Marine Biology (2006) 149: 201–211
DOI 10.1007/s00227-005-0178-z

RESEARCH ARTICLE

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

Received: 15 August 2005 / Accepted: 1 November 2005 / Published online: 6 December 2005
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Abstract In this paper a comparison is made between the growth and morphology of barred mudskippers (*Periophthalmus argentilineatus*) from six mangrove forests along the coast of Tanzania. The fish populations from unpolluted sites consisted of different size classes, whereas only small sized fish were present in the polluted Mtoni mangroves. Age estimation based on the 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. The occurrence of unilateral anophthalmia in the Mtoni mudskippers suggested that these fish were affected by pollutants during early development. The study showed that the 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 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 (Engdahl et al. 1998; de Wolf et al. 2001; Machiwa 1992). 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 which 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 (Robertson and Duke 1987; Nagelkerken and van der Velde 2004). Fish are sensitive to environmental pollutants, particularly during their early development since pollutants may interfere with important developmental processes (Von Westernhagen et al. 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 (Swedmark and Granmo 1981; Cameron and Berg 1992; Klumpp and Von Westernhagen 1995; Kingsford and Grey 1996). The effects which 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 et al. 1988).

Whereas many fish species spend only specific life stages in the mangrove habitat, the barred mudskipper

Communicated by O. Kinne, Oldendorf/Luhe

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Periophthalmus argentilineatus is one of the resident mangrove species. Subsequently, all effects of pollution found in this fish can be attributed to exposure in the mangroves. Hence, 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, the input of anthropogenic wastes into coastal ecosystems also results in an increase in the stable nitrogen isotope signatures of these systems, i.e. eutrophication (Spies et al. 1989; Savage et al. 2004). In this 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.

Because previous observations suggested that size differences between mudskippers in the populations of different mangroves were present, otoliths were used to evaluate the growth patterns of the fish. 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 (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.

Materials and methods

Sampling areas

This study was carried out in the mangrove forests at Mtoni, Kunduchi, Mbweni, Chwaka, Makoba and Rufiji along the coast of Tanzania (Fig. 1).

The Mtoni sampling site is the most polluted site in this study (according to Machiwa 1992; Engdahl et al. 1998) 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

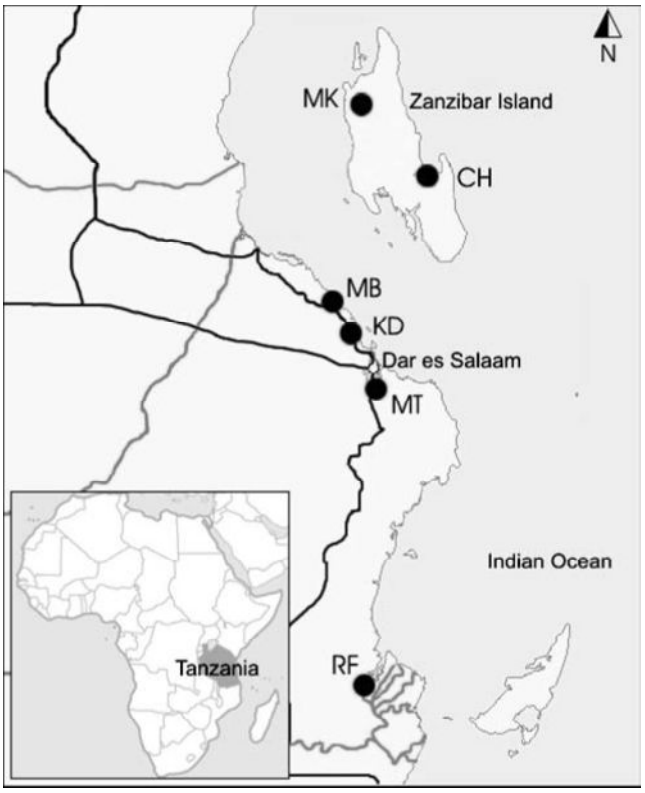


Fig. 1 Locations of the six sampling sites on the coast of Tanzania

Kunduchi and Mbweni according to a study by de Wolf et al. (2001).

