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Ecology and toxicology of mangrove fauna in Tanzania
With particular reference to the mudskipper
Ecology and toxicology of mangrove fauna in Tanzania
with particular reference to the mudskipper

Een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen,
op gezag van de Rector Magnificus prof. dr. C.W.P.M. Blom
volgens besluit van het College van Decanen
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door

Guus Kruitwagen
geboren op 30 maart 1977
te Rheden
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**On the artwork on the frontcover**

The artwork on the cover was painted by Katongo, who is one of the followers of the Tingatinga painting style that was locally developed. This style was named after Edward Saidi Tingatinga, a self-taught painter who used brightly coloured bicycle-paints. Tingatinga paintings often depict everyday Swahili scenes and have become a medium that is used to raise public awareness for issues of general concern.

The painting on the cover of this thesis depicts Tanzanian scenes in relation to environmental pollution. The signposts on the painting are typical writings that can be found all over the city of Dar es Salaam. From top to bottom they state:

- **Usiweke takataka eneo hili** (Do not leave your waste in this spot)
- **Weka jiji lako safi** (Keep your city clean)
- **Usikae eneo hili kama si muhusika** (Do not sit here if you are not authorised)
- **Usichafue mazingira** (Do not pollute the environment)
## CONTENTS

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>General introduction</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 2</td>
<td>Relative importance of mangroves as a feeding habitat for nekton from adjacent mudflats in a Tanzanian estuary</td>
<td>17</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>Influence of morphology and amphibious lifestyle on the feeding ecology of the mudskipper (<em>Periophthalmus argentilineatus</em>)</td>
<td>35</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>Status of pollution in mangrove ecosystems along the coast of Tanzania</td>
<td>51</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Changes in morphology and growth of the mudskipper (<em>Periophthalmus argentilineatus</em>) associated with coastal pollution</td>
<td>67</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Developmental toxicity in zebrafish embryos (<em>Danio rerio</em>) exposed to textile effluents</td>
<td>85</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>General discussion</td>
<td>101</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>109</td>
</tr>
<tr>
<td>Summary / Samenvatting / Muhtasari</td>
<td></td>
<td>121</td>
</tr>
<tr>
<td>Acknowledgements / Dankwoord / Shukrani</td>
<td></td>
<td>129</td>
</tr>
<tr>
<td>Curriculum vitae</td>
<td></td>
<td>133</td>
</tr>
</tbody>
</table>
Chapter 1

General introduction
GENERAL INTRODUCTION

Mangrove forests form an important ecosystem from a biological as well as an economic point of view. The ecosystem is, however, experiencing increasing pressures from human activities. This thesis describes how and to what extent the ecology and physiology of the mudskipper (Periophthalmus argentilineatus Valenciennes), a characteristic species of the mangroves of Tanzania, is affected by coastal pollution.

Mangroves

Approximately 35 percent of the world’s original mangrove cover has been lost due to conversion for aquaculture, salt production, residential, industrial or tourist development, logging for timber, fuel wood, charcoal production, and pollution (Wells et al., 2006). At present the total area of mangrove forest worldwide is estimated between 167,000 km$^2$ (Valiela et al., 2001) and 181,000 km$^2$ (Spalding et al., 1997).

The mangrove ecosystem is among the most valuable ecosystems in terms of economical benefits expressed per square kilometre: in a recent report of the United Nations Environment Programme it is estimated that the mangrove ecosystem represents an annual value of 200,000 to 900,000 US dollars per square kilometre of mangrove forest (Wells et al., 2006). The indirect benefits of mangrove forests are diverse; they provide shoreline protection (regulation of tidal/wave action) and are of cultural importance (e.g. tourism) (Wells et al., 2006). Mangrove forests also support other important ecosystems, such as coral reefs, by providing protection and feeding and breeding opportunities both to mangrove residents and temporary migrants from adjacent coastal ecosystems, as is illustrated by the functioning of the mangrove habitats as a nursery for juveniles of reef and seagrass species (Laegdsgaard and Johnson, 1995; Robertson and Blaber, 1992). The ecological function of the mangroves is also thought to reach beyond the boundaries of the mangrove forests through the export of nutrients from the forests via water movements (Lee, 1995; Werry and Lee, 2005). More direct functions of the mangrove forests for the local populations mostly concern the provisional functions of small scale fishery and the supply of natural products, such as wood, tannins for treatment and conservation of products, and traditional medicines (Hogarth, 1999; Lugo and Snedaker, 1974; Semesi, 1998).

The mangroves in the intertidal zone are dominated by specialised trees (in East-Africa the main species are Avicennia marina, Brugheria gymnorrhiza, Ceriops tagal, Rhizophora mucronata, and Sonneratia alba), which have adapted to brackish waters and to sediments that are waterlogged and hypoxic as a result of prolonged tidal inundation (Hogarth, 1999). These mangrove species have developed specialised root structures: some mangrove species have stilt- or prop-roots, which support them on the semi-liquid sediments, while others have pneumatophores, erect root elements that facilitate aeration of the roots in the hypoxic substrate (Hogarth, 1999; Lugo and Snedaker, 1974). Mangrove roots and pneumatophores also trap floating detritus and slow water movements to the point where suspended particles from circulating surface waters set out (Wolanski et al., 1992; Woodroffe, 1992). This effect is further enhanced
by root hairs below the muddy surface, which bind and stabilise particles in the finely
grained organic mangrove substrate (Harbison, 1986; Wolanski et al., 1992; Woodroffe,

While the basis of the mangrove ecosystem is formed by the mangrove trees,
mangrove communities also contain rich assemblages of invertebrate and vertebrate
species, both terrestrial and aquatic (Hogarth, 1999; Kathiresan and Bingham, 2001).
Among these species is a number of mangrove residents that show ecological,
physiological and morphological adaptations to the variable conditions in the intertidal
zone of the mangroves, including fiddler crabs and mudskippers (Clayton, 1993;
Hogarth, 1999). The latter will be described in more detail below.

**Mangroves and pollution**

Mangroves are particularly at risk of accumulating pollutants, as rivers and streams
intersecting the coastal areas carry large quantities of liquid wastes, and to a lesser
extent solid wastes, into the fragile coastal mangrove ecosystem. In addition, the
“muddy” and “smelly” mangroves are often considered unimportant by the local
population, which leads to the deliberate dumping of anthropogenic wastes directly
into the mangroves (Semesi, 1998).

The mangroves and their muddy sediments not only provide a physical trap for
fine particles, but also a chemical trap for dissolved pollutants, which is enhanced by
the presence of a rich bacterial flora that favours precipitation and immobilisation of
pollutants from overlaying waters. These sediments are predominantly anoxic and fine
particulate, have a high pH, and are rich in organic matter, sulphides, as well as iron
complexes (Hogarth, 1999; Kathiresan and Bingham, 2001; Tam and Wong, 1999; Tam
and Yao, 2002). Organic pollutants such as polychlorinated biphenyls, organochlorine
pesticides, and petroleum hydrocarbons, are characterised by a low solubility and high
hydrophobicity. The fine particulate sediments and organic matter in the mangrove
environment provide extensive surfaces for adsorption of these organic pollutants,
leading to accumulation in sediments and biota (Ke et al., 2005; Tam and Yao, 2002).
The absorption of (heavy) metals, on the other hand, is favoured by the anoxic
conditions that prevail in the mangrove substrates; in an anoxic situation metals rapidly
bind to sulphates to form sulphide complexes that precipitate. Consequentially,
pollutants from wastewaters, which are released directly into the mangroves, imported
via freshwater systems or via tidal action, are retained and accumulated in the
mangrove sediments (Chu et al., 1998; Clough et al., 1983).

Several researchers have quantified specific pollutants in African coastal
ecosystems (e.g. Biney and Ameyibor, 1992; Engdahl et al., 1998; Hamza-Chaffai et al.,
1996; Kamau, 2001; Wandan and Zabik, 1996). In these studies elevated concentrations
of pollutants have been found in sediments in association with urban and industrialised
areas. Few studies have given attention to the accumulation effects in organisms (e.g.
Guyonnet et al., 2003; Okoye, 1991; Otchere, 2005; Marshall and Rajkumar, 2003). The
implications of pollution levels along the African coasts for ecological and physiological
functioning of organisms have received even less attention (an exception is formed by Guyonnet et al., 2003).

**Tanzania**

Tanzania’s relatively pristine coastline at the Western Indian Ocean is formed by sandy beaches, coral reefs, seagrass fields and mangrove forests; the mangroves cover about 1,272 square kilometres (Taylor et al., 2003). The attraction of the natural riches of the coastal ecosystems has led to the development of human settlements along the coast. In recent years, the coastal region of Tanzania in general and the country’s largest city Dar es Salaam in particular, has experienced increasing urbanisation and industrialisation (National Bureau of Statistics, 2002). This clustering of human activities increases pressure on the natural systems in the region by exploitation of natural resources, conversion of lands to urban areas and agricultural lands, and the production of increasing amounts of anthropogenic wastes. In the last two decades the urbanised area of Dar es Salaam has seen a substantial increase in pollution levels of soil, water and air (Bennet et al., 2005; Mashauri and Mayo, 1989; Mayo and Mashauri, 1992). Pesticides have for example been found to accumulate in agricultural products (Ndengerio-Ndossi and Cram, 2005), while heavy metals have accumulated in mangrove sediments and resident fauna along the coast of Tanzania (De Wolf et al., 2001). The abundant small-scale industries are generally characterised by low levels of protection for personnel and environment and have been associated with health problems of workers and the surrounding communities (Rongo et al., 2004; Yhdego, 1992; Yhdego, 1995).

Consequences of pollution for flora and fauna are, however, very difficult to predict from contamination levels alone, since the bioavailability and toxicity of contaminants is highly dependent on the presence of other chemicals and environmental parameters such as temperature, salinity, pH, conductivity, and oxygen levels (Hall and Anderson, 1995; Merian, 1991). Therefore, detailed investigations on the occurrence of pollution effects in biota are required. This is particularly relevant in the intertidal mangrove forests, which are characterised by large chemical and physical variations in space and time (Kathiresan and Bingham, 2001), and where for example physical disturbances such as bioturbation, can result in oxidation of sediments and subsequent remobilisation of precipitated metals from the surface sediments (Harbison, 1986). In situations where contamination sources are diffuse or unknown, assessment of the condition of resident biota can be a helpful and comprehensive indicator of the toxicity of the total mixture of major pollutants and micro-contaminants present in an ecosystem.

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1 The toxicity of metals depends on the chemical speciation, i.e. the form of appearance of the metal, with reduced forms being more bioactive/dissolvable and hence more toxic than oxidised forms (Merian, 1991).
Mudskippers

The peculiar-looking mudskippers are amphibious fish that are characteristic for mangrove forests and mudflats (figure 1). These species have adapted their morphology and physiology to life in the changeable mangrove habitat and occur in mangroves throughout the Indo-Pacific and along the coast of West-Africa. The barred mudskipper (Periophthalmus argentilineatus Valenciennes) is the most common mudskipper species in Tanzania. This mudskipper is an ideal bioindicator for effects on the mangrove ecosystem due to its highly specialised life style and the fact that its dispersion is not influenced by tides, season, or life stage; it is one of the few vertebrate-species that completes its whole reproductive cycle in the mangrove system. Hence, mudskippers are potentially exposed to contaminants in the mangrove habitat throughout their life via ingestion of contaminated sediments or food items, diffusion across respiratory surfaces, and through body surfaces that are in direct contact with contaminated sediments or water. The mudskippers are assumed to be a top predator, so it is highly likely that any accumulation effects of pollutants will appear in this species. As this species has no commercial value, it is not likely to be affected by local fisheries and therefore its distribution will be largely determined by environmental factors.

Mudskippers have developed their skin into an important accessory air-breathing organ by enhanced vascularisation of the dorsal and buccopharyngeal skin (Schöttle, 1932; Suzuki, 1992). While mudskipper gills follow the basic pattern of the gills of teleost fishes (‘bony fish’), the filaments are shorter than in fully aquatic species, presumably to avoid coalescence of the gill lamellae in air (Low et al., 1990). The pelvic and pectoral fins are modified to facilitate locomotion on dry land: the pelvic fins stand in one plane and have partially been joined to form a disc that provides suction to cling to surfaces, while the pectoral fins have a strong musculature that allows the fish to stand on the fins. The fish uses the fins in a crutching locomotion on dry land, but can also “skip” over land or water surfaces by twitching its tail (Stebbins and Kalk, 1961; Swanson and Gibb, 2004). In mudskippers the swimbladder, which provides buoyancy in most fishes, is absent to enable the benthic existence. Consequently, mudskippers sink when they stop swimming, unless they fill their mouth cavity with air, enabling them to float (see figure 1) A prominent modification concerns the eyes, which are placed closely together and are dorsally protruding. The eyes are spherical and contain a flattened lens to prevent the vision becoming myoptic in air (Graham and Rosenblatt, 1997).

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2 Mudskippers, just as most modern amphibious fishes, belong to the subclass Actinopterygii and the suborder Gobioidei. This suborder appeared during the Eocene (37-54 million years ago), relatively recent from an evolutionary point of view (Graham, 1997). Mudskippers are phylogenetically unrelated to the lobe-finned Sarcopterygian fishes (Long and Gordon, 2004). The first invasion of land by fishes was led by Sarcopterygians in the late Devonian (~360 million years ago) that developed four limb-like extremities for locomotion. Nowadays, the coelacanth (Latimeria chalumnae) is one of two known species of the lobe-finned Sarcopterygian fishes that are still alive.
Eye position and shape provide good vision over a large angle when the fish is active on land (Clayton, 1993; Stebbins and Kalk, 1961).

Mudskippers reside on the muddy mangrove sediments that are exposed during low tide and are highly territorial (Brillet, 1975). They actively dig tunnels and burrows that can be up to 1 m in depth (Brillet, 1976). During high tide the mudskippers take refuge in these deep burrows from predators and currents. When the outgoing tide retracts the water from the mangroves, the fishes appear in the turrets that form the entrances to the burrows. If the conditions are favourable, the mudskippers leave the turrets to actively hunt prey on the exposed muddy sediments (Colombini et al., 1996). At the lower part of the burrow a breeding chamber is created, where eggs are attached to the ceiling. In these submerged breeding chambers the eggs are protected and nurtured by the male (Brillet, 1976). Only after onset of the larval-juvenile metamorphosis, during which the eyes migrate to a dorsal position and the strong musculature of the pectoral fins develops, do young mudskippers become amphibious and emerge from the burrows (Brillet, 1976).
Outline of this thesis

The aim of this research is to get insight in the effects of anthropogenic pollution in mangrove forests along the coast of Tanzania by an ecotoxicological approach (see box 1). To get a broad overview of impact of pollution on the mangrove fauna, investigations were aimed at different levels of biological organisation of the barred mudskipper (*P. argentilineatus*). In addition, ecological relationships were studied to assess potential risks for other mangrove biota.

In chapter 2 stable isotope techniques are used to investigate trophic relationships within the Mtoni mangrove community.

To obtain detailed knowledge on the feeding ecology of the mudskipper and the relations with other species in the same feeding guild, the gut contents and stable isotope signatures of mudskippers are studied in chapter 3.

In chapter 4 the levels of different pollutant classes in sediments and fish tissues are quantified. The potential environmental impact of the concentrations of the pollutants is discussed.

The growth and morphology of mudskippers from different sampling sites are compared in chapter 5 to investigate effects of pollution on individual mudskippers and on population level.

Chapter 6 focuses on the specific effects of an industrial effluent on the early development of fish in a standardised bioassay. The aim of this chapter is to verify whether observed effects in the field can be attributed to characteristics of this specific effluent.

The observed effects of pollution in the mudskipper populations are summarised in chapter 7 and discussed in relation to the ecology of the mudskippers and mangrove communities.
When organisms come into contact with chemical pollutants, toxic effects are not always inevitable as such effects can be prevented or restricted through rapid excretion, biological transformation, or sequestration of the pollutant in tissues with limited metabolic activity (Lawrence and Hemingway, 2003). If contaminants cannot be excluded or excreted, undesirable effects can occur at all levels of biological organisation, ranging from the molecular to the ecosystem level. The level(s) on which pollution effects occur(s) is among others dependent on pollutant concentrations, duration and history of exposure, type and complexity of contaminants, and the mode of uptake in biological systems (Connell et al., 1999). The sensitivity of biota to environmental contaminants is highly dependent on the developmental, reproductive and nutritional status of an organism (Connell et al., 1999, Lawrence and Hemingway, 2003). The range of levels at which pollution effects can occur is illustrated by the figure below. It shows that toxicological effects of pollutants are initially restricted to low levels of biological organisation. When exposure time increases and compensatory responses on the lower levels have failed, toxicological effects can be manifested on higher levels (Bickham et al., 2000, Connell et al., 1999). When adverse effects extend to higher levels of biological organisation, the biological response is more complex, more variable, and eventually becomes prominent at the level of the ecosystem (Connell et al., 1999).
Chapter 2

Relative importance of mangroves as a feeding habitat for nekton from adjacent mudflats in a Tanzanian estuary

G. Kruitwagen, I. Nagelkerken, B.R. Lugendo, Y.D. Mgaya, S.E. Wendelaar Bonga
ABSTRACT Mangroves are assumed to support adjacent coastal habitats through outwelling of nutrients. Proof to support this hypothesis is, however, scanty and only concerns a limited number of regions worldwide. In this study, stable isotope signatures are used to determine the contribution of food sources from a fringing mangrove on the coast of Tanzania to the foodweb of adjacent mudflats. It is shown that a clear differentiation exists between mangrove and mudflat signatures at the level of the primary producers. The stable isotope signatures of the invertebrates reveal a gradient in dependency on mangrove and mudflat sources, which is strongly connected to the ecology of the different species. Due to the high tidal range in the Western Indian Ocean, the amphibious mudskipper is the only fish species to which the mangroves are accessible during low tide. As a consequence, the stable isotope results show that the mangroves have limited importance to mudflat fishes as a feeding habitat. It is concluded that carbon from the fringing mangroves of the Tanzanian coast has minor contribution to the functioning of the mudflat community.

INTRODUCTION

Mangrove forests are found in estuaries and on sheltered mudflat habitats along tropical and subtropical shores. These intertidal forests are considered to be closely connected to adjacent coastal habitats through the exchange of resources and biota (Hogarth, 1999). The highly productive mangrove habitats are thought to export nutrients to other habitats, where they are available for assimilation in the local foodweb (Connolly et al., 2005; Guest et al., 2004). Mangrove leaves, zooplankton, and detritus, that are carried away from the mangroves by the water currents are thought to be an important source of carbon for the adjacent habitats (Bouillon et al., 2000; Lee, 1995). This net outflow of resources is generally referred to as outwelling (Lee, 1995). The extent to which the mangroves are connected to the adjacent ecosystems via these exchange processes is the focus of an ongoing discussion among marine biologists (see e.g. Rodelli et al., 1984; Lee, 1995; Marguillier et al., 1997; Bouillon et al., 2000; Bouillon et al., 2004; Connolly et al., 2005).

Mangroves are also linked to adjacent systems through migration of fishes and crustaceans, which are thought to move to the mangroves in search of food and shelter. Studies on the abundances of fishes have shown that particularly juvenile specimens can be found in higher densities in the mangroves than in other (estuarine) habitats (Nagelkerken and van der Velde, 2002; Robertson and Blaber, 1992; Robertson and Duke, 1987). Based on these observations it has been suggested that mangroves provide juveniles of reef and seagrass species with abundant food sources, shelter, protection from predators, and the possibility of ontogenetic migration to adult habitats (Beck et al., 2001; Robertson and Blaber, 1992). Hence, by offering these benefits during the early life stages of fishes the mangrove habitat is assumed to have a nursery function by increasing chances of survival and enhanced production of species (Beck et al., 2001; Robertson and Blaber, 1992). Yet, the value of the different functions differs between species (Laegdsgaard and Johnson, 2001; Verweij et al., 2006). The difference in functions provided by mangroves may influence migration of nekton between coastal habitats and thus influence the exchange of carbon and nutrients.
The connectivity between mangroves and other coastal habitats through outwelling and migration depends on abiotic factors including the tidal amplitude, geomorphology, and hydrology of coastal habitats (Lee, 1995; Nagelkerken and van der Velde, 2004a), as well as on biotic factors such as species richness and mangrove forest structure (Lee, 1995). Consequentially, the extent of connectivity of habitats through both processes may differ between coastal ecosystems (Lee, 1995; Nagelkerken and van der Velde, 2004b; Robertson and Blaber, 1992).

The Mtoni Estuary on the Indian Ocean shores of Tanzania is subject to diurnal tides ranging up to 5 m in height (Richmond, 1997). As a result of this large tidal amplitude, the water retracts completely from the fringing mangroves with outgoing tides. While this tidal movement of water potentially forms a vector for outwelling from the mangroves, the same movement restricts the use of the mangrove habitat by benthic species, which may influence the value of the mangrove habitat for these species (Baker and Sheaves, 2005; Sheaves, 2005).

In this study we make use of stable carbon and nitrogen isotope analysis to assess the magnitude of connectivity of the mangroves in the Mtoni Estuary with the adjacent mud flat habitat. Stable isotope signatures of organisms give insight in the food sources that have been assimilated in the preceding period ranging from weeks to months (Gearing, 1991). Carbon signatures provide a means to determine the habitat from which a species predominantly derives its food sources, while nitrogen signatures reflect the trophic level at which an organism is found in a foodweb. Consequently, combined use of carbon and nitrogen isotopes enables reconstruction of the relationships within the foodweb and assessment of linkages between habitats and foodwebs.

**MATERIALS AND METHODS**

The study was executed in the Mtoni Estuary near Dar es Salaam (figure 1). The central part of the estuary is a shallow basin (ca. 1 m deep at low tide), which has a narrow opening to the Western Indian Ocean and receives freshwater from two small streams, the Kizinga and the Mzinga. The estuary is eutrophicated as a result of input of nutrients from Dar es Salaam. The area has a high diurnal tidal amplitude of 5 m. Consequentially, all brackish water is drained from the intertidal fringing mangrove forests as the tide goes out. Extensive mangrove stands are found along the estuarine sections of the Kizinga and Mzinga Streams and narrow mangrove fringes occur along the estuary basin. The sediments of the mangroves consist of a very fine muddy substrate, while the sediments of the estuary basin and its mudflats have a larger grain size and are mostly sandy. The mangrove stands are dominated by *Sonneratia alba*, *Avicennia marina* and *Rhizophora mucronata*. 
Fishes were collected in the basin of the estuary during day-time from November 2003 to January 2004. At low tide, the inundated part of the estuary was very restricted in size, and was the only place where fishes utilising the mangroves at high tide could go to for refuge. Each month at spring low tide, four replicate hauls with a beach seine net were made at each of three sites in the estuary basin (figure 1). The beach seine net measured 10 m in length and 3 m in height, had a stretched mesh size of 1.8 cm, and was used as a purse seine net to catch the fish. Within the mangroves, four replicate hauls per day were done with the same beach seine during the incoming spring high tide on two consecutive days in January 2004. The mangroves form a 10 m wide fringe at the point where the Kizinga and Mzinga Streams confluence, which widens into a 250 m wide mangrove forest towards the Mzinga Stream (figure 1). The beach seine was hauled over a distance of about 25 m over a sandy substratum in a narrow mangrove channel that stretched from the edge of the mangrove fringe to the high shores.

All fish caught were counted, weighed, and identified using Fischer and Bianchi (1984), Bianchi (1985) and Smith and Heemstra (1995). Total length of each fish was
measured to the nearest millimetre. From each fish, a sample of muscle tissue was taken from section of the tail below the lateral line. This sample was used for stable isotope analysis.

A wide range of potential food items (benthic macro-invertebrates, seagrasses and algae; 2-9 replicate samples per species per habitat) were collected in the estuary basin and in the mangroves (on the substratum as well as on the prop-roots) in January 2004. Particulate organic matter was sampled from the water column in the estuary basin by filtering several litres of seawater over a Whatman GF/C glass fibre filter. Macrofauna was identified using Richmond (1997). For all collected food items the soft body tissues were sampled for stable isotope analysis.

