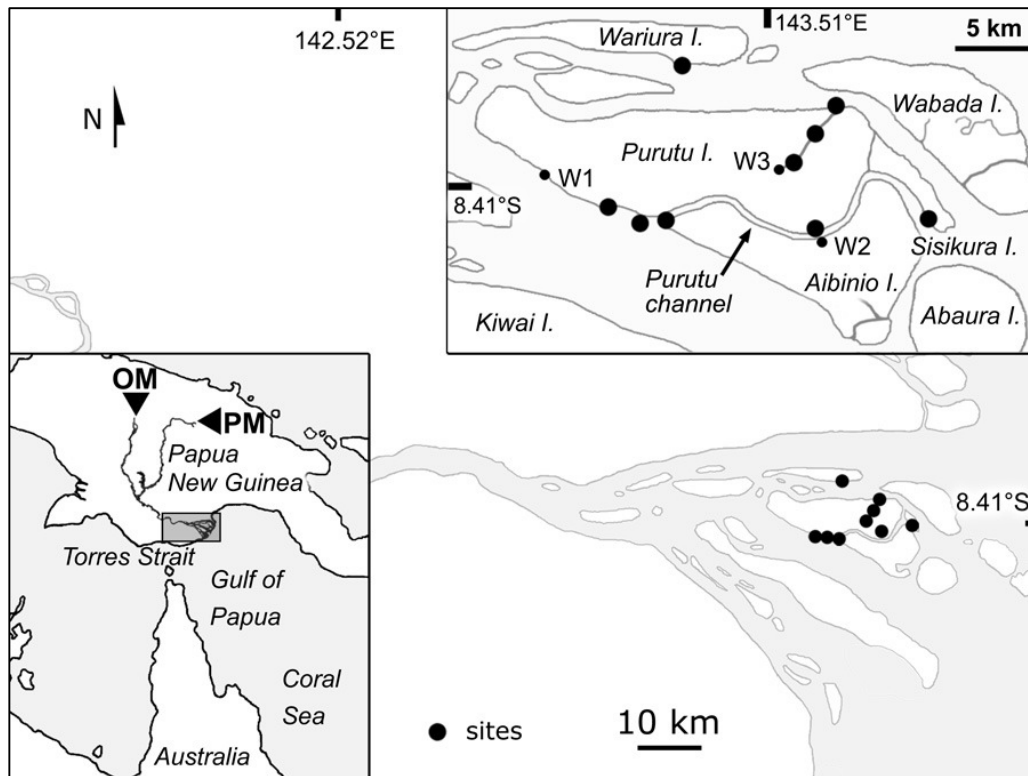
The Fly River is one of the largest basins in the Indo-West Pacific region: in terms of discharge, it ranks with the top ten tropical rivers (Alongi 1990). Its delta is tide dominated, with a minimal wave influence, except during monsoons (Dalrymple *et al.* 2003). These conditions are ideal for the formation of tidal mudflats, which in the northern side are colonised by extensive mangrove forests, which exhibit a marked zonation (Robertson *et al.* 1991).

The middle Fly River had been chronically and heavily impacted by intense mining activities, determining severe environmental issues of international concern (Van Zyl *et al.* 2002a,b). Peculiar geochemical conditions apparently reduce the biological availability of toxicants in the river's waters, and mining apparently impacts most on sedimentological and flooding cycles (Roberts 1999; Van Zyl *et al.* 2002a,b; Townsend & Townsend 2004). The lower Fly River and delta are much less affected, and maintain large and diverse mudskipper communities (Polgar *et al.* 2010). Nonetheless, mudskippers' peculiar behaviour and ecology makes them particularly prone to long-term accumulation of toxicants, due to their intimate contact with HM contaminated soft sediments. Mudskippers are also a consistent part of the traditional fishery of villages in the Fly delta, being utilised as baits and food. Therefore, it is suggested that the risk of bioaccumulation of toxic HM in mudskipper tissues in this area and potential health risks for human consumption cannot be ignored, and investigations are needed to fill this knowledge gap.

### *Material and Methods*

Several field surveys were made during September 2007 in the estuarine reaches of the Fly River and its delta, Western Province, Papua New Guinea (Fig. 2; Polgar *et al.* 2010). Mudskippers were collected from their burrows by hand, or with a handnet from the banks of rivers, creeks, and inside forests. The specimens were preserved in formalin and identified according to the available taxonomic keys (Larson & Takita 2004; Jafaar & Larson 2008; Murdy 1989).

Finally, interviews of people from Wapi village (Fig. 2), and observations of daily fishing activities documented the human use of several mudskipper species.