Chwaka and Makoba are both located in the rural areas of Zanzibar Island. This island is located 30 km from the mainland coast and has few industries. Chwaka and Makoba are therefore presumed to be little affected by pollutants. 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 the 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 (UNEP 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 expected to be relatively clean.

Fish

Barred mudskippers (*P. argentilineatus*) were randomly collected with scoop nets on the exposed mudflats during low tide. The fish were measured for total length (in cm) and weight (in g). The sagittal otoliths were removed from the auditory bulla, measured for length and 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 5 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 (δ^{15}N) 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 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.

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 2 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 a 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 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 400× 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 ($F=2.660$, $P=0.105$, ANOVA), 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

$$L_t = L_1^b + \left(L_2^b - L_1^b\right) \times \left[\frac{1 - e^{-a(t-t_1)}}{1 - e^{-a(t_2-t_1)}}\right]^{\frac{1}{b}}, \text{ where } L_2 > L_1 \text{ and } t_2 > t_1,$$

where 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 mean lengths of the fish at age t_1 and t_2 and a and b are the relative growth rates.

Table 1 Readability of growth zones in burnt and unburnt otoliths, illuminated by transmitted or direct light

Readability	Unburnt		Burnt	
	Direct light	Transmitted light	Direct light	Transmitted light
1	26	0	28	2
2	11	10	8	14
3	0	16	1	16
4	0	11	0	5
Total	37	37	37	37

Readability is expressed in numbers from 1 (least readable) to 4 (best readable)

The Von Bertalanffy growth model is described by the Schnute model where $b=1$ and is reduced to the familiar form of

$$L_{\infty} = L_t\left(1 - e^{-k(t-t_0)}\right),$$

where L_{∞} 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 Fig. 2. 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 ($n=245$, Fig. 3b–d), but there were no apparent skeletal deformities. In all instances the deformities concerned the absence of the left eye (anophthalmia). The right eye appeared properly formed in all cases. No eye deformities were found in any fish in the other mangroves (Fig. 4).

Fish size

The overall length–weight relationship of all fish was found to be allometric and could be expressed as

$$W = 0.0171L^{2.8292} \quad (n = 1,052),$$

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

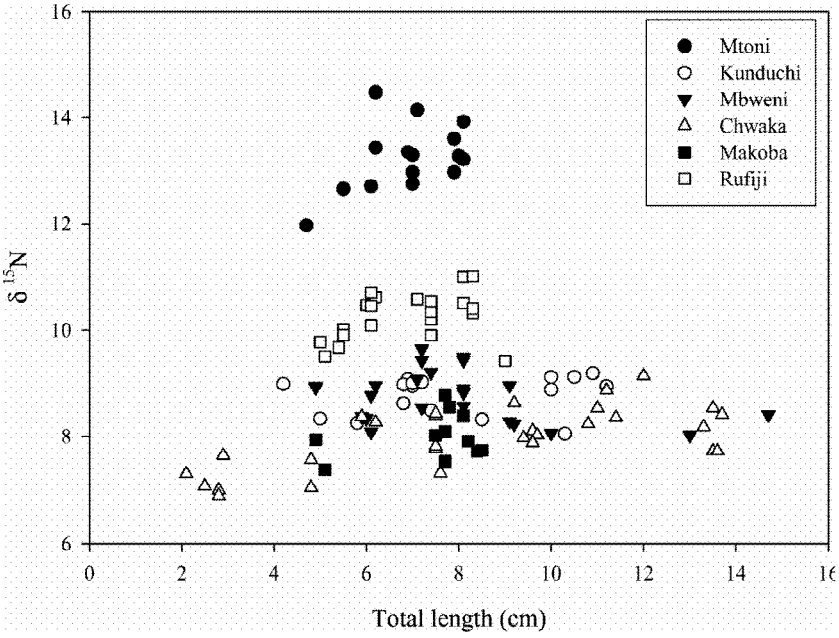


Fig. 2 Stable nitrogen isotopic signatures for mudskippers from all six localities