Tissue of the fishes and the potential food items were dried for 48 hrs at 70 °C and ground to a fine powder using a mortar and pestle with liquid nitrogen. For fishes only muscle tissue was used because of its slow turnover rate, resulting in a history of food assimilation over periods of months, while excluding short-term variability (Gearing, 1991). For mangroves, the first leaves following the terminal bud were used, whereas for seagrasses all green leaves of the entire plant were used, since no variation has been found between young and old leaves or between the tip and base of the leaf (Gearing, 1991). Food items containing calcium carbonate were decalcified to allow focusing solely on organic carbon (Nieuwenhuize et al., 1994). This was not done for the nitrogen isotope analysis, since nitrogen content is affected by acid washing (Bunn et al., 1995). Carbon and nitrogen stable isotope compositions were measured with a Carlo Erba NA 1500 elemental analyser (Carlo Erba, Milan, Italy) coupled on-line via a Finnigan Conflo III interface with a ThermoFinnigan DeltaPlus mass-spectrometer (Thermo Fischer, Waltham, MA, USA). Carbon and nitrogen isotope signatures are expressed as ratios between the isotopes \( \frac{{^{12}C}}{{^{13}C}} \) and \( \frac{{^{14}N}}{{^{15}N}} \) respectively and are noted in the delta notation (\( \delta^{13}C, \delta^{15}N \)) in parts per thousand deviation from the isotopic ratios in the international standard reference samples Vienna PDB (Craig, 1957; Gonfiantini, 1984) and atmospheric nitrogen (Mariotti, 1983). Vienna PDB is the reference material of the International Atomic Energy Agency (IAEA) that is replacing the original reference material (PDB) from a pulverised belemnite fossil from the Upper Cretaceous Pee Dee formation in South Carolina since the supply of the original reference material was exhausted.

Stable isotope ratios in animals reflect those of their diet, with an average of 1‰ carbon enrichment (indicative of food source) and 3‰ nitrogen enrichment (indicative of trophic level), although nitrogen enrichment can vary between approximately 2‰ and 5‰ (Cabana and Rasmussen, 1994; Deniro and Epstein, 1981; Fry, 1988; Fry and Arnold, 1982; Minagawa and Wada, 1984; Rau et al., 1983). To illustrate the contribution of different food sources to the diet of the zoobenthivorous fishes, the Isosource mixing model was used in this study. This model examines feasible contributions of multiple sources to the diet and predicts both the frequency and the range of potential source contributions (Phillips and Gregg, 2003). For the seven zoobenthivorous fish species that were collected in both habitats (Albulla glossodonta, Gerres acinaces, G.
filamentosus, G. oyena, Leiognathus equulus, Sillago sihama, and Terapon jarbua) and for one species occurring only in the mangrove habitat (Periophthalmus argentilineatus) the average isotopic signature was used in the model. Potential contributions of food sources that were selected were based on dietary studies (Crabtree et al., 1998; DeTroch et al., 1998; Hajisamae et al., 2004; Lugendo et al., 2006; see also chapter 3), and were calculated for the zoobenthivores with an increment of 2% and tolerance of 0.1‰, in which the increment indicates the distance between possible combinations of source proportions and the tolerance indicates the difference that is allowed between predicted mixture signatures and the observed mixture signatures (Phillips and Gregg, 2003). To correct for the average enrichment in carbon and nitrogen in the predator relative to its prey, respectively 1‰ and 3‰ was subtracted from the isotopic signature of the fish.

Levene’s test was used to test for homogeneity of variances. Differences in stable carbon and nitrogen isotopic signatures between species were tested with a 1-way ANOVA in combination with a Tukey’s post-hoc test, while differences between habitats were tested with independent t-tests. Correlations between isotopic signatures and fish length were tested with independent t-tests followed by Pearson’s correlations. All statistical tests were performed using SPSS© version 11.5. Significant differences were accepted at the \( P \leq 0.05 \) level.

RESULTS

Plants and algae

The isotopic signatures of the mangrove tree Sonneratia alba and the seagrass Halophila ovalis (figure 2) showed that a clear distinction exists between the mangrove and mudflat habitat at the level of primary producers.

At the base of the estuarine foodweb the particulate organic matter (POM) from the estuary and detritus from within the mangroves were relatively depleted in \( \delta^{13}C \) (\(< -21.0\‰\)) which showed that POM and detritus were highly influenced by mangrove sources (figure 2). Benthic microalgae from the mangroves and brown algae from the mudflats showed similarly depleted \( \delta^{13}C \) values as the POM, suggesting an influence from the mangroves. Only the green algae from the mudflats showed more enriched \( \delta^{13}C \) values indicating a lower mangrove influence. Even though the green and brown algae belong to the primary producers, they were enriched in \( \delta^{15}N \) compared to the other plants from the bay.

The segregation of all primary producers, POM, and detritus between mangroves and the mudflats provided the opportunity to determine the extent to which the biota that occurred in the habitats depend on either habitat for their carbon supply.
MANGROVES AS A FEEDING HABITAT

Figure 2 Average $\delta^{15}$N and $\delta^{13}$C values for food web samples collected from the mudflats (filled symbols) and mangroves (open symbols). Error bars represent standard errors. The circles indicate the species of the genera Sesarma (A), Uca (B), and Portunus and Thalamita (C). 1 Sonneratia alba; 2 detritus; 3 Sesarma guttatus; 4 Sesarma sp.; 5 brown algae; 6 Littoraria scabra; 7 POM; 8 S. leptosoma; 9 Uca lactea annulipes; 10 U. dussumieri; 11 benthic algae; 12 Metopograpsus thukuhar; 13 polychaetes; 14 green algae; 15 U. chlorophthalmus; 16 bivalve sp.2; 17 Thalamita sp. 1; 18 hermit crab; 19 oysters; 20 bivalve sp. 1; 21 Bulla sp.; 22 Cerithidea decollata; 23 Ulva sp.; 24 anemone sp.1; 25 anemone sp.2; 26 Balanus sp.; 27 Solen sp.; 28 Portunus pelagicus; 29 Thalamita sp. 2; 30 shrimp sp.1; 31 mantis shrimp; 32 isopods; 33 amphipods; 34 shrimp sp.2; 35 Dotilla fenestrata; 36 Halophila ovalis.

Macro-invertebrates

The $\delta^{13}$C showed that the sessile filter-feeding macro-invertebrates from the mangroves (anemones, bivalves, oysters, and Balanus barnacles) were among the most carbon-enriched organisms in the mangroves, which suggests a high influence of mudflat resources (figure 2). Their nitrogen signature places these sessile filterfeeders on the first trophic level above the primary producers. The mangrove gastropods Littoraria scabra and Cerithidea decollata were found at the same trophic level as the filterfeeders. C. decollata, which feed on microalgae and detritus on muddy surface of the mangrove sediments (Richmond, 1997), was enriched in $\delta^{13}$C relative to L. scabra, which feed on algae on the mangrove stems (Reid, 1986). The difference between the $\delta^{13}$C of L. scabra and C. decollata reveals that the algae on the surface of mangrove sediments experience a lower influence from the mangroves than the algae on the trees, but are mostly influenced by carbon from mudflat sources instead.

Small crustaceans (e.g. isopods and amphipods) and shrimps were the most enriched macro-invertebrates from the estuary (figure 2) and appeared to depend
almost exclusively on mudflat sources. In contrast, the stable isotope signatures of the larger-sized decapod species showed clearly that the dependency on mangrove and mudflat sources varied, both at genus and species level. The crabs that were found in the mangroves belong to the families of the Grapsidae (*Metopograpsus*, *Sesarma*), Ocypodidae (*Uca*, *Dotilla*), and Portunidae (*Portunus*, *Thalamita*). Of these groups, the *Sesarma* crabs were most depleted in carbon, the Portunidae the most carbon-enriched, while the $\delta^{13}C$ values of the *Uca* crabs were intermediate to those of the other two groups (figure 2). This differentiation resulted in many significant differences between the three groups (table 1). Within the multiple-species genera *Sesarma*, *Uca*, and *Thalamita* no significant differences existed, but at family level there were differences: within the family of the Grapsidae, *Metopograpsus thukuhar* was enriched in carbon relative *Sesarma* sp. and *Sesarma guttatum* (table 1). Within the family of the Ocypodidae, *Dotilla fenestrata* was significantly enriched in carbon relative to all three *Uca* species, but the species was also significantly enriched compared to the Grapsidae.

**Table 1** Statistical differences (1-way ANOVA and Tukey's post-hoc test) between the carbon signatures of mangrove and mudflat crab species. The upper-right panel shows the $P$-values for the statistical differences. In the lower-left panel differences are indicated with symbols.

<table>
<thead>
<tr>
<th></th>
<th>Grapsidae</th>
<th>Ocypodidae</th>
<th>Portunidae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Sesarma sp. 1</em></td>
<td><em>Sesarma guttatum</em></td>
<td><em>Sesarma leptosoma</em></td>
</tr>
<tr>
<td><strong>Sesarma sp. 1</strong></td>
<td>-</td>
<td>1.000</td>
<td>0.783</td>
</tr>
<tr>
<td><em>Sesarma guttatum</em></td>
<td>n.s.</td>
<td>-</td>
<td>0.381</td>
</tr>
<tr>
<td><em>Sesarma leptosoma</em></td>
<td>n.s.</td>
<td>n.s.</td>
<td>-</td>
</tr>
<tr>
<td><em>Metopograpsus thukuhar</em></td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Dotilla fenestrata</strong></td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td><strong>Uca chlorophthalinus</strong></td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td><strong>Uca dussumieri</strong></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Uca lactea annulipes</strong></td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Thalamita sp. 1</strong></td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td><strong>Thalamita sp. 2</strong></td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td><strong>Portunus sp.</strong></td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

n.s. = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
**Fishes**

Frequency distribution of stable carbon signatures reveals that there was little difference between the isotopic signatures of the fishes from the mudflats and from the mangroves (figure 3). Table 2 shows that the $\delta^{13}C$ signatures of individual species that occur in both habitats are not significantly different. The $\delta^{13}C$ values suggest that fishes from both habitats have isotopic signatures that are derived from mudflat food sources. The median of the frequency distribution is formed by the $\delta^{13}C$ groups of -16 to -15‰ and -15 to -14‰. This means that the average food of these fish would have a $\delta^{13}C$ value between -17 and -15‰, which lies within the range of most mudflat food sources (figure 2). *Siganus sutor* from the mudflats and the amphibious *P. argentilineatus* from the mangroves were the only species with depleted carbon signatures (figure 3), which indicates a high contribution of mangrove food sources in their diets (table 2, figure 2).

Table 3 shows that only four out of 39 correlations showed a significant positive correlation between the isotopic signatures and the total length of fish species, which suggests that differences in fish size had not interfered with the foodweb analysis.

The results of the Isosource model point out that isopods, shrimps, and amphipods are the most likely of the selected food sources to be consumed by the zoobenthivorous fishes from the mangroves as well as from the mudflats (table 4). The isopods and amphipods were both mudflat food sources, while the shrimps occurred both on the mudflats and in the mangroves. *P. argentilineatus* living permanently within the mangroves is the only zoobenthivorous fish for which the likelihood of the consumption of isopods, amphipods, and shrimps was lower than that of polychaetes, and *Uca* and *Sesarma* crabs. The potential consumption of the latter sources was low in all other zoobenthivorous species that had to leave the mangroves at low tide. Thus, the Isosource results suggest that the species bound to tidal migrations between the two habitats mostly feed on mudflat sources, whereas the amphibious *P. argentilineatus* mostly feeds on typical mangroves sources.

**DISCUSSION**

**Macro-invertebrates**

The stable isotope signatures of the macro-invertebrates from the estuary showed that a clear connection exists between the lifestyle of species and their degree of dependence on mangrove or mudflat sources. *Littoraria* gastropods and crabs of the genus *Sesarma* and *Uca* are all terrestrial species for which the data showed that they entirely depend on mangrove sources. However, whereas *Littoraria* lives on the mangrove trees above the high tide level where it grazes on algae (Reid, 1986), the mangrove crabs feed on mangrove leaves and detritus (Dahdouh-Guebas et al., 1999; Icely and Jones, 1978).
Figure 3 Frequency distributions of carbon signatures of fishes caught in (a) mudflats ($n = 138$) and (b) mangroves ($n = 70$). The shaded areas represent the contributions of *Siganus sutor* ($n = 6$) and *Periophthalmus argentilineatus* ($n = 15$) to fish assemblages of the mudflats and mangroves, respectively.
Table 2 Stable carbon and nitrogen isotope signatures (‰) and average length of fishes (TL in cm) collected in the mangroves and the mudflats of the Mtoni Estuary. The t-test results (P-values) show the differences of the $\delta^{13}$C and $\delta^{15}$N signatures between mangrove and mudflat fishes. Feeding guilds: D = detritivores, H = herbivores, O = omnivores, P = piscivores, ZB = zoobenthivores and ZP = zooplanktivores.

<table>
<thead>
<tr>
<th>Species</th>
<th>Feeding guild</th>
<th>Mangrove $\delta^{13}$C</th>
<th>Mangrove $\delta^{15}$N</th>
<th>Mangrove TL</th>
<th>Mangrove n</th>
<th>Mudflats $\delta^{13}$C</th>
<th>Mudflats $\delta^{15}$N</th>
<th>Mudflats TL</th>
<th>Mudflats n</th>
<th>P-value $\delta^{13}$C</th>
<th>P-value $\delta^{15}$N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albula glossodonta</td>
<td>ZB</td>
<td>-14.4 ± 0.2</td>
<td>13.4 ± 0.2</td>
<td>4.7</td>
<td>6</td>
<td>-14.7 ± 1.1</td>
<td>13.1 ± 1.6</td>
<td>9.5</td>
<td>3</td>
<td>0.826</td>
<td>0.892</td>
</tr>
<tr>
<td>Apogon sp. 1</td>
<td>ZP/ZB</td>
<td>-16.9 ± 0.4</td>
<td>14.3 ± 0.3</td>
<td>7.1</td>
<td>4</td>
<td>-16.6 ± 0.6</td>
<td>15.2 ± 0.1</td>
<td>6.3</td>
<td>3</td>
<td>0.860</td>
<td>0.249</td>
</tr>
<tr>
<td>Apogon sp. 2</td>
<td>ZP/ZB</td>
<td>-16.4 ± 0.1</td>
<td>13.9 ± 0.6</td>
<td>5.7</td>
<td>2</td>
<td>-16.6 ± 0.4</td>
<td>13.0 ± 0.2</td>
<td>14.1</td>
<td>6</td>
<td>0.205</td>
<td>0.032</td>
</tr>
<tr>
<td>Arothron immaculatus</td>
<td>O</td>
<td>-16.3 ± 0.2</td>
<td>11.8 ± 0.3</td>
<td>5.5</td>
<td>6</td>
<td>-16.3 ± 0.2</td>
<td>11.8 ± 0.3</td>
<td>5.5</td>
<td>6</td>
<td>0.358</td>
<td>0.177</td>
</tr>
<tr>
<td>Atherinidae</td>
<td>ZP</td>
<td>-15.1 ± 1.0</td>
<td>13.6 ± 1.0</td>
<td>3.9</td>
<td>6</td>
<td>-15.1 ± 0.1</td>
<td>14.4 ± 0.2</td>
<td>8.5</td>
<td>6</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Atherinomorus duodecimalis</td>
<td>ZP</td>
<td>-15.0 ± 0.2</td>
<td>12.0 ± 0.5</td>
<td>8.1</td>
<td>6</td>
<td>-15.0 ± 0.2</td>
<td>12.0 ± 0.5</td>
<td>8.1</td>
<td>6</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Callionymes sp.</td>
<td>ZB</td>
<td>-16.7 ± 0.5</td>
<td>9.7 ± 1.6</td>
<td>4.0</td>
<td>3</td>
<td>-17.4 ± 0.2</td>
<td>9.6 ± 0.1</td>
<td>5.2</td>
<td>5</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Carangidae</td>
<td>ZB/P</td>
<td>-14.9 ± 0.1</td>
<td>13.2 ± 0.1</td>
<td>5.5</td>
<td>5</td>
<td>-13.9</td>
<td>14.0</td>
<td>9.0</td>
<td>1</td>
<td>0.205</td>
<td>0.032*</td>
</tr>
<tr>
<td>Gerres acinaces</td>
<td>ZB</td>
<td>-14.8 ± 0.2</td>
<td>12.7 ± 0.1</td>
<td>4.7</td>
<td>6</td>
<td>-15.3 ± 0.3</td>
<td>13.7 ± 0.3</td>
<td>6.6</td>
<td>6</td>
<td>0.358</td>
<td>0.177</td>
</tr>
<tr>
<td>Gerres filamentosus</td>
<td>ZB</td>
<td>-15.1 ± 0.2</td>
<td>12.9 ± 0.1</td>
<td>4.3</td>
<td>6</td>
<td>-14.5 ± 0.5</td>
<td>13.6 ± 0.4</td>
<td>6.1</td>
<td>6</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Gerres oyena</td>
<td>ZB</td>
<td>-15.3 ± 0.3</td>
<td>13.1 ± 0.3</td>
<td>7.6</td>
<td>6</td>
<td>-15.3 ± 0.3</td>
<td>13.1 ± 0.3</td>
<td>7.6</td>
<td>6</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Gobiidae sp. 1</td>
<td>ZB</td>
<td>-14.8 ± 1.0</td>
<td>14.0 ± 0.2</td>
<td>3.8</td>
<td>6</td>
<td>-14.8 ± 0.1</td>
<td>14.0 ± 0.2</td>
<td>3.8</td>
<td>6</td>
<td>0.358</td>
<td>0.177</td>
</tr>
<tr>
<td>Gobiidae sp. 2</td>
<td>ZB</td>
<td>-14.6 ± 0.2</td>
<td>13.9 ± 0.2</td>
<td>6.9</td>
<td>5</td>
<td>-14.6 ± 0.2</td>
<td>13.9 ± 0.2</td>
<td>6.9</td>
<td>5</td>
<td>0.144</td>
<td>0.002**</td>
</tr>
<tr>
<td>Gobiidae sp. 3</td>
<td>ZB</td>
<td>-14.7 ± 0.4</td>
<td>12.3 ± 0.2</td>
<td>5.4</td>
<td>2</td>
<td>-14.7 ± 0.4</td>
<td>12.3 ± 0.2</td>
<td>5.4</td>
<td>2</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Leiognathus equulus</td>
<td>ZB</td>
<td>-16.1 ± 0.4</td>
<td>13.2 ± 0.1</td>
<td>5.0</td>
<td>2</td>
<td>-15.4 ± 0.6</td>
<td>13.2 ± 0.3</td>
<td>5.1</td>
<td>6</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Lethrinus lentjan</td>
<td>ZB</td>
<td>-16.8 ± 0.5</td>
<td>12.4 ± 0.1</td>
<td>5.6</td>
<td>6</td>
<td>-16.8 ± 0.5</td>
<td>12.4 ± 0.1</td>
<td>5.6</td>
<td>6</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Lutjanus fulviflamma</td>
<td>ZB</td>
<td>-14.9 ± 0.3</td>
<td>13.9 ± 0.3</td>
<td>7.6</td>
<td>6</td>
<td>-14.9 ± 0.3</td>
<td>13.9 ± 0.3</td>
<td>7.6</td>
<td>6</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Mugil sp. 1</td>
<td>D/ZB</td>
<td>-14.7 ± 0.6</td>
<td>12.2 ± 0.2</td>
<td>7.8</td>
<td>5</td>
<td>-14.3 ± 0.4</td>
<td>12.0 ± 0.3</td>
<td>7.7</td>
<td>5</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Mugil sp. 2</td>
<td>D/ZB</td>
<td>-15.7 ± 0.1</td>
<td>12.0 ± 0.3</td>
<td>6.8</td>
<td>6</td>
<td>-15.7 ± 0.1</td>
<td>12.0 ± 0.3</td>
<td>6.8</td>
<td>6</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Periophthalmus argenteineatus</td>
<td>ZB</td>
<td>-18.9 ± 0.4</td>
<td>13.2 ± 0.3</td>
<td>6.9</td>
<td>5</td>
<td>-13.9 ± 0.2</td>
<td>13.0 ± 0.4</td>
<td>19.0</td>
<td>6</td>
<td>0.615</td>
<td>0.989</td>
</tr>
<tr>
<td>Platycephalus indicus</td>
<td>ZB/P</td>
<td>-14.7 ± 0.6</td>
<td>12.2 ± 0.2</td>
<td>7.8</td>
<td>5</td>
<td>-14.3 ± 0.4</td>
<td>12.0 ± 0.3</td>
<td>7.7</td>
<td>5</td>
<td>0.615</td>
<td>0.989</td>
</tr>
</tbody>
</table>
### Table 2 [Continued]

<table>
<thead>
<tr>
<th>Species</th>
<th>Feeding guild</th>
<th>Mangrove</th>
<th>Mudflats</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>δ¹³C</td>
<td>δ¹⁵N</td>
<td>TL  n</td>
</tr>
<tr>
<td><em>Sardinella albella</em></td>
<td>ZP</td>
<td>-16.6 ± 0.3</td>
<td>13.1 ± 0.2</td>
<td>8.0 6</td>
</tr>
<tr>
<td><em>Saurida gracilis</em></td>
<td>ZB/P</td>
<td>-14.6 ± 0.2</td>
<td>12.1 ± 0.1</td>
<td>10.8 6</td>
</tr>
<tr>
<td><em>Scarus russelii</em></td>
<td>H</td>
<td>-16.1 ± 0.3</td>
<td>12.4 ± 0.1</td>
<td>6.9 6</td>
</tr>
<tr>
<td><em>Siganus sutor</em></td>
<td>H</td>
<td>-20.6 ± 0.2</td>
<td>13.4 ± 0.2</td>
<td>7.9 6</td>
</tr>
<tr>
<td><em>Sillago sihama</em></td>
<td>ZB</td>
<td>-14.4 ± 0.3</td>
<td>13.3 ± 0.1</td>
<td>5.7 6</td>
</tr>
<tr>
<td><em>Terapon jarbua</em></td>
<td>ZB</td>
<td>-15.2 ± 0.3</td>
<td>13.2 ± 0.2</td>
<td>5.8 6</td>
</tr>
</tbody>
</table>

* = significant at $P \leq 0.05$; *** = significant at $P \leq 0.00$

### Table 3

Correlations between isotopic signature and fish length (Pearson’s correlation). Only significant correlations are shown.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Isotope</th>
<th>P-value</th>
<th>df</th>
<th>$r^2$</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>mudflat</td>
<td><em>Gerres filamentosus</em></td>
<td>δ¹³C</td>
<td>0.030</td>
<td>5</td>
<td>0.729</td>
<td>0.854</td>
</tr>
<tr>
<td>mudflat</td>
<td><em>Mugil sp. 2</em></td>
<td>δ¹⁵N</td>
<td>0.003</td>
<td>5</td>
<td>0.966</td>
<td>0.983</td>
</tr>
<tr>
<td>mudflat</td>
<td><em>Sillago sihama</em></td>
<td>δ¹⁵N</td>
<td>0.048</td>
<td>5</td>
<td>0.665</td>
<td>-0.815</td>
</tr>
<tr>
<td>mudflat</td>
<td><em>Sardinella albella</em></td>
<td>δ¹³C</td>
<td>0.049</td>
<td>5</td>
<td>0.663</td>
<td>0.814</td>
</tr>
</tbody>
</table>
Table 4 Minimum and maximum contribution of various food sources to the total diet of zoobenthivorous fish (in percentages). The selection of food items is based on fish diet studies of (1) Lugendo et al., submitted, (2) chapter 3, (3) Crabtree et al., 1998, (4) De Troch et al., 1998 and (5) Hajisemae et al., 2004. For the crabs of the genera *Uca* and *Sesarma* the average value of their different species was used. mf = mudflat origin; mg = mangrove origin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Amphipods</th>
<th>Isopods</th>
<th>Polychaetes</th>
<th>Shrimps</th>
<th>Detritus</th>
<th>Sesarma crabs</th>
<th>Uca crabs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Albula glossodonta</strong></td>
<td>mf</td>
<td>0.78</td>
<td>0.86</td>
<td>0.30</td>
<td>0.58</td>
<td>0.14</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>0.70</td>
<td>0.90</td>
<td>0.28</td>
<td>0.58</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Gerres acinaces</strong></td>
<td>mf</td>
<td>0.44</td>
<td>0.82</td>
<td>0.16</td>
<td>4.76</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>0.74</td>
<td>0.84</td>
<td>0.36</td>
<td>0.68</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Gerres filamentosus</strong></td>
<td>mf</td>
<td>0.52</td>
<td>0.78</td>
<td>0.50</td>
<td>0.80</td>
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<tr>
<td><strong>Leiognathus equulus</strong></td>
<td>mf</td>
<td>0.70</td>
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Sessile anemones, barnacles, bivalves, and oysters were also found in the mangroves, but these species occurred at the seaward fringe of the mangrove trees where they are submerged during high tides. The carbon isotopic signatures showed that these filter-feeding mangrove inhabitants derive much of their carbon from the water from the estuary basin, which can only be filtered during high tide.