**Figure 2.** The Fly delta: sites visited and sampled. Upper panel: W3 was the most recent settlement of the Wapi village at the time of the study. It was located in the middle of a lowland forest, with easier access to the fresh water table, and at the end of a tidal creek. Two older settlements are present (W1, W2), which were gradually abandoned due to the excessive frequencies of tidal submersions and crocodile attacks (*Crocodilus porosus*). Lower panel: The Ok Tedi-Strickland-Fly river system and the locations of the two mines; shaded rectangle: area of study. OM: Oktedi Mine; PM: Porgera Mine.

#### *Human use of the mudskippers of the lower Fly River and delta*

Ten mudskipper species were collected. These were *Oxuderces wirzi* (Koumans, 1938); *Scartelaos histophorus* (Valenciennes, 1837); *Boleophthalmus caeruleomaculatus* McCulloch & Waite, 1918; *Periophthalmodon freycineti* (Quoy & Gaimard, 1824); *Periophthalmus darwini* Larson & Takita, 2004; *Periophthalmus novaeguineensis* Eggert, 1935; *Periophthalmus takita* Jafaar & Larson, 2008; and *Periophthalmus weberi* Eggert, 1935. Two more species, *Boleophthalmus* sp. and *Periophthalmus* sp., did not correspond to any key and are currently being described.

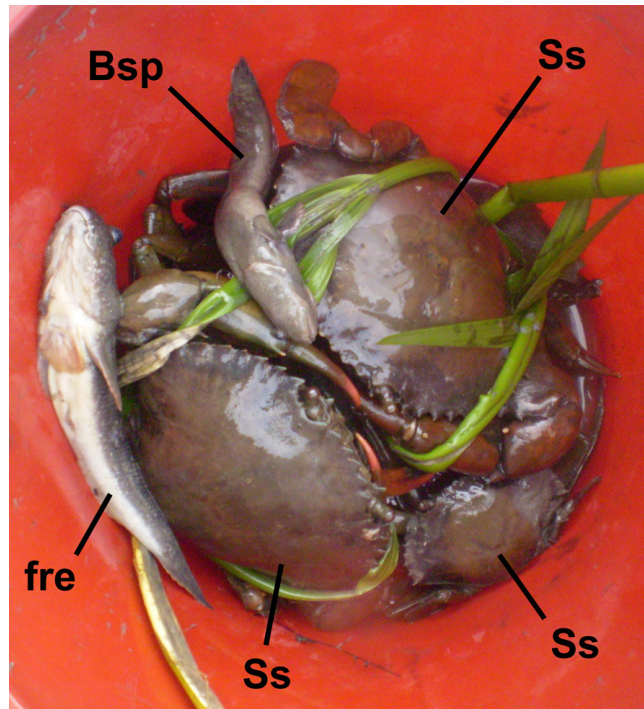
Interviews and analysis of several fish catches from the Fly River delta (Wapi villages, Fig. 2) showed several mudskipper species were extensively used as food items or as baits (Table 1).

**Table 1.** Human use in the Wapi village. Vernacular names of mudskipper species used by the people of the Wapi village. TL<sub>max</sub>: maximum recorded total length; Food or bait: usage by the Wapi people: F = used as food; B: used as bait; N = not used.

Species	Vernacular name	TL <sub>max</sub> (cm)	Food or bait
<i>Periophthalmus darwini</i> Larson & Takita, 2004	sakomo	5‡	N
<i>Periophthalmus novaeguineensis</i> Eggert, 1935	sakomo	5*	N
<i>Periophthalmus</i> sp.	sakomo	5§	N
<i>Periophthalmus takita</i> Jaafar & Larson, 2008	nebesokera	9†	N
<i>Periophthalmus weberi</i> Eggert, 1935	paraguamo#	12§	N
<i>Periophthalmodon freycineti</i> (Quoy & Gaimard, 1824)	genora	28§	F
<i>Boleophthalmus caeruleomaculatus</i> McCulloch & Waite, 1918	ebanea	20**	F/B
<i>Boleophthalmus</i> sp.	poti	15§	F/B
<i>Scartelaos histophorus</i> (Valenciennes, 1837)	seekakowea	17¥	N
<i>Oxuderces wirzi</i> (Koumans, 1937)	canipo	10**	F

#the courting male of *P. weberi* is named ‘blue paraguamo’. ‡Larson & Takita 2004 and Jaafar & Larson, 2008; \*Larson & Takita 2004; †Allen *et al.* 2002; §G. Polgar, this study; \*\*Murdy 1989; ¥Rainboth 1996.

Fishermen capture them on mudflats, mud banks, and in front of the fringing forest along the coast, removing them from their burrows by hand, as they do for the mud crab *Scylla serrata* (Forsskål, 1755) (Fig. 3). The 1:1 correspondence between several vernacular names and scientific species is noticeable, particularly in the case of the discrimination between *P. weberi* (sexes are identified as separate species) and other species of *Periophthalmus* (Table 1). Fishermen are familiar with the species-specific habitat distribution of the mudskippers.