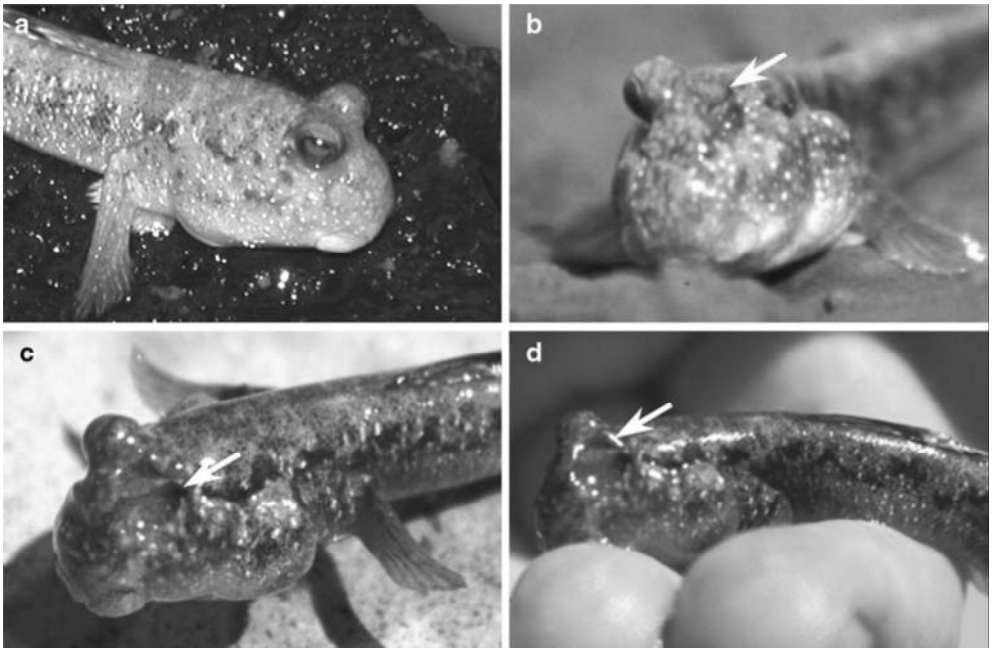


Fig. 3 Pictures of mudskippers with normal eye development (**a**) and fish with left-sided anophthalmia (**b–d**)

Age estimation

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

$$y = 1.671x + 10.599 \quad (r^2 = 0.882,\ n = 89,\ \text{Fig. 4}).$$

The mean 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.

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–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.

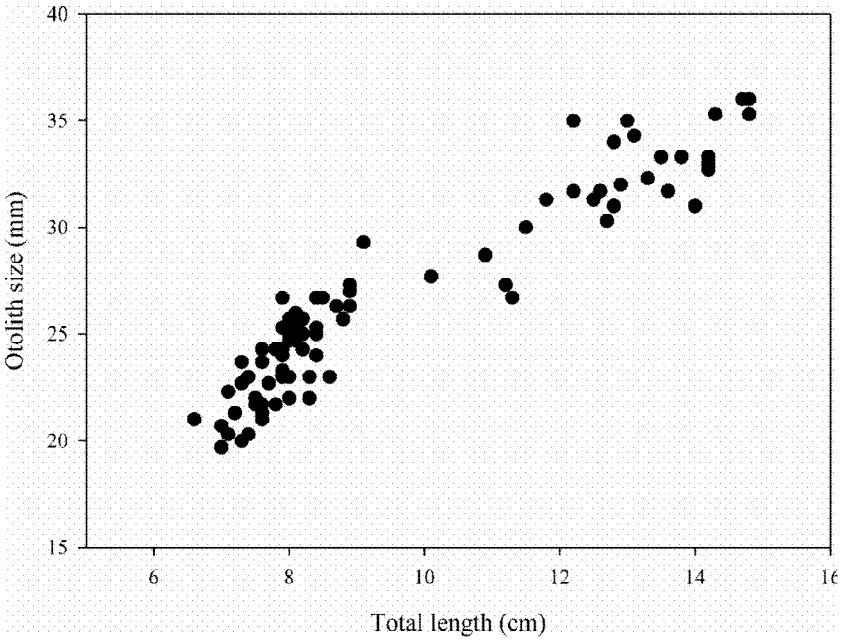


Fig. 4 Relation between the size of the otoliths and the total fish length

Table 2 Mean number of micro-increments on mudskipper otoliths of three age classes