Benthic macro-invertebrates of the mudflats, i.e. swimming crabs, shrimps, crabs, gastropods, polychaetes, isopods, and amphipods, were enriched in their carbon isotopic signatures, which shows that these groups do not feed in the mangroves. Swimming crabs and shrimps that were collected in the mangroves were also enriched, thus indicating that these species have no dependency on the mangroves for feeding. Presumably, these species only use the mangroves as shelter from predation by fishes during high tide.

Besides this differentiation between macro-invertebrate groups, differences in the use of resources were also found between related species in the partitioning within these groups. This resource partitioning was illustrated in the Mtoni Estuary for the crabs, which were among the most abundant large-sized macro-invertebrates. The
genus *Sesarma* consists of crabs which are found in the higher mangrove sections. The species that belong to this family are all known to feed on mangrove leaves, but the contribution of leaves to the total diet differs between species (Dahdouh-Guebas et al., 1999). In the mangroves of the estuary this was demonstrated by the isotopic signatures as *S. guttatum* (feeding mostly on leaves) which was more depleted in carbon than *S. leptosoma*, which also feeds on more enriched macro-algae. The crabs of the genus *Uca* were enriched in $\delta^{13}C$ relative to the Sesarmid crabs because they do not feed on leaves but on algae and diatoms from the sediments (Icely and Jones, 1978); an observation which is supported by the present study. Since the diet of the different *Uca* species is similar, interspecific differences in carbon isotopes in the present study illustrate preferences for different mangrove zones (Icely and Jones, 1978). Thus, the preference of *U. chloropthalmus* for the wetter mangrove zone is reflected here by a higher contribution of mudflat sources (i.e. an enriched $\delta^{13}C$ value) to the diet than for *U. lactea annulipes* and *Uca dussumieri*, which occur in higher mangrove sections (Icely and Jones, 1978). The swimming crabs of the family Portunidae differ from the *Sesarma* and *Uca* crabs in that they mostly have to remain submerged implicating that in Mtoni they have to move to the subtidal mudflats or remain in tide pools when the tides goes out. The necessity to remain submerged thus brings the swimming crabs more frequently into contact with mudflat sources and explains their less depleted carbon signatures. Within the Portunidae, the higher depletion in carbon signature of *Thalamita* sp. 1 indicates that this species depends more on mangrove sources than *Thalamita* sp. 2 and *Portunus pelagicus*. It can be concluded that the differentiation in stable isotope signatures among decapod crabs shows that these crabs are found along a gradient of microhabitats in the mangrove and the mudflat habitats.

The isotopic signatures of the macro-invertebrates from the Mtoni Estuary showed that the species can be subdivided into different ecological groups on the basis of the micro-habitats where they feed: species that are more depleted in their carbon signatures than $-19.5\%$ were collected within drier parts of the mangrove stands, species with carbon signatures between $-19.5\%$ and $-16.1\%$ were collected either on the intertidal mudflats or in the wetter parts of mangroves and typically belong to the intertidal zone, while species that were either more enriched than $-16.1\%$ were collected on the subtidal mudflats or were species that cannot stay out of water for prolonged periods of time (except *D. fenestrata*).

**Fishes**

The stable isotope data of this study shows that fishes in the shallow estuary of Mtoni have a very limited connection to the mangroves as a feeding habitat. A part of the fish species caught on the subtidal mudflats was also represented in the catches at high tide in the mangrove forest, which shows that some species migrate over distances of several hundreds of metres into the mangrove forest when these submerged during high tide. The stable isotope signatures of the fishes caught on mudflats did not differ significantly from those caught in the mangroves, which was also illustrated by the
frequency distribution of isotopic carbon signatures. Thus, it appears that these migrating fish species do not depend on the mangrove habitat for their food provision despite their temporal presence in this habitat. Either these fish feed only on the mudflats, or when feeding in the mangroves at high tide, they select only food sources which are transported into the mangroves by the tides and originate from the mudflats. This was also pointed out for the zoobenthivorous fishes by the Isosource model. For all fish species caught in both habitats, the model showed that the relative contribution of mangrove sources to the total diet was not altered to a great extent by residence in the mangroves, revealing that these species do not feed substantially in the mangroves despite their temporary residence there.

Among the zoobenthivorous fishes, only *P. argentilineatus* mainly utilised mangrove food sources, as a result of its amphibious life style ([chapter 3](#)). Among the mudflat fishes, *S. sutor* is the only fish species for which the stable isotope data pointed out that it possibly enters the mangroves at high tide to feed, on basis of the depleted carbon values relative to that of the other mudflat fishes. The fish could either feed on mangrove sources or on brown algae from the mudflat that were more depleted than other mudflat food sources.  

**Habitat connectivity**  
Since the fringing mangroves of Mtoni are drained completely by outgoing tides, only terrestrial and amphibious macro-invertebrate and fish species could constantly use the mangroves for shelter and food. The fishes which had temporal access to the mangroves during the high tides did not use the mangrove resources to a high degree, with the possible exception of *S. sutor*, while the macro-invertebrate species from the mudflat made only limited use of these resources. The sessile filterfeeders in the mangroves formed the only group that largely depended on the resources from the mudflat habitat, and thus these are the only species that really depend for their feeding on the connectivity between the two habitats.  

Despite the weak connection on the basis of feeding, the presence of macro-invertebrates and fishes in the mangroves of the Mtoni Estuary during high tide shows that a number of species move between the mudflat and the mangroves with the tides. Such movements between intertidal and subtidal habitats can be motivated by increased food availability, minimisation of predation, or in some species benefits for reproduction ([Gibson, 2003](#)). The isotopic signatures of the fishes in this study reveal that tidal migration into the mangroves is not motivated by feeding. Since the tidal migrants were mostly juvenile fish, their movements are not driven by reproductive benefits either. Therefore, it is most likely that these fishes frequent the mangroves to find refuge from predators that enter the mudflat habitat with rising tides. The presence of such migration suggests that the structural complexity of the Mtoni mangroves offers more protection than the mudflats, despite the fact that predation by macro-crustaceans and birds might be substantial in the mangroves ([Sheaves, 2001](#)).
CONCLUSIONS

It is demonstrated here that the carbon outflow from the Mtoni mangroves to the adjacent estuarine foodweb is very limited. The dependence on mangrove-derived carbon differs among macro-invertebrates at species level and results in differentiation in resource use and micro-habitat preferences. In contrast, the dependence on mangrove carbon is very small among fishes that migrate into the mangroves at high tide. Consequentially, the fringing mangroves do not constitute an important feeding habitat for these fishes, and the value of these mangroves is probably restricted to functioning as a temporary shelter for juvenile fish.
Chapter 3

Influence of morphology and amphibious lifestyle on the feeding ecology of the mudskipper *Periophthalmus argentilineatus*

G. Kruitwagen, I. Nagelkerken, B.R. Lugendo, H.B. Pratap, S.E. Wendelaar Bonga

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ABSTRACT The potential feeding advantages that are offered to the barred mudskipper by its amphibious lifestyle were investigated. To this end the feeding ecology of these mudskippers, the position these fish occupy in the mangrove food web, and the importance of different morphological factors for their diet were studied. The analysis of gut contents showed that the major component of the mudskipper diet changes during growth from small crustaceans (e.g. copepods, amphipods), via polychaetes to mangrove crabs. This dietary shift was found to be facilitated through enlargement of the mouth, as well as elongation of the gut. The use of stable isotope analysis revealed that mudskippers had a similar trophic position as other zoobenthivorous fish species. Their diet did, however, not appear to be affected by competition for food with other zoobenthivores. The amphibious habits of the mudskipper provide specific advantages to its feeding ecology, such as access to an abundant food source of mangrove crabs which are mostly inaccessible for other fish species.

INTRODUCTION

In the mangrove forests of the Indo-Pacific region fishes belonging to the mudskipper family Periophthalmidae occupy a special niche in the intertidal zone. The physiology and morphology of these fishes have evolved to enable an amphibious existence in this zone with highly variable environmental conditions (Clayton, 1993). Their amphibious lifestyle provide mudskippers with the possibility to dwell on exposed mudflats, whereas other fish species are forced to retreat to deeper waters with outgoing tides. Mudskippers are thought to feed predominantly when they are out of the water during low tide (Colombini et al., 1996; Nieder, 2001).

Partitioning is an important ecological mechanism to optimise the use of the same resources by different competitors and resource partitioning between species may occur in e.g. spatial and time dimensions (Piet, 1998; Ross, 1986). Nevertheless, in marine fish communities partitioning mostly occurs in the trophic dimension (Ross, 1986; Sibbing and Nagelkerke, 2001).

The characteristics that influence the use of resources in individual species are often subject to change during the ontogeny of an individual (Werner and Gilliam, 1984). In fishes, growth is often accompanied by changes in morphology and ecology, which enables them to use resources not previously utilized, and consequentially, enable them to shift their preference to food sources with a higher energy yield per unit effort (Wainwright and Richard, 1995).

The most important morphological characteristic defining resource use is the gape, or maximal mouth dimensions, of a species (Piet, 1998). The gape puts restrictions on the dimensions of prey that can be ingested, while the morphology, physiology and biochemical characteristics of the gastrointestinal tract define to which extent the food items can be processed and digested after ingestion (German et al., 2004).

Periophthalmidae are generally considered to have a zoobenthivorous diet, but the contribution of different food items to the total diet differs between studies based on gut content analysis (Clayton, 1993; Colombini et al., 1996; Stebbins and Kalk, 1961; Mhaisen and Al-Maliki, 1996; Udo, 2002). Insights into the diet, based on gut content analysis, may be skewed since food items may be unintentionally ingested or digested.
at different rates, and soft tissue food items may be indistinguishable for identification in the stomach. These uncertainties can be reduced by combining gut content analysis with stable isotope analysis. This technique provides a average value of the diet over a period of weeks to months (Gearing, 1991).

In this study we assessed the correlation between size, and subsequent morphological differences, and the dietary preference of the barred mudskipper (Periophthalmus argentilineatus Valenciennes) by a combination of gut analysis and stable isotope composition. Moreover, the role of partitioning in space and time due to the amphibious behaviour of the mudskippers in competition with other zoobenthivorous fishes in the mangrove habitat was determined.

**MATERIALS AND METHODS**

**Study areas**

The fieldwork for this study was performed in the mangroves of Chwaka Bay and Mtoni in Tanzania (figure 1). These mangroves were selected, because the difference in hydrology between the locations may be reflected in the diet of mudskippers and other zoobenthivorous fishes.

Chwaka Bay is a shallow bay of approximately 35 km² at the east coast of Unguja Island (Zanzibar), which is located 30 km from the Tanzanian mainland. The bay opens to the Indian Ocean at the northern end where it is fringed by coral reefs. The southern end of Chwaka Bay consists of a fossil limestone reef that is intersected by deep channels and smaller creeks and is covered by large mangrove forests. The mangrove channels have a tidal amplitude of only 1 m due to the geomorphology of the bay and retain water during low tide (Wolanski, 1989).

The Mtoni mangroves are situated in the shallow estuary of the confluence of the Kizinga and Mzinga streams near the city of Dar es Salaam. These mangroves are subject to a tidal amplitude of 4 m (Richmond, 1997). As a result, all water is drained from the mangroves with the outgoing tides, while all water remaining in the habitat during low tide consists of small channels of surfacing ground water.

**Sample collection and analysis**

Barred mudskippers (P. argentilineatus) were collected at random with scoop nets on the exposed mudflats and in the creeks of Chwaka Bay and Mtoni estuary in January 2004. After collection, fish were placed in ice water to anaesthetise the fish and to stop digestive activities in the gut. Body weight (to the nearest 0.1 g) and total body length (to the nearest mm) were measured for each specimen. Mouth width (maximum lateral measure of mouth gape), mouth height (maximum dorso-ventral measure of mouth gape), and gut length (from oesophagus to anus) were recorded to the nearest mm from dead specimens without stretching. The mouth dimensions and gut length were standardised to fish size by dividing these morphometric dimensions by the total
length of each individual fish. After dissection complete guts were stored in ethanol (70%) until analysis.

Food items in the guts were identified with a stereomicroscope. The quantity of each food item was estimated as a percentage of its volume relative to the total volume of ingested food items in the gut. The food items from the digestive tract were classified to either class level (for Polychaeta, Bivalvia, and Insecta) or order level (for Isopoda, Amphipoda, Copepoda, Diptera, Tanaidacea, Arachnidae, and Mysidacea), while fish and detritus were classified as such. Ingested crabs and crab parts were identified to the lowest possible level (family, subfamily or species level) and their carapace width and merus (the fourth segment of a crustacean pereopod) length were measured. The merus was used as a measure of crab size because this pereopod segment appeared to be most abundant in undamaged state in the gut contents.
Based on Colombini et al. (1996) the most important food items of the mudskipper were collected in the mangroves at both locations simultaneously with the mudskippers: crabs were collected from the subtidal mudflats, exposed intertidal mudflats, and mangrove trees; amphipods and isopods were collected from the seagrasses on the intertidal mangrove flats, and polychaetes were sieved from the sediment.

At both sites fishes were collected with a beach seine net that measured 10 m in length and 3 m in height, had a stretched mesh size of 1.8 cm. In Chwaka, zoobenthivores belonging to the families Gerridae, Lethrinidae and Lutjanidae, zoobenthivores/insectivores from the family Hemiramphidae, and omnivorous fish from the family Monodactylidae were collected. In Mtoni, Albulidae, Callionymidae, Gerridae, Gobiidae, Leiognathidae, Lethrinidae, Teraponidae, and Lutjanidae were caught.

The diet of the mudskippers in Chwaka was compared with the diets of the fish species in the Chwaka mangroves that were reported by Lugendo et al. (2006). The diet of the mudskipper was tested for similarity with the diets of fishes from both the creek and the channel with a Bray-Curtis cluster analysis that was performed with BioDiversity Pro© version 2. In the cluster analysis, data on the gut contents were included for *Gerres filamentosus* Cuvier, *Gerres oyena*, *Lethrinus lentjan*, *Lutjanus fulviflamma*, *Monodactylus argenteus*, *P. argentilineatus*, and *Zenarchopterus dispar*. The length classes of the mudskipper, *P. argentilineatus*, were clustered in three length ranges (2-5, 6-9, and 10-13 cm) and average gut contents were calculated for each of these ranges for use in the cluster analysis. This analysis was not done for Mtoni, as the diet of the fish fauna of Mtoni was not determined (except for *P. argentilineatus*).

For stable isotope analysis, samples were taken from the fish muscle tissue from the section of the tail below the lateral line. From the crabs all soft body tissues in the carapax were used. For small food items the whole body was processed for smaller food items. Samples for stable isotope analysis were dried at 70 °C for 48 h and ground to a fine powder. The carbon and nitrogen stable isotopic composition was determined using a Carlo Erba NA 1500 element analyser (Carlo Erba, Milan, Italy) coupled on-line via a Finnigan Conflo III, with a Thermo-Finnigan DeltaPlus mass-spectrophotometer (Thermo Fischer, Waltham, MA, USA). Carbon and nitrogen isotope signatures are expressed as ratios between the isotopes $^{12}\text{C}/^{13}\text{C}$ and $^{14}\text{N}/^{15}\text{N}$ respectively and are noted in the delta notation ($\delta^{13}\text{C}, \delta^{15}\text{N}$) in parts per thousand deviation from the isotopic ratios in the international standard reference samples Vienna PDB (Craig, 1957; Gonfiantini, 1984) and atmospheric nitrogen (Mariotti, 1983). Vienna PDB is the reference material of the International Atomic Energy Agency (IAEA) that replaces the original reference material (PDB) from a pulverised belemnite fossil from the Upper Cretaceous Pee Dee formation in South Carolina since the supply of the original reference material was exhausted.

To indicate potential food sources for the mudskippers on basis of the stable isotope results we used the range of both isotope values by taking the minimal and
maximal isotopic ratios found for mudskippers, and subtracting 1‰ carbon and 3‰ nitrogen to correct for enrichment as a result of increase in trophic level (Minagawa and Wada, 1984, Rau et al., 1983). If the presence of only a single food source was assumed, the calculated range shows the signatures of potential food items.

**Statistical analysis**

Morphometric data are noted as average ± SD. Pearson’s correlations were used to study the correlation between fish total length and morphological data. Data were tested for normality and homoscedasticity. One-way ANOVA, followed by Tukey’s post-hoc test, was used to test for differences between the stable isotopic signatures of the different mudskipper size classes and populations. All statistical analyses were performed with the software-package SPSS® 11.5 and statistical significance was accepted at \( P \leq 0.05. \)

**RESULTS**

**Morphometry**

The mudskippers that were collected from Chwaka Bay \( (n = 73) \) had an average length of 8.4 ± 3.3 cm, whereas the mudskippers collected in Mtoni \( (n = 107) \) had an average length of 7.3 ± 0.5 cm. The average length of the gut (from oesophagus to anus) found in mudskippers from Chwaka \( (n = 58) \) was 12.5 ± 6.7 cm. In mudskippers from Mtoni \( (n = 46) \) the average gut length was 7.2 ± 1.5 cm.

The total length of the mudskippers showed a strong correlation with mouth width, mouth height, and gut length (figure 2; Pearson’s correlations, mouth width: \( r^2 = 0.925, n = 119, P < 0.001; \) mouth height: \( r^2 = 0.925, n = 119, P < 0.001; \) gut length: \( r^2 = 0.895, n = 101, P < 0.001). The ratio between gut length and total length ranged from 0.38 to a maximum value of 1.95, with a average of 1.14 ± 0.03 \( (n = 101) \).

**Gut content analysis**

The diet of individuals from Chwaka Bay displayed a clear shift with body size, with their prey type increasing in size (figure 3a). The small size classes (20-50 mm) in Chwaka had a diet mainly consisting of copepods and amphipods, while copepods were not found in the gut of mudskippers larger than 35 mm. Medium-sized fish (60-90 mm) had a diverse diet, with the contribution of amphipods decreasing and that of polychaetes and crabs increasing with body length. In the largest size classes (100-130 mm) crabs were the principal component of the diet. Specimens of the genus *Uca* were the predominant crabs found in guts of all fish size classes.

In Mtoni, polychaetes, amphipods, insects, and fish formed the main components of the diet of the mudskippers for the size classes of 40-70 mm (figure 3b). Larger mudskippers were not present in the Mtoni mangroves due to decreased growth and life span (chapter 5).
In the guts of mudskippers that had consumed crabs, leg parts rather than complete crabs were most abundant. The average number (figure 4a; Pearson’s correlation, \( r^2 = 0.235, P < 0.05 \)) as well as length (figure 4b; Pearson’s correlation, \( r^2 = 0.075, P < 0.05 \)) of ingested crab legs increased significantly with fish size, but also the range in values recorded for both variables increased with fish size.

Cluster analysis of the diet of the zoobenthivorous, insectivorous, and omnivorous fishes in the channel and creek of the mangroves of Chwaka Bay on the basis of gut content analysis revealed \( \leq 23\% \) similarity between the diet of the mudskipper and the diets of other fish species captured in the same area (figure 5).

**Stable isotope analysis**

The mudskippers at Chwaka Bay and Mtoni had a \( \delta^{15}\text{N} \) signature that was similar to that of other zoobenthivorous fish (figure 6). However, there was a difference in \( \delta^{13}\text{C} \) between the mudskippers and the other zoobenthivores, with the mudskippers from Chwaka Bay being significantly enriched in \( \delta^{13}\text{C} \) by 1.9‰ (\( P = 0.031 \)), and the mudskippers in Mtoni being significantly depleted by 3.6‰ (\( P < 0.001 \)), relative to the other zoobenthivores. The \( \delta^{13}\text{C} \) signatures of the mudskippers from Chwaka and Mtoni did not differ significantly (\( P = 0.996 \)). Stable nitrogen isotope ratios of mudskippers were about 5‰ lower in Chwaka Bay than in Mtoni (figure 6).
Figure 3 Food items ingested by mudskippers from Chwaka (a) and Mtoni (b). The numbers in the horizontal bar above the graphs represent the number of fish per size class. Food items that comprised less than 5% of the gut contents of a size class were grouped as miscellaneous food items.
Figure 4 Number of crab legs (meri) ingested (a) and average length of the merus of ingested crab legs (b) as a function of the total length of the mudskippers. Vertical lines indicate the range of number and size of meri ingested, respectively.
Figure 5 Similarity among diets of fishes from the Chwaka Bay mangroves collected in the mangrove channel or creek. Mudskippers were only collected in the creeks. 1 G. filamentosus (creek); 2 G. filamentosus (channel); 3 G. oyena (channel); 4 M. argenteus (creek); 5 L. fulviflamma (channel); 6 M. argenteus (channel); 7 Z. dispar (creek); 8 Z. dispar (channel); 9 P. argentilineatus (20-59 mm); 10 P. argentilineatus (60-99 mm); 11 P. argentilineatus (100-139 mm); 12 L. lentjan (channel).

The relatively large variation in stable carbon isotopic signatures of individual mudskippers from Chwaka Bay resulted in a large range for the signature of potential food items (figure 6a). The predicted range showed an overlap with the isotopic signatures of crabs, insects and shrimps. In Mtoni, the predicted range in stable isotopic values of potential food items showed an overlap with the signatures of polychaetes and Uca crabs (figure 6b).

The $\delta^{15}N$ signatures of mudskippers showed a significant increase with fish size in Chwaka Bay (figure 7; Pearson’s correlation, $r^2 = 0.442$, $n = 29$, $P < 0.001$). In the population of Mtoni there was no significant relationship between fish length and $\delta^{15}N$ signatures (Pearson’s correlation, $r^2 = 0.202$, $n = 15$, $P > 0.05$), which was probably due to the small size range of fish at this location. No relationship was found between $\delta^{13}C$ and fish size in Chwaka Bay or Mtoni (Pearson’s correlations, Chwaka Bay: $r^2 = 0.183$, $n = 29$, $P > 0.05$; Mtoni: $r^2 = 0.017$, $n = 15$, $P > 0.05$).
DISCUSSION

The analysis of the morphometry of the mudskipper showed that mouth height, mouth width, and gut length increased with body growth. Increase in mouth dimensions is a common feature during the ontogeny of fishes in general (Luczkovich et al., 1995; Wainwright and Richard, 1995), whereas the relative gut length differs between feeding guilds. Generally, the highest relative gut lengths occur in herbivores, lowest relative lengths in carnivores, and intermediate relative lengths in omnivores, because the digestion of plant materials takes more time than digestion of animal tissues. Shifts to a more herbivorous diet result in elongation of the gut, while a carnivorous diet results in a shorter gut (Drewe et al., 2004; Piet, 1998). Thus, changes in relative gut length may occur when the diet shifts during growth, or in species that are dependent on energy-poor food resources (Gutowska et al., 2004; Piet, 1998). In addition, diet-induced elongations can be accompanied by changes in the activities and levels of different groups of digestive enzymes (Drewe et al., 2004; German et al., 2004).

The results show that the mudskipper diet shifts from small zooplankton towards large decapod crabs during body growth. During the life stages when mudskippers prey on decapod crabs the range of crab sizes that are targeted increased and larger crabs were included in the diet. Small decapod crabs continue to be part of the diet, presumably due to the limited size range of Uca crabs (Litulo, 2005). Simultaneously with the shift in diet, the relative gut length increased. The manifestation of both changes in the mudskipper contradicts the general guild-based pattern of gut lengths (Piet, 1998), while changes in other gobiid fishes are consistent with the pattern (Clayton and Snowden, 2000; Geevarghese, 1983; Yang et al., 2003).

The dietary shift in the present study towards larger crustaceans also means a shift to consumption of crustaceans having exoskeletons with higher chitin content, which are more difficult to degrade and require more chitinolytic (i.e. chitin-digesting) activities than the exoskeletons that are ingested by juvenile mudskippers. The chitinolytic enzymes in fish comprise chitinases for initial breakdown of chitin skeletons into chitobiose dimers, and chitobiases for further breakdown (Fänge et al., 1979). It has been found previously that fish with short guts have high stomach chitinase activities to disrupt the exoskeletons in order to gain access to the nutrient-rich inner tissues of crabs, while fish with longer guts have both chitinase and high chitobiase activities to degrade the exoskeletons completely and to use the exoskeletons themselves as a source of nutrients (Gutowska et al., 2004). Although no enzyme activities were analysed in our study, the data on the mudskipper morphology and diet reveal that elongation of the mudskipper gut coincided with an increase in chitin content of the diet and suggest that the elongation facilitates digestion by prolongation of gut evacuation times.
Figure 6 Stable carbon and nitrogen isotope values of macrofauna and zoobenthivorous fish in Chwaka (a) and Mtoni (b). The colours of the symbols indicate whether species were collected from the subtidal mudflats (black), intertidal mudflats (white), or mangrove trees (grey). The shaded boxes show the isotopic range of potential food items for the mudskippers (based on 100% consumption of a single food source) for both sites. 1 P. argentilineatus; 2 zoobenthivorous fishes (creek); 3 zoobenthivorous fishes (channel); 4 amphipods; 5 Dotilla fenestrata; 6 hermit crabs; 7 insects; 8 isopods; 9 Macrophthalmus sp.; 10 Metopograpus sp.; 11 polychaetes; 12 portunid crabs; 13 Sesarma guttatum; 14 Sesarma sp. 1; 15 Sesarma sp. 2; 16 shrimps; 17 Uca dussumieri; 18 Uca sp.
The changes in morphometry of the mudskipper appear to be closely connected to changes in the diet. While the mouth dimensions define the maximum size of prey that can be handled during early life stages, the length and possibly the biochemistry of the gastrointestinal tract define the type of prey that can be digested. Consequently, dietary changes in growing mudskippers appear to be controlled by both ingestion and digestive capacities.