**Figure 3.** A typical catch by Wapi fishermen on a mudflat in front of a mangrove forest (Sisikura I.). Bsp: *Boleophthalmus* sp.; fre: *Periophthalmodon freycineti*; Ss: *Scilla serrata*. Notice how the crabs are tied up with grasses, to be safely transported.

#### *Pollution and potential health risk issues*

The Ok Tedi Mine (Ok Tedi Mining Limited: OTML) has been active since 1984, initially mining for gold, and then copper (from 1987; Van Zyl *et al.* 2002a). The mine is located on Mount Fubilan, in the Star mountain range, about 1,800 m above sea level, close to the headwaters of the Ok Tedi River, a tributary of the Fly River. The Ok Tedi joins the Fly River at about 840 river km from the delta (Fig. 2). About 80,000 metric tonnes of mine tailings, toxic effluents and 120,000 metric tonnes of waste rocks are released daily into the Ok Tedi River (Van Zyl *et al.* 2002a). As a consequence, the suspended load of the Ok Tedi increased from 100 ppm to 450-500 ppm since the beginning of mining activities (Minerals Council of Australia 2000).

The Porgera Gold Mine is located in Enga Province, about 130 km north-west of Mount Hagen, and has been active since 1990. It discharges its tailings (about 15,500 tons per day) and rocks from erodible waste dumps (30,000-40,000 tons per day) in the Porgera-Strickland River System (Van Zyl *et al.* 2002b). The Strickland River is another important tributary of the Fly River. In 1999, at the confluence with the Tumbudu River, the mine sediment load accounted for about 25-33% of its total sediment load (PJV 1999).

As a comparison, the average total suspended sediment discharge of the Fly River was estimated to be about 345,000 tons per day (Salomons & Eagle 1990).

Mine tailings contain toxic compounds, mainly heavy metals (HM), such as copper, arsenic, lead, zinc, and mercury. According to the Minerals Council of Australia (2000), pollution effects

are buffered both by the alkaline waters of these rivers, which would prevent release of HM from particulates, and by their high organic carbon content, which would make HM not bio-available. Studies conducted in the environment and on finfish, bivalves and crustaceans, showed that HM levels in the animals' tissues would not be at dangerous levels (World Health's Organisation guidelines) for human health in the lower Fly River and its delta (Minerals Council of Australia 2000).

OTML reports that the highest impact of mining activity is sediment accumulation on the river bed (aggradation), which increases the frequency and duration of inundations of flood plains and lowlands by over-bank flooding (BHP 1999). This results in hundreds of km<sup>2</sup> of diebacks of the flood plain vegetation in the Ok Tedi and middle Fly catchments (Van Zyl *et al.* 2002a). According to these reports, this impact is highest in the Ok Tedi River and middle Fly River, lower in the lower reaches of the Fly and much less significant in the delta.

Nonetheless, other reports documented cases of periodical or exceptional releases of significant amounts of particularly dangerous pollutants in the river caused by mining activities or accidents (Roberts 1999), such as cyanides, used to extract gold and other precious metals. Cyanides neither biomagnify nor transfer through food webs, and are seldom persistent in the environment. Nonetheless, fishes are extremely sensitive to cyanides, with both acute and chronic effects, such as teratogeny, yolk sac dropsy, malformations, liver necrosis, behavioural and reproductive impairment, increased susceptibility to predation, osmoregulatory disturbances, and altered growth patterns (Eisler & Wiemeyer 2004). Mudskippers like *Boleophthalmus boddarti* are more tolerant to cyanides, due to a surplus of cytochrome oxydase, the capability to accumulate ammonia and inducible detoxifying mechanisms (Chew *et al.* 1998).

In the lower Strickland, 7-10 fold increases of the concentrations of zinc, arsenic and copper were found in catfish tissues; those of the zinc exceeded the 1989 Australian National Health and Medical Research Council maximum residue limit (PJV 1999). Higher mercury concentrations were found in consumers of fish from Lake Murray, at the confluence of the Strickland River with the Fly River, relative to non-fish consumers from the more polluted areas of the upper Strickland River (Abe *et al.* 1995), suggesting that bioaccumulation takes place also in relatively unpolluted waters. Moreover, Acid Mine Drainage (AMD), created by the leaking of sulphide-rich orebody on failing waste dumps and dredge materials, may rapidly release drastic amounts of toxic heavy metals into ground and surface waters (OTML PRG 2000).