Age	Mean number of increments	Standard deviation	*n*
1	291	26.6	7
2	388	14.1	2
3	840	0.0	1

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 and 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 largely similar, and are illustrated in Fig. 5. 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 Fig. 5 clearly show how the length-at-age estimates for Mtoni fish differ from those for fish of the other sampling sites.

Discussion and conclusions

The assessment of the stable nitrogen signature of mudskippers demonstrated elevated nitrogen signatures in some mangroves. Since the mudskipper populations occupy the same trophic levels in the mangroves of Mtoni and Chwaka (unpublished results), 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 in this study. The high input of anthropogenic nutrients in the Mtoni estuary corresponds with the higher pollution levels that have been reported for the area by Machiwa (1992) and Engdahl et al. (1998). In the Rufiji sampling site the input of nutrients is much lower than in Mtoni and most likely consists of input from the Rufiji River.

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

Locality	Number examined	Number aged successfully	Percentage rejected
Mtoni	68	63	7
Kunduchi	23	22	4
Mbweni	47	43	9
Chwaka	17	14	18
Makoba	46	43	7
Rufiji	66	60	9

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 the embryonic development (Kimmel et al. 1995), and the occurrence of serious eye defects indicates the interference of pollutants with these early inductive processes (Weis and Weis 1977; Nakayama et al. 2005). 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 also appeared normal. In contrast to the findings in our study unilateral anophthalmia in the medaka embryos occurred on either sides at equal frequencies (Y. Ishikawa, personal communications). 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 been described in the literature.

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 the deformities encountered are not the results of infections, nor are they inborn aberrations. Moreover, the anophthalmic individuals were collected at various times during the study period, suggesting that the occurrence is not related to any 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 which survived

Table 4 Age–length keys for mudskippers from the six mangrove localities and all localities combined

Age/length	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.9	14.0–14.9	15.0–15.9	16.0–16.9	Total
Mtoni														
1			1	11	6									18
2				16	26									42
3														0
4														0
5														0
6														0
Total	0	0	1	27	32	0	0	0	0	0	0	0	0	60
Kunduchi														
1	1	2		5	1									9
2				1	2			1	1					5
3					2			1	3					6
4														0
5								1						1
6										1				1
Total	1	2	0	6	5	0	0	3	4	1	0	0	0	22
Mbweni														
1		1	5	2										8
2			2	13	8									23
3				1	2	4								7
4									2	1				3
5											1	1		2
6														0
Total	0	1	7	16	10	4	0	0	2	1	1	1	0	43
Chwaka														
1														0
2						1	1							2
3							1	1	1	2				5
4								1	3	1	1			6
5										1				1
6														0
Total	0	0	0	0	0	1	2	2	4	4	1	0	0	14
Makoba														
1	1			2	1									4
2				1	5									6
3				3	5			2	1	8	1			20
4					1				3	3	3			10
5										1	2			3
6														0
Total	1	0	0	6	12	0	0	2	4	12	6	0	0	43
Rufiji														
1		10	14	4										28
2			3	12	11									26
3					4	2								6
4														0
5														0
6														0
Total	0	10	17	16	15	2	0	0	0	0	0	0	0	60
All localities														
1	1	13	20	24	8									66
2			5	43	52	1	1	1	1					104
3				6	11	6	3	6	2	10	1			45
4					1			1	8	5	4			19
5								1		2	3	1		7
6										1				1
Total	1	13	25	73	72	7	4	9	11	18	8	1	0	242

until adulthood form only a small percentage of the total number of mudskippers that were malformed during development.