The nitrogen isotopic signatures show that the mudskippers belong to the trophic level of the secondary consumers in both ecosystems. The $\delta^{15}N$ of the mudskippers in Chwaka Bay further show a slight enrichment with fish growth, which indicates that the mudskippers shift their diet to species from higher trophic levels. This is supported by the observation of a dietary shift in the gut contents. Overall, elevated $\delta^{15}N$ values in Mtoni relative to Chwaka resulted from high input of foreign nutrients into the Mtoni Estuary (Mashauri and Mayo, 1989), but this did not result in changes in foodweb structure (Chapter 5).

The combination of $\delta^{13}C$ and $\delta^{15}N$ values shows that the mudskippers in both Chwaka and Mtoni mostly prey on species related to the mangroves, while the origin of the resources for the other zoobenthivorous fishes differs between the two sites. In Mtoni, the zoobenthivores were much more enriched in carbon than the mudskippers, indicating that the zoobenthivores had a higher dependence for food on bay habitats than on mangroves. In contrast, the zoobenthivorous fishes in Chwaka were slightly more depleted than the mudskippers, which indicates that the zoobenthivores have a higher dependence on food from the mangroves than the mudskippers. Since the mudskippers had full access to the mangroves at both sites in this study at all times, accessibility did not affect their carbon signatures. The accessibility of the mangroves for other zoobenthivorous fishes was subject to the tidal amplitude and was thus translated into differences in carbon isotopic signatures: only fishes with continuous mangrove access (e.g. in Chwaka) could utilise resources from the habitat to a high degree, whereas fishes with only temporal access to mangroves (e.g. in Mtoni) could consume less food from these areas. The influence of tidal regime on the value of the mangroves as a feeding habitat was also found in a study by Nagelkerken and Van der Velde (2004a), in which only mangrove residents were found to feed inside the mangroves.

During their juvenile stages, the mudskippers used food resources (a.o. copepods and amphipods) that are also commonly used as food items by other zoobenthivorous fishes in tropical coastal habitats (Kamukuru and Mgaya, 2004; Nagelkerken et al., 2000). Minimisation of competition pressure through temporal resource partitioning, which is provided by the amphibious habits of the mudskippers in combination with the tidal regime, may facilitate the use of the same food sources by juvenile mudskippers and other zoobenthivores, when assuming that food is a limiting factor.
During later life stages the fish shift to a diet of mangrove crabs, which are largely unavailable to other zoobenthivores because these crabs shelter at high tide when the other zoobenthivorous fish feed in this habitat. This dietary shift could theoretically be driven by competition with other zoobenthivorous fishes. However, comparison of the diet of different size ranges of mudskippers with the diet of other zoobenthivorous fishes in Chwaka revealed few similarities between the diets. This shows that at this site there is little interspecific competition for the food sources that are used by the mudskipper. The cluster analysis also revealed that the dietary shift that is observed in the mudskipper diet does not result in differences in similarity between the diets of mudskippers and other fish species. It can subsequently be concluded that the dietary shift does not decrease competition pressure and is therefore unlikely to be the driving force behind the shift in the diet of the mudskipper. The comparison of the mudskipper diet with the diets of other zoobenthivorous fishes could only be made for Chwaka, but since no indications of interspecific competition or predation as driving forces behind the observed dietary changes were found, similar changes in diet can also be expected to occur in other mudskipper populations.

Diet changes were likely to be driven by the increasing energetic needs and mechanical capabilities of growing mudskippers that were limited to available food resources in the intertidal mangrove habitat (Kathiresan and Bingham, 2001). Feeding in the subtidal waters to supplement increasing demands is not considered to be a likely option for the mudskippers, because they are thought to have poor eyesight.

Figure 7 Stable nitrogen isotope values of mudskippers in Chwaka (closed circles) and Mtoni (open circles).
during submergence due to adaptations for aerial vision (Colombini et al., 1996; Nieder, 2001, Stebbins and Kalk, 1961). Hence, mudskippers may have adapted to food sources (i.e. crustaceans) that may be difficult to digest and require special digestive adaptations, but which are readily available in the intertidal habitat. As a result, the feeding behaviour of the amphibious mudskipper results in a spatial and temporal resource partitioning from other zoobenthivores.

The combination of morphological data, gut content and stable isotope analysis indicates that the amphibious habits of the mudskippers provide them with specific advantages over other zoobenthivorous fishes. The data show that despite some overlap in the diet, competition with other zoobenthivores can be minimised during juvenile life stages through temporal resource partitioning. Morphological changes in combination with the amphibious life style subsequently facilitate a dietary shift to food sources (i.e. mangrove crabs) that are generally unavailable for potentially competing zoobenthivorous fishes, indicating also a spatial resource partitioning.
Chapter 4

Status of pollution in mangrove ecosystems along the coast of Tanzania

G. Kruitwagen, H.B. Pratap, A. Covaci, S.E. Wendelaar Bonga
ABSTRACT In an assessment of pollution the concentrations and distribution of heavy metals, DDT, PCBs, HCH and organotins in mangrove sediments and tissues of the barred mudskipper were analysed in six mangrove forests along the coast of Tanzania. The results revealed that the distribution of industrial pollutants is mostly restricted to the mangroves of Mtoni and Kunduchi, both located in the urban area of Dar es Salaam, whereas agricultural pollutants were found in all mangroves. The distribution of pollutants reveals that they are limited to the mangroves in which the waterway that carries the pollutants empties. This may follow from the level of pollution which is generally low compared to areas with a higher level of industrialisation with exception of a hotspot of heavy metal pollution in the Mtoni Estuary. The hotspot suggests that an increase in the number of industries may cause severe pollution if no adequate treatment methods are applied.

INTRODUCTION

Worldwide mangrove forests are under threat of the accumulation of pollutants, which may be imported into mangrove ecosystems through waters from rivers and streams, but also from adjacent coastal ecosystems via tidal exchange. The distribution, behaviour, and accumulation of these imported chemicals in the ecosystem is largely defined by the hydrology of the mangroves, the geochemical properties of sediments, and the class of pollutants (e.g. heavy metals, organochlorine pesticides, polychlorinated biphenyls, organotins). The properties of the mangrove sediments provide good binding opportunities for a number of these pollutant classes: hydrophobic organic pollutants adsorb to the extensive surfaces that are provided by the fine particulate sediments of estuaries and mangroves. Metals are trapped in mangrove sediments through the formation of complexes with sulphides (Lacerda et al., 1991), particulate organic carbon, or iron oxyhydroxides (Chapman et al., 1998). As a consequence, anthropogenic pollutants are filtered from the water layer and accumulate in the sediments of estuaries and mangroves (Bayen et al., 2005; Bhattacharya et al., 2003; Tam and Wong, 1995; Tam and Yao, 2002). Depending on the speciation of chemicals, the pollutants can accumulate in the tissues of biota.

Along the coast of Tanzania mangrove stands are found in most river mouths, estuaries, and sheltered bays. Due to this position and the capacity of mangrove sediments to trap chemicals, the pollutant levels in the mangrove ecosystem are likely to reflect the use of pollutants in the coastal area and the hinterlands. A study on heavy metals in brown algae in the Zanzibar Channel revealed that the highest concentrations occurred near river mouths on the Tanzanian mainland (Engdahl et al., 1998). Among the waterways in the urban area of Dar es Salaam, the Msimbazi River has been identified as heavily polluted (Ak’habuhaya and Lodenius, 1988; De Wolf et al., 2001; Machiwa, 1992b), but also the harbour of Dar es Salaam, located within the Mtoni Estuary, has been reported to contain elevated levels of pollutants (Machiwa, 1992a; Mwevura et al., 2002). Only de Wolf et al. (2001) have recently determined the level of pollution in mangroves along the coast of Tanzania. In this study heavy metal concentrations in
sediments and in the periwinkle *Littoraria scabra* were analysed. The Msimbazi River as identified as the most polluted sampling site, but no correlations were found between pollution levels and differences in morphology.

In the present study an assessment was made on the levels of multiple anthropogenic pollutants in sediments and tissues of the barred mudskipper (*Periophthalmus argentilineatus* Valenciennes) in six mangrove stands on the Tanzanian coast. The mangroves are located at various distances from Dar es Salaam, the largest city of Tanzania, and were expected to differ as to the influence of industrial and agricultural activities. In this assessment quantifications were made of heavy metals since these natural elements are used in high concentrations in many industrial processes. Polychlorinated biphenyls (PCBs) were included in this study as key representatives of industrial persistent organic pollutants (POPs). Hexachlorobenzene (HCB) was included since it is known to be a by-product of a wide variety of industrial processes and in incomplete combustion, but it is also present in some pesticides (Bailey, 2001). Dichlorodephenyl-trichloroethane (DDT) and hexachlorohexane (HCH) were included as representatives of persistent agrochemicals.

**MATERIALS AND METHODS**

**Study areas**

Samples were collected in the mangroves of Mtoni, Kunduchi, Mbweni, Chwaka, Makoba, and Rufiji along the coast of Tanzania (figure 1). Though heavily polluted (de Wolf et al., 2001) the mangrove stand at the mouth of the Msimbazi River was not included in this study, because the mudskipper is absent from this sandy river basin.

The harbour of Dar es Salaam is located in the Mtoni Estuary, a large estuary that receives water from two tributaries, the Kizinga and the Mzinga. The Kizinga Stream is the largest tributary (30 km long) and is suspected to carry mixed wastes from household, agricultural, as well as industrial origin. The Kizinga reaches the Mtoni Estuary as a narrow stream. After passing a small bridge the stream widens into a large mangrove stand in which no fish were found. The stream winds through the Mtoni mangroves and passes over the mudflat before the Mzinga joins the Kizinga stream. After the junction of both streams, about 2 km downstream from the bridge, a mangrove stand is found in which mudskippers were present. The different sampling locations in the Mtoni Estuary will be referred to as Mtoni (1) to Mtoni (5) (figure 1).

At 25 and 40 km north of the Dar es Salaam harbour, respectively, the mangroves of Kunduchi and Mbweni are located along the Zanzibar Channel. The area surrounding the Kunduchi mangrove is currently being developed as the northernmost suburb of the city of Dar es Salaam. Several hotels and some industries are located in the proximity of the mangroves. The Mbweni mangrove is located north of Kunduchi, next to a small fishing village with no apparent influences of the city of Dar es Salaam.
The sites of Chwaka and Makoba are both located in rural areas of Unguja Island (Zanzibar), which is located 30 km from the mainland coast and has little industry. Chwaka Bay is a large shallow bay on the east of the island, which is bordered by small fishing villages. A large mangrove forest fringes the closed southern end of the bay. The Makoba mangroves are located in a bay in the northwest of Unguja Island. The Makoba Bay is fed by two streams, the Zingwezingwe and Mwanakombo. In the proximity of the estuary some agricultural farms and mariculture ponds are located.

The Rufiji site is located 100 km south of Dar es Salaam city in the wide delta of the Rufiji River. This river has a catchment area of 177 400 km² and is over 640 km long (United Nations Environment Programme, 2001). In the upstream part of its catchment area some small towns are located. The downstream part is free of industries, but in the delta various pesticides are being used in mangrove sections that have partially been cleared for rice cultivation (Taylor et al., 2003). Samples were collected from a rice paddy and the adjacent mangrove creek.
Sampling

Samples analysed for organochlorine pollutants, heavy metals, and organotins were collected in May 2003, June 2004, and June 2005, respectively.

Samples for analysis of organochlorine pollutants and heavy metal were collected from fine grained mud in the major mangrove stands. For organotin analysis sediment samples were collected over a transect in the Mtoni Estuary. In Mbweni a sample was collected to be used as a control. The sampling effort focused on Mtoni, since this is the only coastal system in this study that is subject to commercial shipping activity that is a potential source of organotin pollution.

Samples were collected from the top 5 cm of sediment. Each sample consists of a pooled sample of five points spaced one meter apart. Sediment samples for analysis of organochlorines and PCBs were air-dried at room temperature. Samples for analysis of organotin compounds were immediately frozen after collection. These sediments were freeze dried and subsequently ground in a mortar and sieved over a 100 µm mesh. Sediments for heavy metal analysis were dried in an oven at 70°C for 24 h. Before analysis all sediments were sieved over a <63 µm mesh to obtain the fine fraction.

Barred mudskippers, *P. argentilineatus* (5.1-13.2 cm), were caught at random with scoop nets during low tide on the exposed mudflats in all mangroves. In Mtoni mudskippers could only be found at site 3 (figure 1). For pesticide and PCB analyses fillets of the muscle tissues of the mudskippers were collected and stored at -20°C until analysis. For heavy metal analysis whole body samples of mudskipper were collected and oven dried at 70°C for 24 h. Prior to heavy metal analysis, the fish samples were grinded to a homogeneous powder in a blender. Muscle tissues and whole body samples were used for analysis because the small size of the individual mudskippers and the small size of the populations did not enable the use of organs in which a higher accumulation rate of pollutants is expected.

In Rufiji rice was obtained that had been harvested from the same rice paddy where sediment and fish were collected for this study. The rice had been dried in the sun and husks had been removed by the local farmers. A sample of the rice was processed and analysed for POPs according to the method described for the mudskipper tissues.

Heavy metals

Sediment and whole body mudskipper samples were digested in a mixture of HNO₃ and H₂O₂ (4:1, v/v) in a Milestone MLS microwave with Teflon bombs and stored at 4°C until analysis. Sediment and whole body samples were analysed for cadmium, chromium, copper, lead, aluminium, zinc, iron, cobalt, tin, and nickel, using a SpectroFlame ICP-AES (Spectro Analytical Instruments, Kleve, Germany). Analytical accuracy was checked with standard reference material for marine sediment (IAEA-356) and mussel tissue (*Mytilus edulis*, BCR278R). Recovery of the reference samples ranged between 85.1% (Co) and 112.7% (Ni) for sediment and between 95.4% (Cu) and 126.7% (Cr) for mussel tissue. Values were corrected accordingly.
Organochlorine pollutants

Samples were analysed for the following organochlorine pesticides: α-, γ- isomers of hexachlorocyclohexane (HCHs), p,p′-DDE, p,p′-DDD and p,p′-DDT (expressed here as DDTs), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs). The following PCB congeners (IUPAC numbers) were targeted: 28, 52, 101, 118, 138, 153, 156, 170, 180, and 187. Internal standards used were PCB 46, PCB 143, and γ-HCH (100 pg/µl). All individual standards of PCBs and pesticides were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany).

The methods for determination of PCBs and OCPs in fish and sediment samples have been previously described and validated by Covaci et al. (2005) and Jacobs et al. (2002). The whole amount of available fish muscle (typically around 4 g) or 3 g sediment were homogenized with anhydrous Na₂SO₄, spiked with internal standards (ε-HCH, PCB 46 and 143) and extracted for 2 h with 100 ml hexane/acetone (3:1, v/v) and an accelerated Soxhlet extractor B-811 (Buchi, Switzerland). Fish extracts were cleaned-up after lipid determination on 8 g acidified silica, while the sediment extract was cleaned-up on 6 g acidified silica topped with 3 g of copper powder. After elution with 15 ml hexane and 10 ml dichloromethane, the cleaned extracts were concentrated. The cleaned extracts were analysed on a Hewlett Packard 6890 GC-µECD (Palo Alto, CA, USA) was equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 (SGE, Zulte, Belgium) 1,7-dicarba-closo-dodecarborane 8% phenyl methyl siloxane capillary column. The identification of OCPs and PCBs was based on their relative retention times to the internal standard used for quantification. The content of organochlorine pollutants could not be analysed in sediment samples from Mtoni (1) due to the nature of the sediments at this location.

The analytical procedures were validated through regular analysis of laboratory reference material (fish tissue) and through participation in Quasimeme interlaboratory tests (PCBs and OCPs determination in sediment and fish). The method limits of quantification (LOQ) for individual compounds ranged between 0.1 and 0.3 ng/g wet weight (ww) for fish and 0.2 ng/g dry weight (dw) for sediment. The analyses were carried out at the Toxicological Centre of the University of Antwerp, Belgium.

The ratio between α-HCH and γ- HCH indicates whether lindane or technical HCH has been used, while the ratio between DDT and its breakdown-products DDE and DDD gives an indication of the moment of use of DDT.

Organotin compounds

Samples of sieved sediments (4-5 g) were acidified with hydrochloric acid, extracted with diethyl-ether/tropolone, derivatized using a Grignard pentylation reaction, purified over deactivated alumina and analysed using gas chromatography with an ion trap mass spectrometric detector (GC-MS and GC-MS-MS; Varian 3400 gas chromatograph; Varian Saturn IV ion trap mass spectrometer; Varian, Walnut Creek, CA, USA) as applied in previous studies (Stäb et al., 1996). Samples were analysed for tributyltin (TBT) and its degradation products dibutyltin (DBT) and monobutyltin (MBT),
tetrabutyltin (TeBT), triphenyltin (TPT) and the degradation products diphenyltin (DPT) and monophenyltin (MPT). Analyses were carried out by the Institute for Environmental Studies (IVM) at the Vrije Universiteit Amsterdam, the Netherlands.

**Statistical analysis**

Differences between the heavy metal concentrations of the various mangroves were analysed by one-way ANOVA. The amount of sediment collected turned out to be insufficient to perform all chemical analyses on all samples. As a result, no statistical analysis could be performed on the results for organochlorine and organotin compounds. All statistical analyses were performed with software-package SPSS© 11.5 and statistical significance was accepted at $P \leq 0.05$. Significant differences between groups are indicated in tables with letters; differences between groups sharing the same letter are not significant.

**RESULTS**

**Heavy metals**

The average metal concentrations for sediment and whole body samples of mudskippers are given in tables 1 and 2, respectively. The data show that the highest average sediment concentrations of zinc, iron, lead, copper, chromium, and nickel were found in Mtoni (1). Particularly, the concentrations of copper, chromium, and nickel were elevated in the sediments of Mtoni (1) compared to the other sampling sites. The concentrations of lead, copper, chromium, and nickel in Mtoni (2) were significantly lower than in Mtoni (1), but still higher than in Mtoni (3), Chwaka, Kunduchi, Mbweni, and Makoba. The concentrations for lead and chromium in the sediments of Rufiji were lower than Mtoni (2), but the concentrations of the other metals were similar to Mtoni (2). The concentrations of aluminium and iron were very high in the sediments of Mtoni (2), Mtoni (1), and Rufiji. The metal concentrations in Chwaka, Kunduchi, Mbweni, and Makoba were generally low.

The concentrations in the whole body samples of mudskippers exhibited only minor variations, but the concentrations were generally low and similar. The fish at Mtoni (3) had higher concentrations of aluminium and iron.

**Organochlorine pollutants**

The concentrations of organochlorine pollutants in sediments and mudskipper muscle tissues are shown in tables 3 and 4, respectively. The concentration of total HCH (γ-HCH and its breakdown-product α-HCH) was highest in the sediments at Mtoni (3) followed by Rufiji. While both γ-HCH and α-HCH were found in Mtoni (3), Chwaka, and Rufiji, only γ-HCH was found in Mtoni (2), Kunduchi, Mbweni, and Makoba. The ratio of γ-HCH/sum HCH was high in the sediments from all sites. In the mudskipper muscle tissues γ-HCH was the only derivate that could be detected: the highest levels were found in the fish...
Table 1 Average metal concentrations in sediments of various sites (µg/g dw ± SE). Sites with different letters are significantly different.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mtoni (1)</th>
<th>Mtoni (2)</th>
<th>Mtoni (3)</th>
<th>Kunduchi</th>
<th>Mbweni</th>
<th>Chwaka</th>
<th>Makoba</th>
<th>Rufiji</th>
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<td>2</td>
<td>1</td>
<td>3</td>
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<td>Aluminium</td>
<td>54258 ± 5791&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61923 ± 4133&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18631 ± 3252&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2373 ± 68&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8151 ± 544&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>10338</td>
<td>13380 ± 2747&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Zinc</td>
<td>2447 ± 101&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178 ± 29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.9 ± 11.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.1 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.4 ± 1.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>18.4 ± 9.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>66.6</td>
<td>57.1 ± 2.5&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>Iron</td>
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<td>28250 ± 499&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8068 ± 1013&lt;sup&gt;c&lt;/sup&gt;</td>
<td>201 ± 141&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>2544 ± 388&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14155</td>
<td>32505 ± 2670&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Lead</td>
<td>384.8 ± 209.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.0 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>23.3 ± 2.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>18.6 ± 1.2 &lt;sup&gt;b&lt;/sup&gt;</td>
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<td>1.7 ± 0.0</td>
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<td>Chromium</td>
<td>6244 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.5 ± 11.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6.4 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>120.0 ± 1.4&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>13.5</td>
<td>32.8 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
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Table 2 Average metal concentrations in fish tissues of various sites (µg/g dw ± SE). Sites with different letters are significantly different.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mtoni (1)</th>
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<th>Mtoni (3)</th>
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<td>Aluminium</td>
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<td>n/a</td>
<td>299.7 ± 91.5</td>
<td>57.4 ± 34.3</td>
<td>26.8 ± 4.1</td>
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<td>172.8 ± 107.3</td>
<td>62.2 ± 14.4</td>
</tr>
<tr>
<td>Zinc</td>
<td>n/a</td>
<td>n/a</td>
<td>218.7 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152.6 ± 21.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>161.3 ± 8.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>164.5 ± 31.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169.6 ± 17.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>148.0 ± 16.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron</td>
<td>n/a</td>
<td>n/a</td>
<td>295.3 ± 42.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.9 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147.2 ± 25.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>168.6 ± 40.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>131.9 ± 9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>141.0 ± 14.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lead</td>
<td>n/a</td>
<td>n/a</td>
<td>8.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3 ± 2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.9 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.9 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cadmium</td>
<td>n/a</td>
<td>n/a</td>
<td>0.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Copper</td>
<td>n/a</td>
<td>n/a</td>
<td>4.9 ± 0.7</td>
<td>3.5 ± 0.5</td>
<td>5.5 ± 1.8</td>
<td>2.9 ± 0.1</td>
<td>5.0 ± 0.7</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>Cobalt</td>
<td>n/a</td>
<td>n/a</td>
<td>0.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chromium</td>
<td>n/a</td>
<td>n/a</td>
<td>3.2 ± 0.2</td>
<td>3.6 ± 0.9</td>
<td>2.0 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>3.1 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tin</td>
<td>n/a</td>
<td>n/a</td>
<td>3.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.6 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nickel</td>
<td>n/a</td>
<td>n/a</td>
<td>2.0 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.3 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.3 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.4 ± 0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.4 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n/a = not applicable
Table 3 Concentrations of organochlorine pollutants in sediments of various sites (ng/g dw ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Mtoni (2)</th>
<th>Mtoni (3)</th>
<th>Kunduchi</th>
<th>Mbweni</th>
<th>Chwaka</th>
<th>Makoba</th>
<th>Rufiji</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>α-HCH</td>
<td>n.d.</td>
<td>0.51 ± 0.07</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.47 ± 0.47</td>
<td>n.d.</td>
<td>0.34 ± 0.09</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>0.53</td>
<td>4.75 ± 0.56</td>
<td>0.24 ± 0.24</td>
<td>0.21 ± 0.21</td>
<td>1.52 ± 0.20</td>
<td>0.84</td>
<td>1.97 ± 0.63</td>
</tr>
<tr>
<td>sum HCH</td>
<td>0.53</td>
<td>5.26</td>
<td>0.24</td>
<td>0.21</td>
<td>1.99</td>
<td>0.84</td>
<td>2.30</td>
</tr>
<tr>
<td>γ-HCH/ sum HCH</td>
<td>1.00</td>
<td>0.91</td>
<td>1.00</td>
<td>1.00</td>
<td>0.77</td>
<td>1.00</td>
<td>0.85</td>
</tr>
<tr>
<td>pp’-DDE</td>
<td>0.66</td>
<td>2.79 ± 0.81</td>
<td>0.13 ± 0.13</td>
<td>n.d.</td>
<td>0.59 ± 0.13</td>
<td>5.17</td>
<td>0.37 ± 0.37</td>
</tr>
<tr>
<td>pp’-DDD</td>
<td>1.30</td>
<td>1.45 ± 0.51</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.14 ± 0.14</td>
<td>2.05</td>
<td>0.71 ± 0.54</td>
</tr>
<tr>
<td>pp’-DDT</td>
<td>0.76</td>
<td>0.46 ± 0.46</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2.49 ± 1.78</td>
<td>1.43</td>
<td>0.58 ± 0.42</td>
</tr>
<tr>
<td>sum DDT</td>
<td>2.72</td>
<td>4.70</td>
<td>0.13</td>
<td>-</td>
<td>3.23</td>
<td>8.64</td>
<td>1.65</td>
</tr>
<tr>
<td>pp’- DDT/sum DDT</td>
<td>0.36</td>
<td>0.12</td>
<td>0.00</td>
<td>-</td>
<td>0.77</td>
<td>0.17</td>
<td>0.35</td>
</tr>
<tr>
<td>CB 52</td>
<td>1.03</td>
<td>0.99 ± 0.02</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.80 ± 0.45</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 101</td>
<td>0.21</td>
<td>0.25 ± 0.03</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 118</td>
<td>0.44</td>
<td>0.48 ± 0.05</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.43 ± 0.02</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 153</td>
<td>0.47</td>
<td>0.49 ± 0.06</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.88 ± 0.50</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 138</td>
<td>0.37</td>
<td>0.34 ± 0.06</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.22 ± 0.22</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 187</td>
<td>0.39</td>
<td>0.21 ± 0.08</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 180</td>
<td>0.35</td>
<td>0.32 ± 0.04</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 170</td>
<td>0.39</td>
<td>0.14 ± 0.09</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>sum PCB</td>
<td>3.65</td>
<td>3.08</td>
<td>-</td>
<td>-</td>
<td>2.33</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n.d. = below detection level

from Mtoni (3), followed by Rufiji and Mbweni. HCB was not detected in any of the sediments (table 3) or fish samples (table 4).