The presence of intensive industrial activities along the river and in peculiar ecosystems where mudskippers live may suggest that consumption of these fishes in the Fly River delta can potentially cause health problems. In particular, the large carnivorous species *Periophthalmodon freycineti* is regularly consumed by inhabitants in the Fly delta, thus being the first candidate for bioaccumulation and potential health issues. Nonetheless, no study ever measured the levels of contaminants in tissues of mudskippers from the Fly River and its delta (OTML, Environment Dep., pers. comm. 2007).

The extensive traditional use of mudskippers as baits and food by villagers in the Fly River delta and the regular release of potentially dangerous contaminants into the Fly River from



intense mining activity upriver suggest that risks of bioaccumulation of pollutants in mudskipper tissues may be present in this area. Other aquatic species which are routinely utilised as biomonitors in eco-toxicological studies (clams, finfish, crustaceans), may actually contain lower levels of HM than in the tissues of mudskippers (Lam & Lam 2004).

## Conclusion

For their abundance and availability, easy and inexpensive maintenance, and considerable tolerance to water quality changes, mudskippers have been frequently used both in aquaculture and in eco-toxicological studies. Therefore, in polluted coastal areas where mudskippers occur the potential for bioaccumulation and human health issues for direct or indirect consumption is reasonably substantial.

Mudskippers can absorb and concentrate many different pollutants released into the environment by industrial, agricultural, domestic and transportation activities, which cause physiological, histological, and embryological damage. In particular, mudskippers are known to accumulate higher concentrations of some toxic compounds (e.g. DDT and some heavy metals) in their tissues, relative to other aquatic and benthic species. In fact, their robustness to environmental stressors and tolerance to many contaminants give them the capacity to be chronically exposed to toxicants without significant acute effects, while their relatively low trophic status makes them less prone to biomagnifying toxicants. These factors make them good models for sub-lethal ecotoxicological studies.

Therefore, apart from the devastating effects of habitat destruction, local extinctions of mudskipper populations exclusively due to moderate levels of pollution are unlikely. On the other hand, better knowledge of the species-specific chronic effects (e.g. bioaccumulation, endocrine and metabolic changes) of different contaminants in this diverse group would assist in better use of representatives of this group as models for ecotoxicological studies of soft bottom intertidal tropical and subtropical systems. In particular, the different trophic characteristics of different mudskipper species (carnivores, omnivores, herbivores) and their habitat differentiation and ecological partition would allow contaminant assessments both along the intertidal gradient, and at different trophic levels.

In fact, like many environmental parameters, such as salinity, sediment grain size and organic matter content, and frequency of submersion (Raffaelli & Hawkins 1999), anthropogenic impacts are likely to vary considerably along the intertidal zone. A study conducted in an intertidal mangrove ecosystem (Kruitwagen *et al.* 2007b) suggested that the hydrophobic fraction of the effluents of a textile industry decreased from land to sea, probably due to adsorption onto the highly organic mangrove sediments (Chapman *et al.* 1998). Since this fraction had higher acute embryotoxicity than the polar fraction (e.g. Kruitwagen *et al.* 2007b), this would suggest a differential impact of this type of pollution along the intertidal zone. Habitat destruction is also invariably more intense from land to sea (Polgar 2008; Polgar & Sasekumar, 2010), while the damaging action of oil spills follows an inverse path, from sea to land (Chaw *et al.* 1993).

In soft bottom tropical and subtropical systems, mudskippers are amongst the very few vertebrate intertidal residents (*sensu* Gibson 1999). They inhabit these habitats over their embryonic and post-larval life, and can live up to 5-7 years (Kruitwagen *et al.* 2006; Nanami & Takegaki 2005; Etim *et al.* 1996). Such characteristics, together with the relatively low trophic status of these mudskippers (secondary consumers: Kruitwagen *et al.* 2007a), suggest that some contaminants released into the sea may have early effects on these fishes (e.g. malformations)

relative to species at higher trophic levels or that spend less time in the intertidal zone (marine and terrestrial visitors). Therefore, in spite of their tolerance to aquatic toxicants, these attributes would make them efficient early warning model species in such ecosystems, prompting remediation before the effects of pollution spread to terrestrial top predators (shorebirds) or adjacent marine ecosystems.

For that which concerns our preliminary survey in the lower Fly River and its delta, we argue that the presence of severe anthropogenic impacts resulting from continued and intense mining activities upriver should prompt periodic assessments of possible health risks to the local people. This can include the use of various biomarker endpoints in mudskippers as part of an environmental monitoring program to assess the effect of heavy metal levels (e.g. As, Hg, Cu, Zn, Pb) on mudskippers inhabiting the Fly delta. Finally, we hope that future OTML reports will be published in peer reviewed journals, thus making these data accessible to the scientific community, to allow for a better understanding of the impacts of anthropogenic pollution on this and other similar ecosystems.

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