The relationship between the length and weight in mudskippers follows the same trend in all mangroves in this study and is comparable 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 have a limited size range in comparison to the other four sites.

Although the increment patterns in mudskippers of the genus *Periophthalmus* are not very clear (Etim et al. 2002), the use of growth zones as annual increments was validated for *P. argentilineatus* in this study. The ageing study revealed that fish communities in the mangrove forests of Chwaka, Kunduchi, Makoba, Mbweni and

Table 5 Parameters of the Schnute or the von Bertalanffy growth equations that best described the growth of the fish in five localities

Locality	Von Bertalanffy			Schnute					
	L_∞	K	t_0	a	b	L_1	L_2	t_1	t_2
Kunduchi (A)	13.55	0.39	−0.76	–	–	–	–	–	–
Mbweni (A)	–	–	–	0.62	2.01	6.57	15.23	1.00	5.00
Chwaka (A)	12.92	1.83	1.26	–	–	–	–	–	–
Makoba (R)	–	–	–	1.08	−3.90	6.55	14.29	1.00	5.00
Rufiji (R)	10.13	0.49	−0.87	–	–	–	–	–	–
All localities (R)	–	–	–	1.02	−3.99	6.48	15.22	1.00	6.00

A Absolute error structure, *R* relative error structure

Table 6 Best-fit model estimates for length-at-age and lower and upper 95% confidence intervals

Locality	Age	Best fit	Lower 95% conf. int.	Upper 95% conf. int.
Mtoni	1	7.72 ± 0.49		
(mean ± SD)	2	8.08 ± 0.42		
Kunduchi	1	6.76	5.82	7.91
	2	8.96	8.26	10.46
	3	10.45	9.47	11.04
	4	11.46	9.76	12.38
	5	12.14	9.79	13.55
	6	12.60	9.79	14.80
Mbweni	1	6.57	6.34	7.07
	2	7.62	7.52	7.79
	3	9.26	8.91	9.33
	4	11.71	10.89	11.87
	5	15.23	14.53	15.88
Chwaka	2	9.61	7.47	11.04
	3	12.39	11.43	12.98
	4	12.84	12.20	13.56
	5	12.91	12.24	14.48
Makoba	1	6.57	4.47	8.78
	2	8.69	7.84	10.76
	3	10.72	8.80	11.44
	4	12.69	10.57	13.50
	5	14.59	11.32	18.18
Rufiji	1	6.14	5.96	6.38
	2	7.70	7.46	7.83
	3	8.66	8.29	9.30
All localities	1	6.48	6.29	6.67
	2	8.27	8.13	8.46
	3	10.36	10.09	10.65
	4	12.49	12.13	12.65
	5	14.20	13.65	14.55
	6	15.22	14.10	16.31

Rufiji consist of several age classes between 1 and 6 years, while the Mtoni population consists 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 disappear from the habitat in high numbers. Because mudskippers do not have a swimming bladder and are not very good swimmers, emigration by adult fishes is not likely. Higher mortality rates after the age of 2 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 differs from the other growth patterns observed in this study. The mean length of the Mtoni fish at the age of 1 year appears to be comparable 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 indicated by the stable nitrogen isotopes. Reductions in fish growth have been described