The highest sum of DDT and its derivatives in sediments was found at Makoba with the highest contribution of DDE (table 3). A similar ratio between the derivatives was found at Mtoni (3). In all fish samples DDE was present in higher concentrations than DDT. The highest sum of DDT was found in fish from Mtoni (3) followed by Makoba.

The PCBs that were quantified in this study could not be detected in the sediments of Kunduchi, Mbweni, Makoba, and Rufiji, nor in the fish muscle tissues from Rufiji (table 3 and 4, respectively). In the sediments of Mtoni (2), Mtoni (3), and Chwaka, the congeners 52, 118, and 153 were most abundant. In the fish tissues from all five sites where PCBs were detected, congeners 153, 138, and 180 were present in the highest concentrations. The total sum of PCBs in fish muscle tissues was highest in Kunduchi, followed by Chwaka and Mtoni (3).
The Rufiji rice sample had a very high concentration of \( \gamma \)-HCH and a low concentration of \( \alpha \)-HCH (table 4), which resulted in a \( \gamma \)-HCH/sum HCH-ratio of 0.99. The sample contained all derivates of DDT in the DDT/sum DDT-ratio of 0.22. A number of PCB congeners could be detected in the rice sample, of which CB 101 was found in the highest concentration.

**Organotin compounds**

The results from the analysis of organotin compounds in the sediments of Mtoni are presented in figure 2. The data show that the concentrations of MBT, DBT, and TBT decreased within the Mtoni Estuary with increasing distance from Dar es Salaam harbour. The highest concentrations of MPT were observed in the proximity of a textile dyeing plant. TeBT was not detectable in any of the sediments that were analysed. DPT and TPT were only detectable in the sample that was collected at Mtoni (4).

**DISCUSSION**

**Distribution of pollutants**

In the present study the main differences in concentration of pollutants between the various sites were observed for the heavy metals. The concentrations of this group of pollutants were highest in the mangroves of Mtoni, where heavy metals occur in concentrations that indicate heavy industrial pollution. The samples taken at different locations within the Mtoni mangroves reveal a concentration gradient that increased in the direction of the Kizinga Stream, where a textile dyeing mill is located nearby. With increasing distance from the effluent discharge pipe of the mill in the downstream direction the concentration of heavy metals in the sediments decreased rapidly. This decrease over a relatively short distance reveals a strong capacity and effectiveness of the sediments to remove heavy metals from the water layer. Mangrove sediments are generally reduced, anaerobic and characterized by high sulphide and iron concentrations (Tam and Wong, 1996). These conditions favour the immobilisation of heavy metals through the formation of metal-sulphide complexes by sulphate-reducing bacteria (Chapman et al., 1998; Marchand et al., 2004), which are abundant in the sediments of the Mtoni mangroves (Lyimo et al., 2002).

In the samples from the mangrove sites other than Mtoni only aluminium, iron and zinc were present in relatively high concentrations. However, these metals are most likely not from anthropogenic origin, but can be attributed to the high content of aluminium and iron oxides in the soils of the East African region (Food and Agriculture Organisation, 1998). These heavy metal concentrations comply with the concentrations that are commonly found in South African coastal sediments (Mzimela et al., 2003).
### Table 4
Concentrations of organochlorine pollutants in fish tissues and a rice sample of various sites (ng/g lipid weight ± SE). Sites with different letters are significantly different.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Mtoni (3)</th>
<th>Kunduchi</th>
<th>Mbweni</th>
<th>Chwaka</th>
<th>Makoba</th>
<th>Rufiji</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fish tissue</td>
<td>fish tissue</td>
<td>fish tissue</td>
<td>fish tissue</td>
<td>fish tissue</td>
<td>rice</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Lipid content (%)</td>
<td>0.46 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.48 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.51 ± 0.03</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>α-HCH</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>2571.4 ± 624.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6 ± 17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>527.1 ± 74.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.2 ± 62.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4 ± 13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>632.8 ± 51.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sum HCH</td>
<td>2571.4 ± 624.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6 ± 17.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>527.1 ± 74.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.2 ± 62.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4 ± 13.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>632.8 ± 51.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>γ-HCH/sum HCH</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>pp'-DDE</td>
<td>799.0 ± 141.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>24.3 ± 17.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.d.</td>
<td>269.3 ± 48.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.9 ± 14.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>pp'-DDD</td>
<td>166.3 ± 37.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>8.5 ± 8.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.d.</td>
<td>103.9 ± 10.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>pp'-DDT</td>
<td>117.5 ± 45.6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sum DDT</td>
<td>1082.8 ± 212.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>36.7 ± 25.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>373.2 ± 49.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.9 ± 14.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>pp'-DDT/sum DDT</td>
<td>0.11</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>CB 28</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 52</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 101</td>
<td>n.d.</td>
<td>n.d.</td>
<td>300.0 ± 146.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>25.8 ± 17.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 118</td>
<td>10.4 ± 10.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136.0 ± 60.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>16.4 ± 10.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
<td>52.0</td>
</tr>
<tr>
<td>CB 153</td>
<td>135.3 ± 12.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>951.8 ± 403.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.9 ± 9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>187.3 ± 81.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.1 ± 11.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 138</td>
<td>98.1 ± 7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>917.0 ± 379.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.8 ± 10.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202.5 ± 90.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.4 ± 10.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 187</td>
<td>44.8 ± 10.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>454.4 ± 193.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>90.6 ± 52.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.1 ± 8.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 156</td>
<td>9.2 ± 9.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>238.2 ± 91.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.</td>
<td>52.1 ± 36.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CB 180</td>
<td>88.9 ± 9.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1330.0 ± 469.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0 ± 7.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>356.8 ± 202.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>126.6 ± 19.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sum PCB</td>
<td>427.5 ± 31.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5039.0 ± 1968.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.7 ± 27.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1146.8 ± 605.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>365.0 ± 58.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

n.d. = below detection level; lipid content concerns the extractable lipids.
Organotin compounds have various applications in industries and agriculture and are mostly from anthropogenic origin (Fent, 1996). In the present study organotin concentrations were only determined in the sediments of Mtoni, where butyltin compounds appeared to be present in a gradient of concentrations that increased from the Kizinga stream in the direction of the harbour. The direction of the gradient suggests that the butyltin compounds in the environment are derived from shipping activities that are concentrated in the estuary mouth. The organotin concentrations decrease over distance due to sorption of organotins to sediments close to their release site (Langston and Pope, 1995). Tidal flushing of the estuary may also contribute to the observed distribution gradient in the estuary. The ratios between the different butyltin compounds suggest that TBT is gradually debutylated within the estuary (Yonezawa et al., 1994) at a rate similar to the release of TBT into the environment. The observed butyltin concentrations correspond to an average shipping density of less than 5 ships of 100 gross tons per day according to the method used by Ten Hallers-Tjabbes (2003) for shipping activity in the North Sea and the Skagerrak, which corresponds to the reported total of 1,039 deep sea ship calls to the Dar es Salaam harbour in the year 2003/2004 (Tanzania Ports Authority, 2006). Monophenyltin was detected in the sediments of the Kizinga Stream in addition to butyltin compounds. Phenyltins are in use as fungicide in agriculture and occur in coastal sediments as a result of run-off (Stäb

Figure 3 Sediment concentrations of organotin compounds in relation to the distance to the Dar es Salaam harbour. Numbers correspond to sampling locations in Mtoni (see figure 2). The sample at 50 km distance was collected in the Mbweni mangroves and was used as a reference sample.
et al., 1996). The presence of monophenyltin in the Kizinga Stream indicates agricultural activities in the hinterland of the stream.

PCBs are the only pollutants typical of industrial pollution that were found outside the city of Dar es Salaam. In Kunduchi a relatively high load of PCBs was found in the muscles of the mudskippers, which is most likely derived from plastics manufacturers that are located in the proximity. The PCBs have, however, not been found in the sediments from Kunduchi. This apparent contradiction might be the result of the low content of organic matter in the sandy sediments of Kunduchi. The presence of PCBs in the mangroves of Chwaka Bay is most likely the result of the use of outboard engines in the semi-enclosed bay since there are no industries in the area.

The presence of HCH and DDT and their derivates are directly related to agricultural activities (Mmochi and Mberek, 1998). HCH was present in the highest concentrations in the sediments and fish samples from Mtoni and Rufiji. In all samples, including the rice sample from the paddy in the Rufiji mangroves, HCH was mostly present as \( \gamma \)-HCH, indicating the use of lindane rather than technical HCH as a pesticide in the rice paddies (Willett et al., 1998). The high content of HCH in the rice sample reveals that this pesticide is used in considerable quantities during the growing season, which is not necessarily reflected by the concentrations in the environment since the pesticide is very volatile and susceptible to breakdown by solar radiation in tropical agriculture (Abdullah et al., 1997; Ritter et al., 1995). DDT is also subject to these processes and may be broken down to the derivates DDE and DDD (Abdullah et al., 1997). The highest total concentrations of DDT and its breakdown-products were encountered in the sediments of Makoba and Mtoni. With exception of the sediments of Chwaka, all analysed samples contained higher concentrations of the breakdown-products DDE and DDD than DDT, which indicates that the DDT derivates in the samples are the result of past use of DDT. Only the ratios between DDE, DDD, and DDT in the sediment samples from Chwaka indicated more recent use.

The variations in pollutant concentrations in the sediments were only reflected to a limited extent in the fish tissues. At all locations where mudskippers were collected, heavy metal levels in the sediments were low and consequently did not result in elevated levels of metals in the mudskipper tissues. The low concentrations of the lipophilic organochlorine pollutants in the fish tissues and the limited variation in the concentrations suggest that these pollutants were only available for uptake in limited amounts. Apart from the low environmental concentrations, the accumulation of lipophilic compounds in the muscle tissues of the mudskipper would also be limited by the low fat content of the mudskipper muscle tissue. The average fat content of 0.49% in the muscle tissues is much lower than for most fish species (Manirakiza et al., 2002), but is similar to fat percentages found in related gobiiid fishes (Mwevura et al., 2002; Voorspoels et al., 2004). The low fat content thus reduces the sensitivity of the mudskipper to accumulation of lipophilic pollutants from the coastal environment in its muscle tissues. It is likely that the analysis of pollutant concentrations in kidneys or livers would have yielded higher concentrations of pollutants than the concentrations in the fish tissues.
that were found in the mudskipper muscle tissues and whole body samples. However, the amount of kidneys and livers that could be collected was too small to enable analysis in these organs.

When the pollutant concentrations in the sediments, fish tissues, and the rice sample are considered, it appears that the distribution of industrial and agricultural pollutants over the mangrove sites closely follows the actual distribution of industries and agriculture: industrial influence is found in Mtoni and Kunduchi, while agricultural pollutants occur in Mtoni, Makoba, Rufiji and Chwaka. Mtoni is not only the site with the highest pollutant concentration, but also the only site where both industrial and agricultural pollutants can be found. In contrast, neither industrial nor agricultural pollutants were found in the samples from Mbweni. Simultaneously, the results show that the geographical range over which the effects of industrialisation and urbanisation are observed is still relatively limited.

**Level of pollution**

Heavy metal concentrations of 65-5300 µg Cu/g and 25-190 µg Pb/g have been found in coastal sediments in Hong Kong (Blackmore, 1998), while concentrations of 131 µg Cu/g and 372 µg Pb/g were present in the sediments of the Tees estuary in Great Britain, an estuary notorious for its high pollutant concentrations in the 1970’s (Jones and Turki, 1997). In the present study only the sediment heavy metal concentrations at the hot spot in the Kizinga Stream reached similar levels. The concentrations at the other sampling locations are also surpassed by the heavy metal concentrations of 50-229 µg Cu/g and 55-165 µg Pb/g in the mangrove sediments of the Makupa Creek in Mombasa, Kenya (Muohi et al., 2003b), but are similar to the concentrations of 2-20 µg Cu/g and 9-46 µg Pb/g found in the mangrove sediments of the relatively pristine Shirazi Creek 70 km south of Mombasa (Muohi et al., 2003a) as well as to the concentrations of < 1-17 µg Cu/g and 3-28 µg Pb/g found in Richards Bay, South Africa (Mzimela et al., 2003). This comparison indicates that the mangrove sediments that were analysed in the present study were relatively unpolluted by heavy metals, with the exception of the Kizinga Stream.

Compared to the concentrations reported for Hong Kong (0.1-2.3 ng HCH/g, 1.4-311 ng total DDT/g, and 0.1-16.0 ng total PCB/g; Hong et al., 1995) and concentrations found in the Scheldt Estuary (< 0.3 ng HCH/g, 11.8-21.0 ng DDT/g, and 167-238 ng PCB/g; Covaci et al., 2005), the levels of organochlorine pollutants in the sediments of the mangroves of Tanzania were relatively limited. Organochlorine concentrations of similar magnitudes were found in the mangroves of Singapore (Bayen et al., 2005) and the Hugli Estuary in India (Guzzella et al., 2005). The organochlorine concentrations that were found in the tissues of the barred mudskipper in the present study are similar to the levels of pollutants in mudskippers in relatively unpolluted areas of Japan and Singapore (Bayen et al., 2005; Nakata et al., 2002).

The concentrations in the sediments that are reported in this study are very similar to the heavy metal concentrations found in previous investigations in the same
study area (De Wolf et al., 2001; Machiwa, 1992a), except for the high heavy metal levels found in Mtoni (1) and Mtoni (2). The concentrations of PCBs in the Mtoni Estuary have decreased during the last decade relative to the concentrations reported by Machiwa (1992a). Concentrations of DDT and its derivatives have decreased as well since the previous investigation, but DDT still shows the same ratio in relation to total DDT-related compounds (Mwevura et al., 2002), indicating that the breakdown of DDT has continued. The concentrations of $\gamma$-HCH found in the present study were higher than the concentration of < 0.8 µg/g reported for the Mtoni Estuary by Mwevura (2002). In 1992, Machiwa anticipated an increase in population and industries and predicted that this would result in an increase in the level of pollution in the Mtoni Estuary (Machiwa, 1992a). Since the study by Machiwa (1992a) the population of Tanzania in general and of the coastal zone in particular has increased (National Bureau of Statistics, 2002), but nonetheless no changes in the general pollution levels were noticed, except for the occurrence of a heavy metal hot spot following the establishment of a textile mill on the banks of the Kizinga stream.

The low concentrations of industrial pollutants suggest that the level of industrialisation along the Tanzanian coast is limited. Industrial activities are concentrated to the urban area of Dar es Salaam, whereas agricultural activities are widespread and are even found within the boundaries of the city of Dar es Salaam. All over the African continent industrialisation is concentrated in the major centres of commerce, which have primarily emerged along the coasts of the Indian and Atlantic Oceans (Biney et al., 1994; Scheren et al., 2002). If control of waste disposal and treatment of wastes are limited, such concentration of industrial activities increases the chances of pollution of the local environment. The establishment of new industries can thus rapidly create hot spots of pollution, as is shown by the heavy metal pollution in Mtoni. This pollution suggests that treatment of the wastes of the textile dyeing mill is insufficient, even though proper treatment methods for such wastewater exist (El Geundi, 1997; Volesky and Holan, 1995). This study reveals that environmental pollution levels along the coast of Tanzania due to industrial waste disposal and agricultural runoff are limited. The pollution hot spot in Mtoni should be considered as an example of the large impact that may be expected to occur if the level of industrialisation of the urban area of Dar es Salaam continues and insufficient measures of waste treatment are taken to prevent increasing environmental impact.
Chapter 5

Changes in morphology and growth of the mudskipper (Periophthalmus argentilineatus) associated with coastal pollution

G. Kruitwagen, T. Hecht, H.B. Pratap, S.E. Wendelaar Bonga

ABSTRACT In this paper a comparison is made between the growth and morphology of barred mudskippers from six mangrove forests along the coast of Tanzania. The fish populations from unpolluted sites consisted of a wide range of size classes, whereas only small-sized fish were present in the polluted Mtoni mangroves. Age estimation, based on examination of otoliths, revealed that the mudskippers followed similar growth patterns in all sites with limited pollution. However, the age estimates from the polluted Mtoni site revealed an abnormal growth pattern. Moreover, the occurrence of unilateral anophthalmia in the Mtoni mudskippers suggested that these fish were affected by pollutants during early development. The study showed that presence of urban and industrial wastes from Dar es Salaam city, as indicated by isotopic enrichment, correlated with abnormal growth and developmental effects in mudskippers. We hypothesize that the pollution might also affect fish species that use the mangroves as a temporary habitat.

INTRODUCTION

During recent years Tanzania has experienced a rapid increase in urbanisation and industrialisation, particularly in the coastal area of Dar es Salaam (National Bureau of Statistics, 2002). These changes have been accompanied by increasing amounts of anthropogenic wastes ending up in the coastal environment (De Wolf et al., 2001). Heavy metal levels have been found to be elevated in the Mtoni Estuary, where the harbour of Dar es Salaam is located (De Wolf et al., 2001; Engdahl et al., 1998; Machiwa, 1992a). The sediments of the estuary were furthermore found to be polluted with polychlorinated hydrocarbons (PCBs), petroleum hydrocarbons (PAHs; Machiwa, 1992), and organochlorines pesticides (OCPs; Mwevura et al., 2002). Pollution studies on the coast of Tanzania have been focussed on the chemical analysis of environmental pollutants, while the effects of the pollutants on marine and estuarine fauna have mostly been ignored.

Many environmental pollutants that are released into the waters of the Tanzanian coastal area pass through mangrove forests before reaching the Indian Ocean coast. The fish assemblages in these intertidal forests encompass early life stages of many fish species, including reef and seagrass species, which find protection against predators in the mangroves, as well as large amounts of organic matter, and associated macrofauna for feeding (Nagelkerken and van der Velde, 2004b; Robertson and Duke, 1987). Fish are sensitive to environmental pollutants, particularly during their early development, since pollutants may interfere with important developmental processes (von Westernhagen, 1988).

Early exposure to pollutants may result in a wide array of specific and non-specific effects in fish, including reduced egg production, delay or failure of hatching, growth reduction, and deformities (Cameron and Berg, 1992; Kingsford and Gray, 1996; Klumpp and von Westernhagen, 1995; Swedmark and Granmo, 1981). The effects that pollutants have on fish strongly depend on the characteristics of the pollutant, the duration of exposure, and the life stage of the organism (von Westernhagen, 1988).

Whereas many fish species spend only specific life stages in the mangrove habitat, the barred mudskipper (*Periophthalmus argentilineatus* Valenciennes) is one of
the resident mangrove species. Subsequently, all effects of pollution found in this fish species can be attributed to exposure in the mangroves. This study evaluated the morphology and growth of the mudskipper in six mangroves along the Tanzanian coast that are expected to vary in the level of pollution.

Nitrogen isotopes accumulate in the foodweb and stable nitrogen signatures consequently indicate the trophic level of an organism (e.g. Minagawa and Wada, 1984). However, since the stable nitrogen signatures of land-based sources are much higher than marine sources, input of anthropogenic wastes into coastal ecosystems also results in an increase in the stable nitrogen isotope signatures of these systems, i.e. eutrophication (Savage et al., 2004; Spies et al., 1989). In the present study the stable nitrogen signatures of mudskippers were used to provide a means for comparison of the general anthropogenic input in the different mangrove sites.

Previous observations suggested that size differences between mudskippers in the populations of different mangroves were present. To evaluate the growth patterns in the mudskippers of the different populations, otoliths were used. Otoliths are bony structures in the inner ear of the fish that grow continuously as a result of deposition of calcium. Endogenous and exogenous rhythms influence the deposition which results in growth marks in the otolith that can be used for age determination (figure 1; see review by Morales-Nin, 2000). Since seasonal changes are less pronounced in tropical than in temperate zones, growth marks can be less clear in tropical fish (Morales-Nin, 1992). Therefore the validity of the use of growth zones in the otoliths of mudskippers as annual increments was tested.

Figure 1 Cross-section of a sagittal otolith of a mudskipper. The radius of the otolith increases from the core outwards as deposition of calcium results in new growth increments (arrow). The inset shows a lateral view of the sagittal otolith.
Figure 2 Locations of the six sampling sites on the coast of Tanzania.

MATERIALS AND METHODS

Sampling areas

This study was carried out in mangrove forests at Mtoni, Kunduchi, Mbweni, Chwaka, Makoba, and Rufiji along the coast of Tanzania (figure 2).

The Mtoni sampling site is the most polluted site in this study (chapter 4, Engdahl et al., 1998; Machiwa, 1992a) and is located in the estuary in which the harbour of Dar es Salaam is situated. The estuary receives water from two tributaries, the Kizinga and the Mzinga. The Kizinga is the largest tributary and is known to carry a mixture of household, agricultural and industrial wastes.

The mangroves of Kunduchi and Mbweni are found along the Zanzibar Channel, about 25 and 40 km north of the Dar es Salaam harbour. The area surrounding the Kunduchi mangrove is being transformed into the northernmost suburb of Dar es Salaam. A large part of the mangrove has been converted to saltpans and some hotels are found in the proximity. The Mbweni mangrove is located next to a small fishing village with no apparent input of pollutants. The concentrations of environmental pollutants are relatively low in both Kunduchi and Mbweni (chapter 4; de Wolf et al. 2001).
Chwaka and Makoba are both located in rural areas of Unguja Island (Zanzibar). This island is located 30 km from the mainland coast and has few industries. Chwaka and Makoba are little affected by pollutants (chapter 4). Chwaka Bay is a large shallow bay on the eastern part of the island, which is bordered by small fishing villages. A large mangrove forest fringes the closed southern end of Chwaka Bay. The Makoba mangroves surround Makoba Bay that is fed by the Zingwezingwe and Mwanakombo streams. Some farms and mariculture ponds are located in the proximity of the Makoba Bay.

The Rufiji site is located 100 km south of Dar es Salaam city in the wide delta of the Rufiji River. This river has a catchment area of 177,400 km$^2$ and is over 640 km long (United Nations Environment Programme, 2001). Several small towns are located in the upper part of its catchment area. The lower part is free of industries and subsequently the Rufiji sampling site is relatively clean (chapter 4).

**Fish**

Barred mudskippers (*P. argentilineatus*) were randomly collected with scoop nets on the exposed mudflats during low tides. The fish were measured for total length (in cm) and weight (in g). The sagittal otoliths were removed from the auditory bulla and cleaned. The maximal width of the otoliths was measured before the otoliths were stored dry in cross-referenced plastic vials.

To avoid too much pressure on the small mudskipper populations, fish for the growth study were collected at intervals of five months between November 2001 and June 2003. For the stable isotope analysis fish were collected in January 2004.

**Stable isotopes**

Samples of muscle tissue from the mudskippers were dried at 70°C for 48 h and ground to a fine powder. The nitrogen isotopic composition was determined with a Carlo Erba NA 1500 element analyser coupled on-line via a Finnigan Conflo III, with a Thermo-Finnigan DeltaPlus mass-spectrophotometer. Nitrogen isotope ratios are expressed in the delta notation (δ¹⁵N) relative to atmospheric nitrogen (Rau et al., 1981).

**Age estimation**

The age of the fish from the various sampling sites was determined by counting growth increments in the otoliths (Morales-Nin, 2000). To test whether burning would improve the readability of growth zones 37 otoliths were burnt either to a medium brown or dark brown state over a low intensity ethanol flame. The burnt otoliths were then embedded, together with the unburnt otolith of the pair, in clear polyester casting resin and sectioned transversely through the nucleus with a double bladed diamond edged saw and mounted on slides with the DPX mountant. An arbitrary 4-point scale of zone clarity was used to determine the effect of burning. Analysis of the results indicated that burning did not enhance readability (table 1). The remainder of the otoliths were embedded in an unburnt state and sectioned as described above.
Table 1 Readability of growth zones in burnt and unburnt otoliths, illuminated by transmitted or direct light. Readability is expressed in numbers from 1 (least readable) to 4 (best readable).

<table>
<thead>
<tr>
<th>Readability</th>
<th>Unburnt</th>
<th></th>
<th>Burnt</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct light</td>
<td>Transmitted light</td>
<td>Direct light</td>
<td>Transmitted light</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>0</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>16</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
</tbody>
</table>

The number of opaque zones in otoliths was read three times with a dissecting microscope under transmitted light without any reference to the size of the fish. If the three readings were the same, the age estimate was accepted. If only two of the readings coincided, the otolith was read a fourth time and the age estimate accepted if three of the readings were the same. Otoliths were rejected, if the growth zones were not readable, or if the three readings differed by more than two years.

Because it was not possible to collect samples on a monthly basis, the commonly used marginal zone analysis technique (e.g. Hyndes et al., 1992) could not be utilised to validate the growth zones as annuli. Instead, the otoliths of 14 fish, < 14.4 cm TL, from the Kunduchi (n = 3), Makoba (n = 5) and Mbweni (n = 6) mangrove swamps were prepared for the analysis of daily growth zones. To this end otoliths were mounted on a slide using Canada balsam (Secor et al., 1992). The otoliths were then ground by hand using fine sanding paper (P400-800), until the core was clearly visible and then polished with a rotary tool (Dremel, the Netherlands) equipped with a 13 mm polishing felt wheel using a 1 µm and then 0.3 µm Alumina paste (Buehler, Lake Bluff, IL, USA). After preparing one side, the otoliths were taken from the balsam and mounted with the polished side down to enable preparation of the other side. Once the growth zones were clearly visible, the otolith sections were made free of balsam and covered with a drop of the DPX mountant. Of the 14 otoliths prepared in this manner, 11 had micro-increments that were readable from the core to the otolith margin. The total number of micro-increments from the core to the edge of the otoliths was read three times at 400x magnification. The average of the three counts was calculated to accept or reject the hypothesis that the first clearly observable growth zone was an annulus, based on the assumption that around 365 daily rings were deposited.

Since there was no significant difference between the length-at-age data for males and females (F = 2.660, P = 0.105) the data for the two sexes were combined.

Length-at-age data were fitted to the Schnute model and the three-parameter specialised von Bertalanffy growth models, with both absolute and relative error structures (Schnute,1981). The Schnute growth model is described by

\[ L_t = L_i + \left( L_L - L_i \right) \cdot \left[ \frac{1 - e^{-a(t-t_i)}}{1 - e^{-a(t_L-t_i)}} \right] \]

72
where \( L_2 > L_1 \) and \( t_2 > t_1 \), \( L \) is the length of the fish at age \( t \), \( t_1 \) and \( t_2 \) are the ages of the youngest and the oldest fish in the sample, \( L_1 \) and \( L_2 \) are the average lengths of the fish at age \( t_1 \) and \( t_2 \), and \( a \) and \( b \) are the relative growth rates. The von Bertalanffy growth model is described by the Schnute model where \( b = 1 \) and is reduced to the familiar form of

\[
L_\infty = L_1 (1 - e^{-k(t-t_0)})
\]

where \( L_\infty \) is the predicted asymptotic length, \( K \) is the Brody growth co-efficient, and \( t_0 \) is the age at “zero” length (Ricker, 1975).

The models were fitted with a downhill simplex search, which is a non-linear minimisation routine to obtain parameter estimates of the selected growth model. For each model and error structure, a non-parametric one-sample runs test was applied to test for randomness. Bartletts test was used to test for homoscedasticity of the residuals. A particular model was chosen only if the residuals were both random and homoscedastic. Variance estimates were calculated with conditioned parametric bootstrap re-sampling (Efron, 1982) with 150 bootstraps. Upper and lower confidence intervals of the predicted length-at-age were constructed from the bootstrap data using the percentile method described by Buckland (1984).

**Statistical analysis**

One-way ANOVA was used to test for differences in isotopic signatures between the six localities. The differences in the length-at-age data of the sexes and log-transformed length data were tested with one-way ANOVAs, followed by Games-Howell post-hoc tests. All statistical analyses were performed with software-package SPSS© 11.5 and statistical significance was accepted at \( P \leq 0.05 \).

**RESULTS**

**Stable isotopes**

The stable nitrogen ratios for mudskippers from all sampling sites are shown in figure 3. The ratios found for mudskippers from Chwaka were significantly lower \( (P < 0.001) \) than in all other sites, except Makoba. The highest ratios were found for the fish in the Mtoni mangrove. The ratios observed in the fish from Mtoni and Rufiji differed significantly \( (P < 0.001) \) both from each other and from the ratios found for the four other sites.

**Malformations**

Malformed eyes were encountered in 3.3% of the adult fish which were collected in the Mtoni mangroves \( (n = 245, \text{figure 4}) \), but there were no apparent skeletal deformities. In all instances the deformities concerned the absence of the left eye (unilateral
anophthalmia). The right eye appeared properly formed in all cases. No eye deformities were found in any fish in the other mangroves (figure 5).

**Fish size**
The overall length-weight relationship of all fish was found to be allometric and could be expressed as

\[ W = 0.0171 \cdot L^{2.8292} \quad (N = 1052) \]

where \( W \) is the weight (in g) and \( L \) is the total length of the fish (in cm).

**Age estimation**
The otolith width/fish length relationship was found to be linear and is best described by the function

\[ y = 1.671x + 10.599 \quad (r^2 = 0.882, n = 89) \]

The average daily micro-increment counts are presented in table 2. The results suggest that the first clear growth zone represents the first annulus. On the assumption that this is a recurring pattern, the subsequent rings were also interpreted as annuli and were used to estimate the age of the fish in years.
**Figure 4** Pictures of mudskippers with normal eye development (upper left), and fish with left-sided anophthalmia.

**Figure 5** Relation between otolith width and total fish length.
**Table 2** Average number of micro-increments on otoliths of mudskippers of three different age classes.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Average number of increments</th>
<th>Standard deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>291</td>
<td>26.6</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>388</td>
<td>14.1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>840</td>
<td>0.0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3** Number of otoliths examined for annuli, the number of otoliths that could be aged successfully, and the percent of otoliths that had to be rejected as unreadable, either because they could not be read at all, or did not fit the predetermined protocol for accepting age estimation.

<table>
<thead>
<tr>
<th>Site</th>
<th>Number examined</th>
<th>Number aged successfully</th>
<th>Percentage rejected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chwaka</td>
<td>17</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Kunduchi</td>
<td>23</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Mbweni</td>
<td>47</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>Makoba</td>
<td>46</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>Mtoni</td>
<td>68</td>
<td>63</td>
<td>7</td>
</tr>
<tr>
<td>Rufiji</td>
<td>66</td>
<td>60</td>
<td>9</td>
</tr>
</tbody>
</table>

**Table 3** shows that the annual growth zones could be read successfully for most otoliths from each of the six mangrove swamps. The annuli consisted of a wide translucent and a narrow opaque zone.

The length-at-age keys are presented in **table 4** for the fish from the six localities separately, and for all fish used for age estimations combined. Given the relatively small sample of fish from each mangrove swamp, and the highly variable numbers per age class, it was decided that any statistical analysis, such as Cerrato’s (1990) likelihood ratio test, could not be used with any degree of confidence or justification.

As indicated in the methods section, both the Schnute and the von Bertalanffy growth equations were fitted to the length-at-age data. **Table 5** illustrates the parameters of the most suitable model for each of the populations. **Table 6** shows the best-fit model estimates for the length-at-age as well as the upper and lower 95% confidence intervals. The growth curves of the various populations are highly similar, and are illustrated in **figure 6**. A total of 58 fish were collected from Mtoni ranging between 6.8 and 8.8 cm TL, consisting of only 1 and 2 year old fish. The length-at-age data from Mtoni were therefore not fitted to either of the two growth models. The open circles in **figure 6** clearly show how the length-at-age estimates for Mtoni fish differ from those for fish of the other sampling sites.
Table 4 Length-at-age keys for mudskippers from the six mangrove localities and all localities combined.

<table>
<thead>
<tr>
<th></th>
<th>Mtoni</th>
<th>Kunduchi</th>
<th>Mbweni</th>
<th>Chwaka</th>
<th>Makoba</th>
<th>Rufiji</th>
<th>All sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0-4.9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>5.0-5.9</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 13</td>
</tr>
<tr>
<td>6.0-6.9</td>
<td>1</td>
<td>5 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 3 20 5</td>
</tr>
<tr>
<td>7.0-7.9</td>
<td>11 16</td>
<td>5 1</td>
<td>2 13 1</td>
<td>2 1 3</td>
<td>4 12</td>
<td>24 43 6</td>
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<td>8.0-8.9</td>
<td>6 26</td>
<td>1 2 2</td>
<td>8 2</td>
<td>1 5 5</td>
<td>11 4</td>
<td>8 52 11 1</td>
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<td>1</td>
<td>4 1</td>
<td></td>
<td></td>
<td></td>
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<td>2 1 6</td>
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<td>11.0-11.9</td>
<td>1 1 1</td>
<td>1 1 2</td>
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<td></td>
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<td>12.0-12.9</td>
<td>1 3</td>
<td>2 1 3 1</td>
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<td>1 2 8</td>
</tr>
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<td>13.0-13.9</td>
<td>1 1</td>
<td>2 1 1 8 3 1</td>
<td></td>
<td>10 5 2 1</td>
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<td>1 1 3 2</td>
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<td>1 4 3</td>
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<td>15.0-15.9</td>
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<td></td>
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</tbody>
</table>

Table 5 Parameters of the Schnute or the von Bertalanffy growth equations that best describe the growth of the fish in five localities. A = absolute error structure, R = relative error structure.

<table>
<thead>
<tr>
<th>Locality</th>
<th>von Bertalanffy</th>
<th>Schnute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>K</td>
</tr>
<tr>
<td>Kunduchi (A)</td>
<td>13.55</td>
<td>0.39</td>
</tr>
<tr>
<td>Mbweni (A)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chwaka (A)</td>
<td>12.92</td>
<td>1.83</td>
</tr>
<tr>
<td>Makoba (A)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rufiji (R)</td>
<td>10.13</td>
<td>0.49</td>
</tr>
<tr>
<td>All localities (R)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
DISCUSSION

The assessment of the stable nitrogen signature of mudskippers demonstrated elevated nitrogen signatures in the mangroves of Mtoni and Rufiji. Since the mudskipper populations occupy the same trophic levels in the mangroves of Mtoni and Chwaka (chapter 3), differences in stable nitrogen isotopes can be attributed to the input of foreign nutrients into the mangroves (Rau et al., 1981), rather than to differences in the trophic position of the fish. Consequently, the higher stable nitrogen signatures of Mtoni and Rufiji fish show that these mangrove systems receive a higher load of foreign nutrients than the other sites studied. The high input of anthropogenic nutrients in the Mtoni Estuary corresponds with the higher pollution levels that have been reported for the area in chapter 4, and by Machiwa (1992a) and Engdahl et al. (1998). In the Rufiji sampling site the input of nutrients was much lower than in Mtoni, indicated by the lower nitrogen isotope signatures, and most likely consists of input from the Rufiji River.

The most conspicuous observation in this study was the absence of the left eye (unilateral anophthalmia) in a part of the mudskipper population in the Mtoni mangroves, while no such abnormalities were found in the other populations. The eyes are formed as outgrowths of the diencephalon during the neurula stage early in embryonic development (Kimmel et al., 1995) and the occurrence of serious eye defects indicates interference of pollutants with early inductive processes (Nakayama et al., 2005; Weis and Weis, 1977). The occurrence of malformed eyes is a phenomenon that naturally occurs at a low frequency during development, but this frequency can increase as a consequence of exposure to a variety of environmental, chemical and genetic agents (Strömland et al., 1991).

Remarkably, the malformations in the Mtoni fish were restricted to left-sided anophthalmia with normal development of the eye sockets on both sides. During normal eye development the chokh/rx3 genes regulate evagination of the retinal progenitor cells into optic vesicles (Fantes et al., 2003; Loosli et al., 2003). Spontaneous mutation of the rx3 gene has resulted in the occurrence of uni- and bilaterally anophthalmic embryos in Japanese medaka (Oryzia latipes) following the failure of optic vesicles to evaginate (Ishikawa et al., 2001). In the medaka embryos the skeletal structures of the empty eye sockets appeared normal, which also appeared normal in the mudskippers. However, in contrast to the findings in our study, unilateral anophthalmia in the medaka embryos occurred on either sides at equal frequencies (Y. Ishikawa, personal communication). Anophthalmia has also been observed as a transgenerational toxic effect in the offspring of female medaka that were experimentally exposed to tributyltin (Nakayama et al., 2005). To our knowledge no previous accounts of anophthalmia in wild fish populations have appeared in literature.
Table 6  Best-fit model estimates for length-at-age and lower and upper 95% confidence intervals.

<table>
<thead>
<tr>
<th>Site</th>
<th>Age</th>
<th>Best fit</th>
<th>Lower 95% confidence interval</th>
<th>Upper 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mtoni</td>
<td>1</td>
<td>7.72 ± 0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.08 ± 0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(average ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kunduchi</td>
<td>1</td>
<td>6.76</td>
<td>5.82</td>
<td>7.91</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.96</td>
<td>8.26</td>
<td>10.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.45</td>
<td>9.47</td>
<td>11.04</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11.46</td>
<td>9.76</td>
<td>12.38</td>
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<td>5</td>
<td>12.14</td>
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<td>6</td>
<td>12.60</td>
<td>9.79</td>
<td>14.80</td>
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<tr>
<td>Mbweni</td>
<td>1</td>
<td>6.57</td>
<td>6.34</td>
<td>7.07</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.62</td>
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<td>7.47</td>
<td>11.04</td>
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<td>12.39</td>
<td>11.43</td>
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<td></td>
<td>4</td>
<td>12.84</td>
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<td></td>
<td>5</td>
<td>12.91</td>
<td>12.24</td>
<td>14.48</td>
</tr>
<tr>
<td>Makoba</td>
<td>1</td>
<td>6.57</td>
<td>4.47</td>
<td>8.78</td>
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<td></td>
<td>2</td>
<td>8.69</td>
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<td>10.76</td>
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<td>5</td>
<td>14.59</td>
<td>11.32</td>
<td>18.18</td>
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<tr>
<td>Rufiji</td>
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<td>6.14</td>
<td>5.96</td>
<td>6.38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.70</td>
<td>7.46</td>
<td>7.83</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.66</td>
<td>8.29</td>
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<tr>
<td>All sites</td>
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<td>6.67</td>
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<tr>
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<td>8.27</td>
<td>8.13</td>
<td>8.46</td>
</tr>
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<td>10.36</td>
<td>10.09</td>
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<tr>
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<td>4</td>
<td>12.49</td>
<td>12.13</td>
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<tr>
<td></td>
<td>5</td>
<td>14.20</td>
<td>13.65</td>
<td>14.55</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.22</td>
<td>14.10</td>
<td>16.31</td>
</tr>
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</table>

The frequency and specificity of eye deformities at Mtoni, in combination with the complete absence of visible deformities in mudskippers from the other mangroves, strongly suggest that these deformities were not the results of infections nor of inborn aberrations. Moreover, since the anophthalmic individuals were collected at various times during the study period, the occurrence was most likely not related to a specific event.

Since the collection of larval or juvenile mudskippers from their underground burrows is very difficult, all observations of anophthalmic individuals in Mtoni
concerned adult fish. Generally, the number of malformed fish that survive decreases with development and few individuals with abnormalities survive until adult stages (Klumpp and von Westernhagen, 1995). Similarly, only few medaka embryos that developed anophthalmia following maternal exposure to tributyltin hatched successfully and none survived for more than a few days (Nakayama et al., 2005). Hence, it is well possible that the anophthalmic mudskippers that survived until adulthood formed only a small percentage of the total number of mudskippers that were malformed during development.

The relationship between the length and weight in mudskippers followed the same trend in all mangroves in this study and was similar to the relationship found for the Atlantic mudskipper, *Periophthalmus barbarus* (Etim et al., 1996). Nonetheless, our data show that the fish in Mtoni and Rufiji had a limited size range in comparison to the other four sites.

Although the increment patterns in mudskippers of the genus *Periophthalmus* are generally not very clear (Etim et al., 2002), the use of growth zones as annual increments could be validated for *P. argentilineatus* in this study. The ageing study revealed that fish communities in the mangrove forests of Chwaka, Kunduchi, Makoba, Mbweni, and Rufiji consisted of multiple age classes between 1 and 6 years, while the Mtoni population consisted merely of 1 and 2 year old fish. The absence of mudskippers older than 2 years of age in the Mtoni population suggests that the fish disappeared from the habitat in high numbers. Because mudskippers do not have a swimming bladder and are not very good swimmers, the emigration by adult fishes is not likely. Higher mortality rates after the age of two years in the Mtoni population in comparison to the other populations are a more likely explanation. Mortality during later life stages can occur as a delayed effect of exposure to pollutants at early age (Heintz et al., 2000).

The length-at-age data do not show any absolute differences between the fish from Mtoni and the other sites, but nonetheless the growth pattern of the Mtoni mudskippers differed from the other growth patterns observed in this study. The average length of the Mtoni fish at the age of one year appeared to be similar to the length of fish in the other populations, but the growth seems to be stunted to the end of the second year.

Growth can be affected by all factors that contribute to habitat quality. However, abiotic factors and pressure from predation and fisheries do not seem to differ between the mangrove sites, whereas the high input of foreign nutrients distinguishes Mtoni from the other sites. The growth effects and the absence of older age classes in Mtoni correlate to the high degree of environmental pollution as found in chapter 4 and as indicated by the stable nitrogen isotopes. Reductions in fish growth have been described for a variety of pollutants (Heintz et al., 2000; Laroche et al., 2002; Makita et al., 2003; Marr et al., 1996).
Figure 6 Growth curves as predicted by the von Bertalanffy growth model for mudskippers from five mangroves separately, and for all sampling locations combined. The solid line shows the predicted growth and the dashed lines depict the 10% and 90% confidence intervals. The open circles in every graph represent the average body length of the Mtoni fish.
In the sediments of the Mtoni Estuary heavy metals, OCPs, PCBs, and PAHs have been found at concentrations of 13.3-44.3 µg lead/g dw, (Machiwa, 1992a), 11.7-43.9 ng/g dw (Mwevura et al., 2002), 9-70 µg/g (Machiwa, 1992a), and 132-164 µg/g dw (Machiwa, 1992a), respectively. These concentrations are below the values reported for highly contaminated sites (e.g. lead 35-160 µg/g dw, Wong et al., 2000; total OCPs 4.45-311 ng/g dw, Hong et al., 1995; total PCBs 1.4-329.6 ng/g dw, Wurl et al., 2005; PAHs 1013-8503 ng/g dw, Wong et al., 2000), but could nevertheless cause adverse effects (Klumpp et al., 2002). Because pollution in the estuary is complex (Machiwa, 1992a) the observed adverse effects in Mtoni might, however, also be caused by pollutant classes which have not yet been analysed. Since both suppression of growth and disturbance of eye development have previously been attributed to the genotoxicity of environmental pollutants (Heintz et al., 2000; Nakayama et al., 2005), this might be the mode of action via which the observed defects in Mtoni have been caused. Further research is needed to identify the cause of the observed effects.

We conclude that there is a correlation between the degree of physiological and morphological deviations reported in this study and the extent of pollution in the different sites as indicated by stable isotope results and the results of chapter 4. The observed effects on growth and morphology suggest that the early stages of embryonic development are the life stages of the mudskippers that are most affected by pollutants. This damage may either have been inflicted by direct exposure of developing fish to pollutants, or may have resulted from parental exposure to pollutants. Since the effects in Mtoni appear to concern the whole population and have been found at different points in time, the anticipated causative agents were either continuously present in the mangroves exposing every generation anew, or damage caused by a past pollution event was passed on from generation to generation. Both scenarios imply that environmental pollutants in the Mtoni Estuary affect the mudskippers on the level of individuals, but also on the population level. Since many fish species, including seagrass and coral reef species, are dependent on the mangroves for parts of their life cycle (Nagelkerken and van der Velde, 2004b), the effects of pollution on mangrove populations deserves more attention. Pollution is likely to reach beyond the strictly mangrove-resident species and might also influence fish populations in other coastal ecosystems.
Chapter 6

Developmental toxicity in zebrafish embryos (Danio rerio) exposed to textile effluents

G. Kruitwagen, V. Scheil, H.B. Pratap, S.E. Wendelaar Bonga

submitted
ABSTRACT An effluent that was collected from the discharge pipe of a textile dyeing mill in Dar es Salaam, Tanzania, was used to investigate the toxicity of the effluent for developing fish in laboratory experiments. A subsample of the raw effluent was separated into fractions that differed in polarity by counter-current chromatography to simulate the separation of compounds in the field following the interaction of the mill effluent with mangrove sediments. The toxicity of the untreated effluent and the polar fractions that were expected to be most environmentally relevant was tested in early life stage tests with zebrafish embryos. The raw effluent was found to cause a delay in gastrulation, a decrease in heart rate, decreased hatching rates and elevated mortality rates at dilutions lower than 1:50. Exposure to the polar fractions resulted in a decrease in heart rate at the lowest dilution, but had no other effects. The results of this investigation indicate that the highest embryotoxicity of the collected effluent is exerted in the upstream sections of the mangrove and that the embryotoxicity subsequently decreases following interaction with the environment.

INTRODUCTION

In the production processes of textile industries vast amounts of water are used that are drawn from nearby rivers and later released as wastewater. Upon release the water is polluted with the waste products from dyeing and finishing processes. Worldwide, research has focussed on the removal of dyes and toxic constituents from textile effluents and a variety of treatment processes for detoxification and decolourisation are currently known (Banat et al., 1996; Crini, 2006; Gottlieb et al., 2003). However, the treatment processes are not always applied to sufficient extent and consequentially heavily stained waters and toxic effects due to the release of textile effluents still occur (Sumathi et al., 2001). Textile effluents have been shown to cause physiological disturbance in fishes such as inhibition of ATPase activity in the liver, brain, and muscle (Chhaya et al., 1997b), induction of CYP1A in the liver (Gadagbui and Goksøyr, 1996), and DNA damage (Sumathi et al., 2001).

In the mangroves of the Mtoni Estuary near Dar es Salaam, Tanzania, we have observed a decreased growth, reduced life span, and a frequent occurrence of anophthalmia in a resident population of barred mudskippers (Periophthalmus argentilineatus Valenciennes; chapter 5). These adverse developments were brought into connection with waste water that is released by a textile dyeing mill and indicated interference of pollutants with embryonic development.