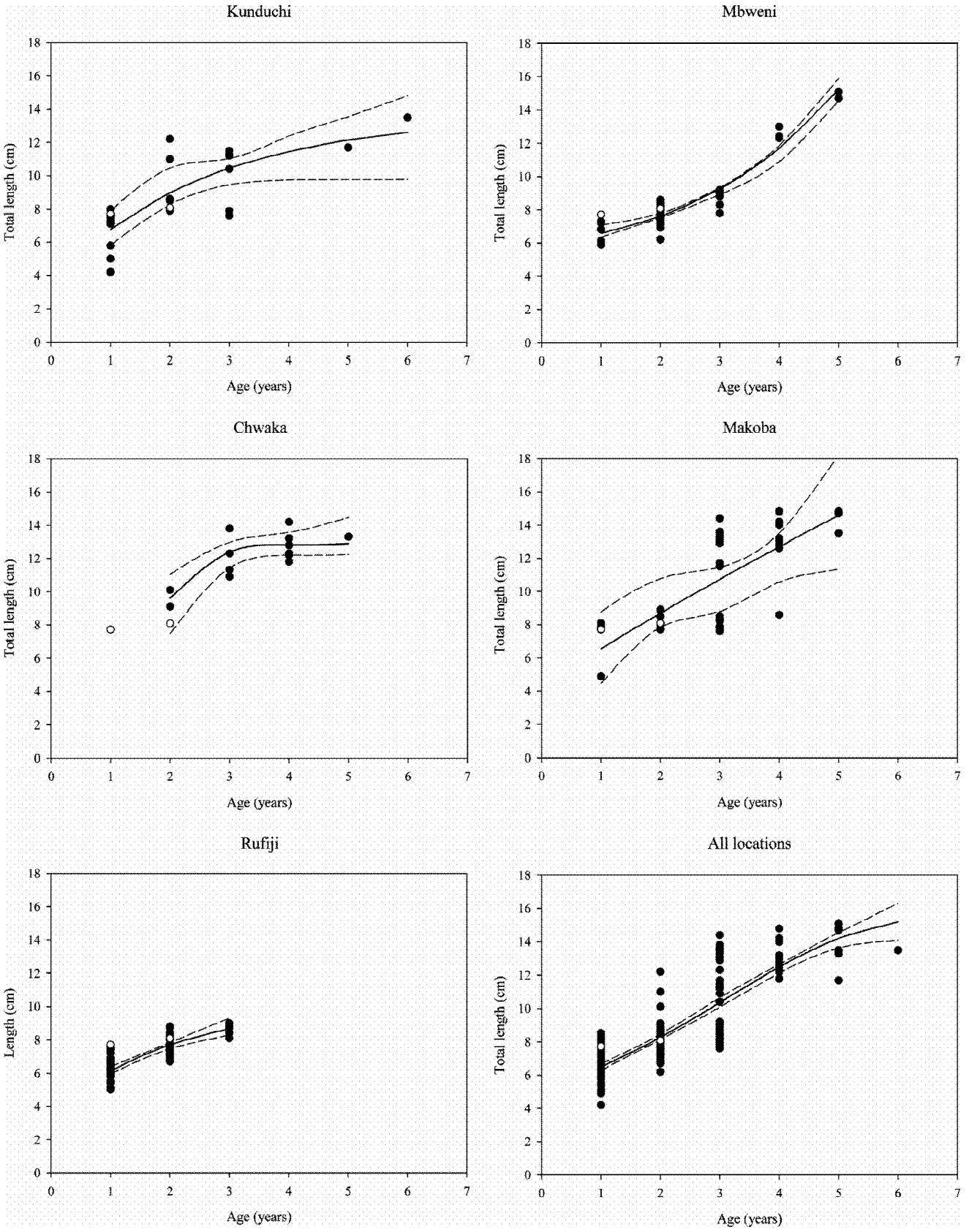


Fig. 5 Growth curves for mudskippers from five mangroves separately, and for all sampling locations combined. The *solid line* shows the predicted growth and the *dashed lines* depict the 10 and 90% confidence intervals. The *open circles* in every graph present the average body length of the Mtoni fish

for a variety of pollutants (Marr et al. 1996; Heintz et al. 2000; Laroche et al. 2002; Makita 2003).

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 1992), 11.7–43.9 ng/g dw (Mwevura 2002), 9–70 µg/g (Machiwa 1992) and 132–164 µg/g dw (Machiwa 1992), 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 1,013–8,503 ng/g dw, Wong et al. 2000), but could nonetheless cause adverse effects (Klumpp et al. 2002). Because pollution in the estuary is complex (Machiwa 1992) 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 reports in the literature. 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 the direct exposure of developing fish to pollutants, or may have resulted from parental exposure to pollutants. Since the effects in Mtoni concern the whole population and have been found at different points in time, the anticipated causative agents are either continuously present in the mangroves and expose every generation anew, or damage caused by a past pollution event that is passed on from generation to generation. Both scenarios imply that environmental pollutants in the Mtoni estuary have effects not only 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 2004), 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.