The embryonic and early larval stages of fishes are most sensitive to environmental pollutants: developmental processes are very vulnerable to disturbance because epithelia and organs are not yet completely differentiated and developing fishes have a higher metabolic rate than later life stages (von Westernhagen, 1988). For this reason fishes in the early life stages are often used in experimental exposure tests to determine and quantify sublethal and lethal effects of xenobiotics. In the present study the toxicity of different fractions of an effluent from a textile dyeing mill in Dar es Salaam was tested in the laboratory on embryos of zebrafish, Danio rerio Hamilton, to investigate whether the released textile effluent could be the causative agent of the defects that were previously observed in the mudskipper (chapter 5). The use of an
early life stage-test (ELS) with zebrafish offers major advantage over the use of mudskippers, because the ELS-test with zebrafish is a widely used (Birge et al., 1985; Luckenbach et al., 2001, McKim, 1977; Suter et al., 1987) and standardised test (DIN 38415-6). Moreover, zebrafish eggs are readily available, and the development and physiology of the zebrafish embryos are well known (Hisaoka and Battle, 1958; Westerfield, 1998). In contrast, the knowledge of the early life stages of mudskippers is very limited and collection of eggs and observation of early development are complicated by the fact that mudskippers breed in burrows well below the surface of the mangrove sediments (Brillet, 1976) and reproduction in captivity is very difficult (Hong and Zhang, 2004; Tsuchako et al., 2003).

**MATERIALS AND METHODS**

**Collection of effluent**

The textile dyeing mill in the Mtoni suburb of Dar es Salaam, Tanzania (figure 1) is located on a hill near the banks of the Kizinga Stream. The discharge pipe of the mill runs downhill and releases wastewater into the stream 300 m before it enters a mangrove stand and widens into an estuarine basin. The mangrove stand stretches for 2 km towards the coast after which the estuary deepens and widens to form a shallow bay. The mudskipper population in which adverse effects of pollution were observed (chapter 5) is found in the mangroves in proximity of the bay, at the far end of the mangroves relative to the textile mill at a distance of approximately 3 km.

The effluent released by the textile factory is estimated to be diluted 1 to 5 times upon entry into the small Kizinga Stream. A narrow stream and surfacing ground water are the only water sources that may cause further dilution of the effluent within the mangroves. The effluent concentration in the surface water upon entry into the bay is expected to be a dilution of 1:5 to 1:50 of the original effluent. Effluents from the textile dyeing factory in Mtoni were collected straight from the discharge pipe in polypropylene bottles (Nalgene, Rochester, USA) on the 25\textsuperscript{th} of October 2005. The bottles were immediately sealed upon collection, frozen at -20°C, and transported to The Netherlands.

**Processing of effluent**

The contents of one bottle were used for chemical processing at the Department of Organic Chemistry of the University of Nijmegen. The compounds of the textile mill effluent were separated by counter-current chromatography using a two-phase solvent system of butanol/acetic acid/water (4:1:5, v/v/v). After 80 cycles of the counter-current, seven separate fractions could be isolated on the basis of colouration (fractions 1 to 7). The first fraction contained stationary polar compounds, while the second fraction contained polar compounds that are considered environmentally relevant at the point of emission in the field, because these fractions are more hydrophilic than the stationary compounds and are subsequently more likely to spread through the
environment in field conditions. The second fraction was separated further on the counter-current chromatograph during 120 additional cycles, resulting in 6 fractions (fractions 2A-F). All isolated fractions of both columns were concentrated by evaporation. The concentrated liquids were subsequently freeze-dried into solid products. The freeze-dried products of the two most polar fractions that were isolated during the second series of cycles, fractions 2A and 2B, were transported frozen to the University of Tübingen (Germany) together with a bottle filled with the untreated raw effluent.

**Figure 1** Map of the Mtoni Estuary near Dar es Salaam, Tanzania. The cross marks the point of effluent release; the fish symbol indicates the location of the mudskipper population; the densely shaded areas represent the mangrove stands; the dashed line indicates the low-tide mark.

**Animals**

Zebrafish embryo tests were conducted at the Animal Physiological Ecology Section of the University of Tübingen (Germany). Adult zebrafish (*Danio rerio*) of both sexes were kept in the laboratory in aerated and filtered aquaria with a minimum of 1 L water per fish, at 26 ± 1°C and a 12:12 hour light:dark cycle. The adult fish were fed twice daily
with TetraMin dry flake food (Tetra, Melle, Germany), alternated with either Artemia
nauplii or with red mosquito larvae from uncontaminated sources. The eggs used in the
test were collected using spawn traps that had been placed at the bottom of each
aquarium the evening of the day before spawning was required. Spawning was
triggered once the light was turned on and was completed within 30 min. All eggs were
transferred immediately into glass Petri dishes containing the different test solutions
(see below). Unfertilized eggs were removed and the fertilized eggs were placed into
new glass Petri dishes (10 embryos per Petri dish, 4 dishes per concentration) with the
respective test solutions. The water for the exposure of the eggs/embryos was prepared
according to ISO-Standard 7346/3 and contained 294 mg/L CaCl₂, 123.25 mg/L MgSO₄,
64.75 mg/L NaHCO₃, and 5.75 mg/L KCl, dissolved in aqua bidest. The water was aerated
to oxygen saturation before addition of the test substances.

The tests were performed in climate chambers at a 12:12 hour light:dark cycle.
Water temperature was maintained at 26 ± 1°C. The Petri dishes were covered to avoid
evaporation.

**Exposure to whole effluent**
The untreated raw textile dyeing effluent was defrosted and diluted with the exposure
water to different concentrations (1:5, 1:10, 1:30, 1:50, 1:100, 1:300, 1:500, and 1:1000). The
test was performed with 8 concentrations of effluent and a negative control
containing pure exposure water. Embryo development was monitored at 0, 8, 12, 24, 48,
60, 72, 84, and 96 hrs after fertilization. Endpoints used for assessing the effects of the
textile dyeing effluent included among others 70% epiboly (i.e. blastoderm enveloping
70% of the yolk sphere; Hisaoka and Battle, 1958), egg and embryo mortality,
gastrulation, somite formation, movement, tail detachment, pigmentation, heartbeat,
blood circulation, hatching time, and hatching success (table 1). Malformations and
delays in development were also noted and described for the developing eggs from
both control and treated groups, using a stereomicroscope.

**Exposure to effluent fractions**
The two freeze-dried fractions of the textile dyeing effluent were mixed and
reconstituted to the original volume of 200 ml with the water that was prepared for the
exposure. The reconstituted fractions of the effluent were further diluted to 1:30, 1:50,
1:100, 1:300, 1:500, and 1:1000. The quantities of effluent fractions were insufficient to
enable testing at dilutions 1:10 and 1:5. The tests with the fractions of the textile dyeing
effluent were conducted as described for the tests with the raw effluent.

**Statistical analysis**
Exposure effects on the delay of epiboly, heart rate, and mortality of the zebrafish
embryos were tested using one-way analysis of variance (ANOVA) and Dunnett t post-
hoc tests. Effects of the textile effluent on hatching rates were analysed with one-way
repeated measures ANOVA followed by Dunnett t post-hoc tests. Due to technical
problems, the control group in the experiment with raw effluents deviated strongly from the accepted response for control groups. For this reason the control group for the raw effluent was excluded from the analysis. Instead results for the raw effluent were tested against the results for the group exposed to a dilution of 1:1000. This approach was accepted since testing against dilution 1:1000 yielded the same results for groups exposed to the polar fractions as testing against the control for the polar fractions. All statistical tests were performed using SPSS© version 11.5. Significant differences were accepted at the $P \leq 0.05$ level.

RESULTS

Field observations at collection site
The raw textile effluent was turbid and dark blue in colour. In the immediate surroundings of the discharge pipe the effluent stained the sediments heavily, which resulted in an indigo colouration. Just before the mangrove stand, about 200 m from the discharge pipe, the sediments and the water were dark green in colour, whereas effluents produced a rusty brown colour in the upstream section of the mangroves, where both water and the top layer of the sediments appeared to be devoid of fauna (i.e. fish and macrofauna). Living fauna was only encountered 1 km downstream into the mangroves where the turbidity of the water had decreased and the water had a dark red colour, but the sediments appeared unstained. At the transition from mangrove to the open bay the water was red-brown. The colour faded upon mixing with tidal waters in the bay.

Chemical processing
The chemical separation of the raw textile effluent on the basis of polarity resulted in 7 fractions, of which fraction 2 was further separated into 6 sub-fractions. The colours of the various fractions resembled the colours that were observed in the Kizinga Stream and the adjacent mangrove forest. Blue and green colours dominated in the apolar fractions (butanol > water), while red and brown colours were found in the more polar fractions (butanol < water).

Exposure to test solutions
The number of embryos that had not reached 70% epiboly after 8 hrs significantly increased with increasing concentrations of raw effluent ($P < 0.05$; figure 2a). However, due to the large variation, Dunnett t post-hoc test did not reveal significant differences between any of the tested dilutions. There was no effect of dilution on epiboly after exposure to the polar fraction of the effluent (figure 2b). The heart rates of zebrafish embryos were significantly correlated with dilution of the raw textile effluent ($P < 0.001$; figure 3a), as well as with dilution of the polar fraction of the effluent ($P < 0.05$; figure 3b).
Table 1 Investigated endpoints during zebrafish egg development (modified after OECD 210 and DIN 38415-6).

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<th>Endpoint</th>
<th>8h</th>
<th>12h</th>
<th>24h</th>
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<th>84h</th>
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<td>Exogastrulated embryo</td>
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Hatching rates of zebrafish embryos exposed to raw textile effluents were significantly affected by dilution of the effluent ($P < 0.001$; figure 4a). Delay in hatching increased with increasing concentrations of the raw effluent. The polar fraction did not affect the hatching rate of zebrafish embryos (figure 4b).

Exposure to the raw effluent resulted in an increase in mortality rates relative to the groups exposed to a dilution of 1:1000 ($P < 0.001$; figure 5a). Exposure to the raw effluent caused 100% mortality within 12 hrs in all groups at concentrations of 1:5, 1:10, and 1:30. Exposure to the polar fractions of the effluent did not affect the mortality rate (figure 5b). Other developmental differences between control and exposed groups were occasionally observed, but their distribution over the various groups appeared to be random (table 2).
**Figure 2** Percentages of zebrafish embryos of which epiboly had not reached 70% after 8 hrs of exposure to raw textile effluent (a) and polar fractions of the effluent (b) at different dilutions. Cross indicates that no delay in epiboly was observed due to 100% mortality before 8 hrs had elapsed. N.D. indicates that epiboly was not determined. Error bars represent standard errors of the mean.
Figure 3 Heart rate of zebrafish embryos subjected to different dilutions of raw textile effluent (a) and polar fractions of the effluent (b) at 48 hrs after fertilisation. Crosses indicate that no heart rates were detected due to 100% mortality before 48 hrs had elapsed. N.D. indicates that heart rate was not determined. Error bars represent standard errors of the mean. Significant differences from the 1:1000 (a) or the control (b) are indicated with asterisks over the bars: * $P < 0.05$, *** $P < 0.001$. 
Table 2 Total number of aberrations observed in 4 replicate groups of 10 zebrafish embryos exposed to different concentrations of raw effluent or polar effluent fractions after 96 hours.

<table>
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<th>1:5000</th>
<th>1:3000</th>
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<th>1:100</th>
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DISCUSSION

The range of colours that were observed in the field indicates that the effluent from the textile dyeing mill at Mtoni in Dar es Salaam is a chemical mixture that is separated into different fractions after release into the environment, probably due to interaction with mangrove sediments. These sediments are known to trap metals from overlaying waters by complexation with sulfides, particulate organic carbon, or iron oxyhydroxides, depending on prevailing physico-chemical conditions (Chapman et al., 1998), while the large quantities of organic matter in the sediments provide extensive binding surfaces to organic pollutants.

In the laboratory, chromatography revealed that the textile effluent was composed of a number of components that could be separated on the basis of polarity (i.e. hydrophobicity), and colour. The colours of the various fractions and their following order had a striking similarity with the colouration of the water and sediments of the mangroves downstream from the discharge pipe of the textile factory: the apolar fractions separated by chromatography had a blue-green colour that was similar to the
Figure 4 Hatching rate of developing zebrafish subjected to different concentrations of raw textile effluent (a) and the polar fractions of textile effluent (b) at various time points after fertilisation. Error bars represent standard errors of the mean. Significant differences from the 1:1000 dilution (a) are indicated with asterisks: * $P < 0.05$, *** $P < 0.001$. 
colour of the sediments in the direct vicinity of the effluent pipe, while the red-brown colours of the hydrophilic fractions that were obtained by chromatography corresponded with the colour of the water in Stream Kizinga at the point of mixing with the water from the bay. Thus, the results of chromatography strongly indicate that the separation that occurs in the field is based on polarity of the constituents of the textile mill effluent.

**Toxicity tests**

The textile effluent had profound adverse effects on the development of the zebrafish embryos from the early stages of development up to the moment of hatching, which strongly depended on the polarity of the fractions. While the raw effluent resulted in a delay of completion of 70% epiboly, decrease in heart rate, delay of hatching, reduction of hatching rate, as well as decrease in the survival of the developing zebrafish, the polar fractions of the effluent only decreased the heart rate. This indicates that the toxicity of raw effluent was much higher than the toxicity of the isolated polar fractions, suggesting that the toxicity of the effluent is mainly derived from relatively apolar, i.e. hydrophobic, fractions, or from other chemicals that rapidly bind to the mangrove sediments in the field situation (e.g. heavy metals; see chapter 4).

The concentration of the textile effluent was a major determinant for the impact on the development and survival of the zebrafish embryos: the lowest observed effect concentration was 1:100 for the raw effluent and 1:30 for the polar fraction of the effluent. The strongest effects were observed for exposure to the raw effluent at concentrations of 1:30, 1:10, and 1:5. Other studies on the toxicity of textile dye mill effluents for fish found similar effects and correlations with dilution. For example, Sakthivel and Sampath (1989) found reductions in growth rates and mortality rates in juvenile carp, *Cyprinus carpio*, that increased with increasing concentration of textile dye effluent. Mortality rates were reached 100% after exposure to an effluent concentration of 15%. The same mortality rate was found in zebrafish embryos in the present study at effluent dilutions of 1:30. In another study on juvenile carp, textile dye effluents were found to be able to exert genotoxic effects on fish tissues (Sumathi et al., 2001). Direct exposure to textile effluents induced enzyme activity (EROD, GST, UDP-GT) in adult tilapia, *Oreochromis niloticus*, at distances of 0.6, 4, and 8 km from the point of effluent discharge (Gadagbui and Goksøyr, 1996). Also Chhaya et al. (1997a) found that textile dye effluents significantly altered acid phosphatase activity in adult mudskippers, *Periophthalmus dipes*, after exposure to concentrations of 0.1, 0.5 and 1%.

**Mtoni mangroves**

After release of the raw textile effluent into the Mtoni mangroves near Dar es Salaam, apolar constituents from the effluent will bind rapidly to organic matter and sediments. Consequentially, these substances will mostly be restricted to the upstream mangrove sections. More polar fractions will remain longer in the water layer, but may be
Figure 5 Mortality rates of zebrafish embryos subjected to different concentrations of raw textile effluent (a) and polar fractions of the effluent (b) at 96 hrs after fertilisation. n.d. indicates that mortality was not determined. Error bars represent standard errors of the mean. Significant differences from the 1:1000 dilution (a) are indicated with asterisks over the bars: * $P < 0.05$, *** $P < 0.001$. 
transported to the sediments further downstream at a distance depending on their polarity. The most hydrophilic components will remain in the water layer throughout the mangroves and will be carried down to the opening into the bay. From our experiments it follows that this gradient in polar fractions in the water layer can result in a gradient in toxicity, because the apolar fractions exerted a higher toxicity than the more polar fractions. As the textile effluent passes through mangrove sections further downstream, the polarity will increase while the toxicity of the effluent fractions in the water layer will decrease. In the direct vicinity of the discharge pipe both polar and apolar fractions will be present in the water. Therefore toxic effects will most likely be more extensive in this mangrove section. The apolar fractions of the textile effluent are likely to have a similar (acute) toxic effect in the field as they had on the zebrafish embryos at the lower dilutions under laboratory conditions, and may be accountable for the apparent, total absence of fauna from the upstream mangrove sections.

In an earlier study we reported on the occurrence of abnormal eye development, decreased growth and decreased longevity in the natural population of mudskippers in the Mtoni mangroves (chapter 5). Other studies reported on the occurrence of similar adverse effects in fish after exposure to textile dyes (Meyer and Jorgensen, 1983, Srivastava et al., 2004) or textile dye effluents (Sakthivel and Sampath, 1989). The present study suggests that the mudskippers were primarily exposed to polar fractions of the effluents at the location where they reside. However, according to this hypothesis, the low toxicity of the polar fractions of the textile dye effluent in the tests with the zebrafish embryos appears to contradict the observed effects in the mudskippers of Mtoni. Several reasons for this discrepancy can be proposed: (1) the concentrations of waste products of the mill in the mangrove environment may vary strongly over space and time following variations in the physical and chemical conditions, as well as variations in release due to alterations in industrial processes; (2) the apolar fractions may have traveled further in the field than expected based on the laboratory tests; (3) the observed effects may be the result of accumulation of compounds from the textile effluent resulting in elevated concentrations (e.g. heavy metals); (4) observed effects in the field are due to chronic exposure, while the embryo tests with polar fractions were aimed at detecting more acute effects.
Chapter 7

General discussion
Introduction

The sediments of mangrove stands are characterised by a high organic content and fine grain size particles (Hogarth, 1999; Woodroffe, 1992). These properties promote the binding and accumulation of organic pollutants and metals from overlaying waters (Bayen et al., 2005; Chapman et al., 1998; Lacerda et al., 1991; Tam and Yao, 2002). In this way the mangrove sediments provide a natural filter for anthropogenic pollutants in waters that pass through the mangroves before reaching the ocean. In coastal areas that are subject to increasing urbanisation and industrialisation, the quantity and variety of pollutants that end up in the mangrove ecosystems are expected to increase rapidly. The subsequent accumulation of pollutants in mangrove sediments and biota will affect the fauna of these ecosystems that are highly productive and show a rich biodiversity.

The work that is presented in this thesis was conducted to study the extent of anthropogenic pollution in mangroves along the coast of Tanzania. This was done by estimating the impact of pollution on the mangrove biota. To this end an ecotoxicological approach was chosen that encompassed both ecological and physiological techniques and in which the barred mudskipper (Periophthalmus argentilineatus Valenciennes) was given a central position. In this chapter the findings of the study are synthesised and discussed.

Potential impact of pollution

Mangrove forests typically harbour large assemblages of juvenile fishes, whereas the number of adult fishes that is present is generally limited (Krumme et al., 2004; Lugendo et al., 2005; Robertson and Duke, 1987; Wakwabi and Mees, 1999). The juvenile fishes are thought to gather in the mangrove habitat in search of suitable food items and shelter from predation and strong currents (Laegdsgaard and Johnson, 2001; Nagelkerken and van der Velde, 2004a). Whereas the mangrove forests may provide physical protection thanks to their structural complexity, the accumulation of pollutants in the mangrove sediments poses a potential risk to the juvenile fishes. This risk is enhanced by the fact that the sensitivity of fishes to pollutants is highest during their embryonal and larval stages (von Westernhagen, 1988).

Within the mangrove community the mudskippers occupy a unique niche. Physiological adaptations to gills, skin, fins, and eyes (chapter 1; Clayton, 1993; Graham and Rosenblatt, 1970; Low et al., 1990) enabled this fish to lead an amphibious existence and to reside in the intertidal zone. As a consequence of the high degree of specialisation the mudskipper is bound to the mangroves during all life stages (Clayton, 1993), which makes these fishes highly vulnerable to changes to the mangrove habitat. Moreover, it can result in a higher risk of direct exposure to pollutants via water and sediments in the mangroves for the mudskipper than for fish species that are only temporarily present in the mangroves. In addition, the intensity of contact with the mangrove sediments is higher in mudskippers than in most fish species due to the use of burrows (Brillet, 1976). The use of deep burrows brings the mudskipper into contact
with the lower sediment layers, which exposes the mudskippers to pollutants in sediment layers from which other fishes are shielded. The risk of direct exposure of fishes to pollutants via the water is generally limited in mangroves following the extensive exchange of water between estuaries and the ocean due to the high tidal amplitude (Richmond, 1997) and the conditions within the estuaries that favour binding of pollutants to organic matter.

In chapter 2 it was found that the fringing mangroves of Mtoni had little importance as a feeding habitat for most zoobenthivorous fish species that were found to enter the mangroves at high tide. The mangroves appeared to be mainly of importance as a feeding habitat to mudskippers and invertebrates in these areas, since these animals reside exclusively within the mangrove habitat. Consequentially, the mudskippers are the zoobenthivorous fishes that run the highest risk to take up pollutants through ingestion in the Mtoni mangroves. The situation is different if pollution occurs in mangroves that retain water during low tide, e.g. Chwaka, since these mangroves have a high significance as feeding habitat to a large number of fish species (Guest et al., 2004; Lugendo et al., 2006; Sheaves, 2005). In these mangroves the risk of uptake of pollutants through ingestion will spread beyond the permanent mangrove residents.

Analysis of the feeding ecology of the mudskipper in both Mtoni and Chwaka revealed that the diet of mudskippers developed following changes in mouth and gut morphology (chapter 3). During the early life stages the mudskippers feed on small crustaceans and polychaetes that are also targeted by other zoobenthivores. However, the amphibious capacities of the mudskipper enable this species to feed at times that the food items are not available to other fish. During the adult life stage the mudskippers feed on crustaceans from higher mangrove sections that are not accessible for other zoobenthivorous fishes. As such, the mudskippers minimise competition with other zoobenthivores through temporal resource partitioning during early life stages and through spatial resource partitioning during the adult life stage. This dietary shift increases the dependency of the mudskipper on the mangroves as a feeding habitat, which enhances the vulnerability of the mudskippers to pollution of mangroves.

Mudskippers and pollution

Out of the mudskipper populations of the six mangrove sites that were included in the present study, only the population of Mtoni revealed abnormalities: the mudskippers of Mtoni had a distorted growth and the population included unilaterally anophthalmic individuals (chapter 5). These abnormalities were likely to be the result of genetic damage that was induced by pollutants. The fact that the stable nitrogen signatures of mudskippers from Mtoni were enriched compared to the mudskippers in the other mangrove sites, supported the hypothesis of pollution as causative agent, since enrichment in nitrogen signatures without changes in trophic level indicates the input of foreign nutrients into the mangroves (Rau et al., 1981). Analysis of pollutant
concentrations in chapter 4 confirmed that the Mtoni mangroves were indeed subject to higher levels of pollution than the other mangrove sites.

The pollutant classes that were encountered in the mangroves (PCBs, organochlorine pesticides, organotin compounds and metals, in particular chromium and copper; chapter 4) can cause a wide array of adverse effects in fishes (Dunier and Siwicki, 1993; Fent, 1996; Hontela et al., 1992; Mallat, 1985; Wendelaar Bonga and Lock, 1992). The levels of pollutants that were found in the mudskipper tissues, and in the mangrove sediments at the locations where mudskippers were collected, were however much lower than the levels of pollution at which adverse effects are normally expected. Only the levels of heavy metals that were found at the mouth of the Kizinga Stream were likely to cause adverse effects in fishes, but since no fish were found at this location no adverse effects could be observed.

The effects that were observed in the mudskipper population of Mtoni (chapter 5) are more severe in type and intensity than expected after exposure to the relatively low levels of pollution in the water and sediments that were found in this area. Adverse effects of the limited degree of pollution found in Mtoni are commonly restricted to a low number of individuals within a population (Lawrence and Hemingway, 2003), but in Mtoni all individuals of the mudskipper population were affected. The adverse effects in the Mtoni population may be the result from exposure to the high concentrations of the pollutant classes in the Mtoni sediments (chapter 4), from the high toxicity of mixtures of these pollutants, or from exposure to (geno-) toxic pollutants that could not be identified in water or sediments. The damage may also have been the consequence of chronic exposure of the mudskippers to the causative pollutants via the water or the mangrove sediment. Alternatively, the mudskippers may have carried the burden of pollution events in the (near or distant) past within the genetic material of the population and passed the damage over to following generations via the gametes. Nevertheless, the specific cause of the observed effects is difficult to determine since genetic damage in general is highly unspecific and can be induced by a large number of compounds (Ohe et al., 2004).

In chapter 6 it was shown that the waste products of the textile dyeing mill at Mtoni were embryotoxic, suggesting that these effluents might have been the causative agent of the genetic damage underlying the effects that were found in the mudskipper population of the mangroves downstream (chapter 5). Textile industries are known as sources of mutagenic waste products (Mathur et al., 2005; Ohe et al., 2004) and large quantities of heavy metals (Mathur et al., 2005; O'Neill et al., 1999). Other studies on textile mill effluents (Gadagbui and Goksøyr, 1996; Sakhivel and Sampath, 1989; Sumathi et al., 2001) have revealed effects that were similar to those that were observed in the mudskippers. In chapter 6 the apolar fractions were found to be highly toxic in the laboratory tests, while the polar fractions had a relatively low toxicity. Yet, these polar fractions were thought to be the only fractions to reach the mangrove stand where the mudskippers reside under field conditions. Nonetheless, it cannot be ruled out that the effects that are observed in the mudkipper population of Mtoni are caused
by exposure to the textile dye mill effluents. Several potential explanations are given in chapter 6, including the accumulation of specific compounds from the effluent.