Acknowledgements We gratefully acknowledge Richard Massinde from the Department of Zoology and Marine Biology, University of Dar es Salaam, for assistance during collection of samples in the field, and Ernst Thompson from the Department of Ichthyology and Fisheries Sciences, Rhodes University, for technical assistance in the processing of otoliths. This is Centre for Wetland Ecology publication number 412.

References

Buckland ST (1984) Monte Carlo confidence intervals. Biometrics 40:811–817

Cameron P, Berg J (1992) Morphological and chromosomal aberrations during embryonic development in dab *Limanda limanda*. Mar Ecol Prog Ser 91:163–169

Cerrato RM (1990) Interpretable statistical tests for growth comparisons using parameters in the Von Bertalanffy equation. Can J Fish Aquat Sci 47:1416–1426

De Wolf H, Ulomi SA, Backeljau T, Pratap HB, Blust R (2001) Heavy metal levels in the sediments of four Dar es Salaam mangroves; accumulation in, and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda). Environ Int 26:243–249

Efron B (1982) The jackknife, the bootstrap, and other resampling plans. In: CBMS-NSF regional conference series in applied mathematics, monograph 38. Society for Industrial and Applied Mathematics, Philadelphia, 92 pp

Engdahl S, Mamboya F, Mtolera M, Semesi AK, Björk M (1998) The brown macroalgae *Padina boergesenii* as an indicator of heavy metal contamination in the Zanzibar channel. Ambio 28:694–700

Etim L, Brey T, Arntz W (1996) A seminal study of the dynamics of a mudskipper (*Periophthalmus papilio*) population in the Cross river, Nigeria. Neth J Aquat Ecol 30:41–48

Etim L, King RP, Udo MT (2002) Breeding, growth, mortality and yield of the mudskipper *Periophthalmus barbarus* (Linneaus 1776) (Teleostei:Gobiidae) in the Imo estuary, Nigeria. Fish Res 56:227–238

Fantes J, Ragge NK, Lynch SA, Mcgill NI, Collin JRO, Howard-Peebles PN, Hayward C, Vivian AJ, Williamson K, van Heyningen V, FitzPatrick DR (2003) Mutations in SOX2 cause anophthalmia. Nat Genet 33:461–463

Heintz RA, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW (2000) Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. Mar Ecol Prog Ser 208:205–216

Hong H, Xu L, Zhang L, Chen JC, Wong YS, Wan TSM (1995) Environmental fate and chemistry of organic pollutants in the sediment of Xiamen and Victoria Harbours. Mar Pollut Bull 31:229–236

Hyndes GA, Loneragan NR, Potter IC (1992) Influence of sectioning otoliths on marginal increment trends and age and growth-estimates for the flathead *Platycephalus speculator*. Fish B-NOAA 90:276–284

Ishikawa Y, Yoshimoto M, Yamamoto N, Ito H, Yasuda T, Tokunaga F, Iigo M, Wakamatsu Y, Ozato K (2001) Brain structures of a medaka mutant, el (eyeless), in which eye vesicles do not evaginate. Brain Behav Evol 58:173–184

Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF (1995) Stages of embryonic development of the zebrafish. Dev Dyn 203:253–310

Kingsford MJ, Gray CA (1996) Influence of pollutants and oceanography on abundance and deformities of wild fish larvae. In: Schmitt RJ, Osenberg CW (eds) Detecting ecological impacts: concepts and applications in coastal habitats. Academic, San Diego, pp 235–255

Klumpp DW, Von Westernhagen H (1995) Biological effects of pollutants in Australian tropical coastal waters: embryonic malformations and chromosomal aberrations in developing fish eggs. Mar Pollut Bull 30:158–165