Effect of pollution beyond the mudskipper population

In chapter 2 it was found that the value of the mangroves as feeding habitat to fishes and the composition of the mangrove foodweb were determined by inundation patterns rather than by the level of pollution. The only difference between the foodwebs of Mtoni and Chwaka that can be related to the higher pollution level in Mtoni, concerned an overall enrichment in nutrient level, which came to expression in a generally higher $\delta^{15}$N. This difference did, however, not appear to have affected the functioning of the foodweb since the composition of the foodwebs of Mtoni and Chwaka were similar (chapter 2; Lugendo et al., 2006). Notwithstanding the impact that pollution had had on the growth and development of the mudskipper population of Mtoni, even the position of the mudskippers in the foodweb and the feeding preference of the mudskippers had not been affected by the pollution (chapter 3). It can thus be concluded that the adverse effects of pollution in the mangrove ecosystem of Mtoni were confined to the mudskipper, at least, as far as fishes are concerned.

Concluding remarks

The research on the mangroves of Tanzania that is presented in this thesis has demonstrated that the adverse effects of pollution in mudskippers were restricted to the mangroves at Mtoni, an area of high economic activities. The distribution of pollutants and their concentrations over the mangroves reflected the intensity and type of economic activities.

The mudskipper population at Mtoni revealed abnormalities in growth and development. Nevertheless, the levels of the most commonly analysed pollutant classes in the downstream section of the Mtoni mangroves, where the mudskippers were collected, were only slightly elevated compared to the levels that were found in the other mangrove sites. Further investigation showed, however, that high heavy metal levels and embryotoxic effluents, potential causative agents of the observed defects in the mudskippers, were present in the upstream sections of the mangroves. Pollution gradients within the Mtoni mangroves revealed that the mangrove sediments readily bind pollutants, which limited the distribution of these pollutants. Most of the pollutants released into the Mtoni Estuary were trapped within the upstream sections of the mangroves, which appeared to be devoid of fish fauna. In the downstream mangrove sections adverse effects of pollution were found in the mudskippers, but no noticeable effects were seen in the fish species that only use the mangroves as a shelter during high tide. Hence, the mudskipper is a good indicator of the effects of pollution on mangrove fauna.

The results of this study indicate that the impact of pollution on the Mtoni Estuary is currently restricted to the mangrove ecosystem, but that the impact on the resident fish within the mangroves is considerable. If the pressure on the ecosystem by
pollution increases and the mangrove vegetation deteriorates, the filtering function of the mangroves will eventually be lost. In that case pollutants and their adverse effects will no longer be confined to the mangroves, but can spread to adjacent ecosystems. This prospect emphasises the value of mangroves as a natural filter and stresses the importance of conservation of the mangrove ecosystem.
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Summary / Samenvatting / Muhtasari
SUMMARY

The increasing urbanisation and industrialisation of Tanzania's coastal zone is likely to result in an increase in the number of pollutants that end up in mangroves, because these forests occupy the transition zone between land and sea in estuaries and bays. The conditions in the mangrove forests favour the trapping of pollutants. This prevents transport to other ecosystems, but potentially increases the risk of pollution effects on mangrove biota. The aim of the research that is described in this thesis was to investigate the extent of pollution along the coast of Tanzania by studying the effect of pollution on the ecology and physiology of a resident mangrove fish, the barred mudskipper (Periophthalmus argentilineatus Valenciennes).

In chapter 2 the significance of the fringing mangroves of Mtoni as a feeding habitat for fishes was investigated. Analysis of stable isotope signatures of fishes and potential food sources revealed that the connectivity between these mangroves and other coastal ecosystems is low. Despite the presence of juveniles of many fish species from adjacent habitats in the Mtoni mangroves at high tide, the isotopic signatures of these fishes indicated that most fishes feed almost exclusively outside the mangroves. To these fishes the mangroves only function as shelter. The mangroves only appeared to present an important feeding habitat for the amphibious mudskippers, which are resident in the mangroves. Consequentially, the carbon sources from the mangroves of Mtoni have a minor contribution to the functioning of the mudflat fish community.

The functioning of the mudskipper within the mangrove communities of Chwaka and Mtoni was investigated by dietary analysis in chapter 3. It was found that the mudskipper has a similar trophic position as other zoobenthivores, but that the amphibious habits of the mudskipper provide this species with the opportunity to minimise competition with other zoobenthivores through resource partitioning. In the fringing mangroves of Mtoni mudskippers benefit from spatial partitioning during all life stages, because they are the only species for which the mangroves are of importance as a feeding habitat. In the mangroves of Chwaka that present a feeding habitat to a wide range of zoobenthivorous fishes, the mudskippers benefit from temporal resource partitioning during their juvenile life stages by feeding during low tide on food items that are preyed upon by other zoobenthivores during high tide. At the same time adult mudskippers prey on food items in higher mangrove sections that are inaccessible for other fish and therefore benefit from spatial resource partitioning. The dietary shift that enables the resource partitioning during all life stages of mudskippers is facilitated by an enlargement of the mouth and an elongation in gut length. Similarity between the diets of the mudskippers of Mtoni and Chwaka indicates that the feeding ecology is not affected by the significance of the mangroves as a feeding habitat to other zoobenthivores.

In chapter 4 the quantification of PCBs, heavy metals, organochlorine pesticides, and organotins in the mangrove sediments and muscle tissues reveals that the distribution of industrial pollutants is restricted to the direct proximity of the city of Dar es Salaam, whereas agricultural pollutants are found in all mangroves. The level of
pollution in the mangroves is generally low. High concentrations of pollutants were only found near the mouth of the Kizinga Stream in the mangroves of Mtoni, and were associated with a nearby textile dyeing mill.

Comparison of the mudskipper populations of six mangroves along the coast of Tanzania in chapter 5 showed that the length distribution of the fish in five populations was similar, but that the fishes from the Mtoni population have a relatively small size. Otolith research showed that this population has an abnormal growth pattern and that mudskippers over 2 years in age are absent from the Mtoni population. The presence of unilaterally anophthalmic individuals in the Mtoni population further distinguishes this population from the other mudskipper populations. The abnormal growth and developmental effects in the Mtoni population corresponds with isotopic enrichment, which suggests that the adverse effects result from pollution.

Chapter 6 presents the results for early life stage tests in which the toxicity of the effluents of the textile dyeing mill at Mtoni to zebrafish embryos, Danio rerio Hamilton, was investigated. The tests reveal that the raw effluent from the textile dyeing mill is highly embryotoxic and, at high concentrations, results in decreased heart rate, decreased hatching rates and elevated mortality rates. The toxicity of the polar fractions of the effluent is much lower and these fractions only resulted in a decreased heart rate at the highest concentration. Since the apolar fractions rapidly bind to the mangrove sediments after release, the most toxic compounds will be restricted to the upstream mangrove sections.

The findings of this study have been summarised and discussed in chapter 7. In the mangroves of Tanzania the mudskippers are most exposed to pollutants from the mangrove through food as well as through polluted sediments, due to their intimate contact with the mangrove sediments and food sources. Therefore, the mudskipper is a good indicator of mangrove pollution. The general level of pollution in the sediments of Mtoni differs little from the sediments of other mangroves, but adverse effects in the mudskippers point at the presence of severe pollution within the Mtoni Estuary. The adverse effects found in the mudskipper population indicate the occurrence of genetic damage. The results of this study reveal that the impact of pollution on the Mtoni Estuary is still confined to the mangrove ecosystem, but that the functioning of the mangrove ecosystem is already stressed by pollution. If the pressure of pollutants on the mangrove system will further increase, the filtering capacities of the mangroves may diminish and adjacent ecosystems will be at risk of pollution. This study emphasises the importance of conservation of the mangrove ecosystem and identifies the mudskipper as a valuable indicator of mangrove pollution.
SAMENVATTING

Door de voortschrijdende industrialisatie en urbanisatie van de kustzone van Tanzania neemt de hoeveelheid vervuilende stoffen die in de mangroves terecht komt waarschijnlijk toe. Mangrove bossen bevinden zich op de overgang tussen land en zee in estuaria en baaien. De heersende condities in de mangrovesedimenten leiden ertoe dat vervuilende stoffen snel gebonden worden. De verspreiding van deze stoffen naar andere ecosystemen wordt zo voorkomen, maar de ophoping van stoffen in de mangroves kan resulteren in schadelijke effecten op organismen in de mangroves. In het onderzoek dat in dit proefschrift is beschreven is onderzocht in welke mate de mangroves langs de kust van Tanzania onderhevig zijn aan vervuiling door de effecten van vervuiling op de ecologie en fysiologie van de slijkspringer (*Periophthalmus argentiineatus*), een aan de mangroves gebonden vis, te bestuderen.

In **hoofdstuk 2** is de waarde van de mangroves bij Mtoni als foerageergebied voor vis onderzocht. Deze mangroves, zogenaamde “fringing mangroves”, nemen slechts een smalle oeverstrook in en hebben slechts een geringe waterdiepte. Tijdens vloed zijn de juvenielen van een groot aantal vissoorten uit nabijgelegen habitats in de mangrove aanwezig. Een analyse van de stabiele isotopen signatuur van deze vissen en potentiële voedselbronnen liet echter zien dat deze vissen vrijwel uitsluitend buiten de mangroves foerageren. De mangrove van Mtoni vormt slechts een schuilgebied voor deze vissen. De mangroves bleken alleen een belangrijk foerageergebied te vormen voor de amfibische slijkspringers. De mate van verbondenheid tussen mangroves en aangrenzende habitats is dus beperkt, evenals de bijdrage van koolstofbronnen in de mangroves van Mtoni aan de visgemeenschap van de aangrenzende habitats.

De positie en het functioneren van de slijkspringer binnen de mangrovegemeenschappen van Chwaka op Unguja eiland (Zanzibar) en Mtoni is in **hoofdstuk 3** onderzocht aan de hand van een analyse van het dieet van de slijkspringer. Uit de analyse kwam naar voren dat de slijkspringer weliswaar een positie op een vergelijkbaar trofisch niveau inneemt als andere zoobenthivore vis, maar dat de amfibische eigenschappen van de slijkspringer deze soort mogelijkheden bieden om de directe competitie voor voedsel met andere soorten te beperken. In de mangroves van Mtoni profiteren de slijkspringers gedurende alle levensstadia van ruimtelijke scheiding van andere vissoorten doordat de slijkspringer de enige soort is waarvoor de mangroves een belangrijk foerageergebied vormen. In tegenstelling daartoe vormen de diepe mangrove kreken van Chwaka een waardevol foerageergebied voor een groot aantal vissoorten. De slijkspringers hebben hier tijdens juveniele levensstadia het voordeel van scheiding van andere soorten in de tijd doordat zij tijdens eb foerageren en voedselbronnen gebruiken die tijdens vloed door andere zoobenthivore vissoorten worden aangesproken. De volwassen slijkspringers maken in Chwaka gebruik van voedselbronnen uit hoger gelegen delen van de mangroves en maken zodoende gebruik van ruimtelijke scheiding van het foerageergebied van andere vissoorten. De verschuiving in het voedselpatroon die de scheiding in voedselbronnen ten opzichte van andere soorten mogelijk maakt wordt gefaciliteerd door een verlenging van de
darm en vergroting van de bek. Uit de gelijkenis tussen de diëten van slijkspringers tussen Mtoni en Chwaka blijkt dat het voedselpatroon van deze vissen niet beïnvloedt wordt door het gebruik van de mangroves als foerageergebied door andere zoobenthivore vissoorten.

In hoofdstuk 4 zijn de gehaltes aan PCBs, zware metalen, gechlorieserde pesticiden en organoot verbindingen in mangrove sedimenten en spierweefsels van slijkspringers bepaald. Uit deze bepaling bleek dat industriële vervuiling beperkt is tot de directe omgeving van Dar es Salaam, terwijl landbouw chemicaliën in alle mangroves aangetroffen zijn. Het niveau van vervuiling is over het algemeen laag. Hoge concentraties van vervuilende stoffen werden alleen bij de monding van de rivier de Kizinga in de mangroves van Mtoni aangetroffen. Deze hoge concentraties worden in verband gebracht met een nabijgelegen textiel fabriek.

Uit een vergelijking van populaties van slijkspringers uit zes verschillende mangroves langs de Tanzaniënaanse kust liet zien dat de lengteverdeling in vijf populaties vergelijkbaar was, maar dat de slijkspringers van de populatie uit Mtoni relatief klein waren (hoofdstuk 5). Onderzoek aan de otolieten toonde aan dat de afwijkende lengte voortkwam uit een verstoord groeipatroon en dat er in de populatie van Mtoni geen slijkspringers van een hogere leeftijd dan twee jaar aanwezig waren. De slijkspringer populatie van Mtoni onderscheidde zich verder van de overige populaties doordat er in de populatie van Mtoni individuen aanwezig waren waarbij slechts één oog tot ontwikkeling was gekomen (anophthalmie). Het optreden van een afwijkend groeipatroon, een ontwikkelingsstoornis en een verrijking in de stabiele isotopen signatuur in de Mtoni populatie ten opzichte van de overige populaties suggereert dat de afwijkingen voortkomen uit vervuiling.

In hoofdstuk 6 is door middel van blootstellingsexperimenten onderzoek gedaan aan de toxiciteit van de effluenten van deze fabriek voor zebravis (*Danio rerio*) embryo’s. De testen toonden aan dat de ruwe effluenten van de textielfabriek zeer giftig waren. Bij hoge concentraties bleek de blootstelling te resulteren in verlaging van de hartslag, een verweerd ... succes en verhoogde sterftecijfers. De geïsoleerde polaire fracties van het effluent bleken veel minder giftig te zijn. Blootstelling aan de hoogste concentratie van deze fractie resulteerde alleen in een verlaagde hartslag. Aangezien de apolaire fracties snel na lozing in de mangrove aan de sedimenten bleken te worden gebonden, bleef de verspreiding van de meest giftige componenten van het effluent beperkt tot de stroomopwaarts gelegen delen van de mangroves.

De bevindingen van dit onderzoek zijn samengevat en bediscussieerd in hoofdstuk 7. Vanwege hun intensieve contact met mangrove sedimenten en de beperking tot voedselbronnen uit de mangroves bleken de slijkspringers van de vissoorten in de mangroves van Tanzania in de grootste mate blootgesteld te worden aan vervuilende stoffen. Hieruit blijkt dat slijkspringers een goede indicator voor vervuiling van de mangroves zijn. Hoewel het gemiddelde niveau van vervuiling in de sedimenten van Mtoni weinig leek te verschillen van het niveau van vervuiling in de overige mangroves, duiden nadelige effecten in de slijkspringer populatie
suggereerden dat genetische schade optreedt als gevolg van vervuiling. De resultaten van deze studie tonen aan dat de vervuiling tot noch is tot de mangroves van Mtoni, maar dat het functioneren van het mangrove systeem in de huidige situatie onder druk staat van vervuiling. Indien de belasting me vervuilende stoffen verder toeneemt, zal de buffercapaciteit van de mangroves aangetast worden waardoor het risico van vervuiling voor stroomafwaarts gelegen habitats toeneemt. Deze studie wijst op het belang van behoud van het mangrove ecosysteem.
MUHTASARI

Kukua kwa mji na viwanda katika ukanda wa pwani ya Tanzania kuna eleke kusababisha ongezeko la idadi ya vichafuzi vinavyoishia kwenye mikoko, kwa kuwa misitu hii hupatikana katika ukanda wa mpito kati ya nchi kavu na bahari kwenye milango mikubwa wa mito na ghuba. Misitu ya mikoko ina tabia ya kunasa vichafufu. Hii huzuia usafiri wa vichafuzi katika mifumo mingine ya ikolojia, lakini huongeza uwezekana wa madhara ya uchafuzi katika viumbe haiwaishio kwenye mikoko. Lengo la utafiti lililobainishwa katika tasnifu hii ni kuchunguza kiasi cha uchafuzi katika pwani ya Tanzania kwa kujifunza madhara ya uchafuzi katika ikolojia na fiziolojia ya samaki aina ya amfibia mkazi wa mikoko. 

Katika sura ya 2, umuhimu wa mikoko ya Mtoni kama eneo la malisho ya samaki ulichunguzwa. Uchanganuzi wa saini za atomi imara aina ya isotopu za samaki, na wa vyanzo wa chakula vinavyoishia ulionesha kuwa uhusiano kati ya mikoko hiya na mifumo mingine ya ikolojia ya Pwani. Mbali na kuwepo, kwa samaki wadogo wa spishi mbalimbali za samaki kutoka mifumo makazi jirani katika mikoko ya Mtoni katika kipindi cha babamvu, saini za ki-isotopu za hawa samaki zinaonesha kuwa karibia samaki wengi hula nje kabisa ya mikoko. Kwa samaki hawa, mikoko hiyo ni kwa ajili ya malazi ya samaki. Mikoko ilione kwa eneo muhimu la malisho kwa samaki hawa aina ya amfibia, ambao ni wakazi wa mikoko. Hivyo, vyanzo vya kaboni kutoka kwenye mikoko ya Mtoni vina mchango mdogo tu kwa utendaji kwa jamii ya samaki wengine wa upwa.

Utendaji za samaki aina ya amfibia katika jamii za mikoko ya Chwaka na Mtoni ulichunguzwa kwa uchanganuza wa chakula katika sura ya 3. Ilidhihirika kuwa samaki amfibia ana nafasi sawa ya kilulali kama samaki wengine wanaoukula wanyama wadogo wadogo wa chini ya bahari. Lakini, tabia ya kiamfibia humpa furasu za mchungaji kushindani rasilimali. Katika kingo za jamii ya mikoko ya Mtoni, samaki amfibia wanafaidika na mgawanyo wa kieno kwa hatua zote za maisha yao kwa kuwa wao ndio spishi pekee ambazo mikoko kwao ni eneo muhimu la malisho. Katika mikoko ya Chwaka ambayo ni eneo la malisho kwa sehemu kubwa ya samaki wengine wanaoukula wadogo wadogo wa chini ya bahari, samaki amfibia hunufaidika kwa muda katika mgawanyo wa rasilimali wakiwa. Wadogo wadogo katika hatua za mwanza za maisha, kwa kula wakati wa maji kupwa vyakula ambayo huliwa na samaki wengine wakati wa maji kujia. Samaki wakubwa aina ya amfibia wao hufaidika kieno kwa kina kwa mgawanyo wa rasilimali wakati wa hatua zote za maisha yao kwa kutumia uchanganzi wa kurefu kwa utumbo na sukari ya mwendo. Kwa samaki amfibia wa Mtoni na Chwaka ulione ambayo mbalimbali za samaki wengine wanaoukula wadogo wadogo wa chini ya bahari.

Mlinganisho wa idadi ya samaki amfibia wa maeneo tofauti sita ya mikoko kando ya pwani ya Tanzania katika **sura ya 5** uameonesha kuwa mwanzo wa uchafuzi wadogo tu karibu na mlango wa kusafishaji. Kwa uchafuzi wa mto Kizinga, idadi ya samaki amfibia wa maeneo tofauti sita ya mikoko ni kidogo. Wingi wa samaki amfibia ulionekana tu karibu na mlango wa kusafishaji. Hii ilihusisha na kiwango cha uchafuzi cha ujumla katika mikoko ya Mtoni.

**Sura ya 6** inawasilishwa matokeo ya majaribio ya maisha ya mwanzo ambapo kwango cha sumu cha maji machafu yatokayo kwafuza kwa ujumla. Utakatika sita ya mikoko ndani ya Mtoni, samaki amfibia yatenendelea kuwa kutoka mwanzo na mto au kwenye matope ya mikoko. Hivyo, majaribio yameonesha kuwa kwango cha uchafuzi cha mwanzo kwa maji machafu yatokayo kwafuza kwa ujumla. Hii ilihusisha na kiwango cha mwanzo cha maji machafu yatokayo kwafuza kwa ujumla, kwa uchafuzi wa mto kwa kwango cha mwanzo cha maji machafu yatokayo kwafuza kwa ujumla. Matokeo ya utafiti huu yaliwekwa muhtasari na kujadiliwa katika **sura ya 7**.

Matokeo ya utafiti huu yaliwekwa muhtasari na kujadiliwa katika **sura ya 7**. Utakatika sita ya mikoko ndani ya Mtoni, samaki amfibia yatenendelea kuwa kutoka mwanzo na mto au kwenye matope ya maji machafu yatokayo kwafuza kwa ujumla. Hivyo, majaribio yameonesha kuwa kwango cha mwanzo cha maji machafu yatokayo kwafuza kwa ujumla.
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It was almost midnight on the 16th of June 2000 when I arrived at Dar es Salaam Airport for the first time. Several months before I had had a meeting with Sjoerd Wendelaar Bonga during which he had asked me whether I liked to do a research project in Tanzania focussing on amphibious fish. I had replied that I was grateful to do so. Having finally arrived in Dar es Salaam, I was very excited, but at the same time very confused by all the new impressions. Fortunately, the confusion did not last long and at the very next daybreak I already started to feel at home in Dar.

After a couple of days of preparation, my local supervisor Pratap, field assistant Richard, and I ventured into the mangroves of Mtoni to search for my first mudskippers ever. They appeared to be extremely hard to find, since mudskippers are dull brown, exactly matching the mangrove mud. We succeeded in finding the mudskippers, but postponed our first attempt to actually catch the mudskippers.

Before our next trip we gathered some metal pieces from a scrap heap and a piece of fine mesh wire curtains and constructed a “Bongo”-net since fishing gear is not available in Dar³. In the field the mud offered the next challenge: with every step we would sink into the mud up to our knees. After we mastered the art of manoeuvring on the muddy surface without sinking into the mud, the biggest challenge remained: closing in on amphibious fish that would casually walk away, jump over the muddy surface, dive into burrows, or just when you thought you almost succeeded, skip away by skimming over the waters’ surface. Thanks to the efforts of my colleagues we succeeded in the end. Some time later I would even be called “Mr. Expert”, referring to my agility in catching mudskippers. Successful collection of mudskippers was only the start of it all.

The excitement about Dar es Salaam, Tanzania, mudskippers, and the Tanzanians has remained until today, but in the mean time we have come a long way. I commenced my journey in completely new world, but found many great people helping me along the way and cooperated and made friends with many people. In this acknowledgement / dankwoord / shukrani I have made an attempt to thank these people for their contribution to my journey and its products: a wonderful time and this thesis:

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³ Dar es Salaam is soothingly nicknamed “Bongoland” by its inhabitants because they say that you need to use your brain (bongo in Swahili) to achieve anything in Dar since money and materials are always scarce.
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Guus
GUUS KRUITWAGEN

GUUS KRUITWAGEN was born on the 30th March 1977 in Velp, the Netherlands. He followed secondary education at the Thomas a Kempis College in Arnhem. In September 1995 he commenced a master in Biology at the University of Nijmegen. During this study he did a research project on stress in halibut at the department of Organismal Animal Physiology of the University of Nijmegen, followed by a research project on the habituation of lowland gorillas via the Tropical Nature Conservation and Ecology of Vertebrates group of the Wageningen Agricultural University. This research focussed on the habituation of lowland gorillas in the Dzanga-Sangha National Park, Central African Republic. After Guus obtained his master degree in January 2000 he cooperated in EU-research programs on stress in fish under aquaculture conditions in Norway and Greece. In May 2000 Guus started the PhD research that is described in this thesis at the department of Organismal Animal Physiology of the University of Nijmegen under supervision of Sjoerd Wendelaar Bonga. He did his research in
cooperation with Harishchandra Pratap of the department of Zoology and Marine Biology. Guus initiated cooperation with Tom Hecht of the department of Ichthyology of Rhodes University in Grahamstown, South Africa, and visited Grahamstown twice for age determination of fishes using otoliths. He also cooperated with the department of Ecophysiology, Biochemistry and Toxicology and the department of Pharmacology of the University of Antwerp and with the department of Physiological Animal Ecology of the University of Tübingen, Germany. Guus presented his results at the “3rd International Conference of Comparative Physiology & Biochemistry in Africa: Animals and Environments” of the Society for Experimental Biology in Louwsburg, South Africa and on the “ECOTOX 2005: Advances and Trends in Ecotoxicology” conference of the Society for Ecotoxicology and Environmental Safety in Brno, Czech Republic. Since December 2005 Guus is employed as ecologist at Witteveen+Bos Consulting Engineers in Deventer.
PUBLICATIONS


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