Klumpp DW, Humprey C, Huasheng H, Tao F (2002) Toxic contaminants and their biological effects in coastal waters of Xiamen, China. II: Biomarkers and embryo malformation rates as indicators of pollution stress in fish. Mar Pollut Bull 44:761–769

Laroche J, Quiniou L, Juhel G, Auffret M, Moraga D (2002) Genetic and physiological responses of flounder (*Platichthys flesus*) populations to chemical contamination in estuaries. Environ Toxicol Chem 21:2705–2712

Loosli F, Staub W, Finger-Baier KC, Ober EA, Verkade H, Wittbrodt J, Baier H (2003) Loss of eyes in zebrafish caused by mutation of *chokh/rx3*. Embo Rep 4:894–899

Machiwa JF (1992) Anthropogenic pollution in the Dar es Salaam harbour area, Tanzania. Mar Pollut Bull 24:562–567

Makita Y, Tanaka A, Omura M, Ogata R (2003) Effects of simultaneous administration of tributyltin (TBT) and *p,p'*-DDE on female offspring of Wistar rats. J Toxicol Environ Health A 66:2337–2347

Marr JCA, Lipton J, Cacela D, Hansen JA, Bergman HL, Meyer JS, Hogstrand C (1996) Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth. Aquat Toxicol 36:17–30

Minagawa M, Wada E (1984) Stepwise enrichment of N^{15} along food chains—further evidence and the relation between δN^{15} and animal age. Geochim Cosmochim Acta 48:1135–1140

Morales-Nin B (2000) Review of the growth regulation processes of otolith daily increment formation. Fish Res 46:53–67

Morales-Nin B (1992) Determination of growth in bony fishes from otolith microstructure. FAO Fisheries Techical Paper No. 322. FAO, Rome, 51 pp

Mwevura H, Othman OC, Mhehe GL (2002) Organochlorine pesticide residues in sediments and biota from the coastal area of Dar es Salaam city, Tanzania. Mar Pollut Bull 45:262–267

Nagelkerken I, Van der Velde G (2004) Relative importance of interlinked mangroves and seagrass beds as feeding habitats for juvenile reef fish on a Caribbean island. Mar Ecol Prog Ser 274:153–159

Nakayama K, Oshima Y, Nagafuchi K, Hano T, Shimasaki Y, Nakayamaj K (2005) Early-life-stage toxicity in offspring from exposed parent medaka, *Oryzias latipes*, to mixtures of tributyltin and polychlorinated biphenyls. Environ Toxicol Chem 24:591–596

National Bureau of Statistics (2002) Tanzania: 2002 population and housing census. Dar es Salaam, Tanzania

Rau GH, Sweeney RE, Kaplan IR, Mearns AJ, Young DR (1981) Differences in animal ^{13}C, ^{15}N and D abundance between a polluted and an unpolluted coastal site: likely indicators of sewage uptake by a marine food web. Estuar Coast Shelf Sci 13:701–707

Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191. Department of the Environment, Fisheries and Marine Service, Ottawa, 382 pp

Robertson AI, Duke NC (1987) Mangroves as nursery sites: comparisons of the abundance and species composition of fish and crustaceans in mangroves and other nearshore habitats in tropical Australia. Mar Biol 96:193–205

Savage C, Leavitt PR, Elmgren R (2004) Distribution and retention of effluent nitrogen in surface sediments of a coastal bay. Limnol Oceanogr 49:1503–1511

Schnute J (1981) A versatile growth model with statistically stable parameters. Can J Fish Aquat Sci 38:1128–1140

Secor DH, Dean JM, Laban EH (1992) Manual for otolith removal and preparation for microsctructural examination. In: Stevenson DK, Campana SE (eds) Otolith microstructure examination and analysis. Canadian special publication of fisheries and aquatic sciences No. 117. Fisheries and Oceans Canada, Ottawa, pp 19–57

Spies RB, Kruger H, Ireland R, Rice DW (1989) Stable isotope ratios and contaminant concentrations in a sewage-distorted food web. Mar Ecol Prog Ser 54:157–170

Strömland K, Miller M, Cook C (1991) Ocular teratology. Surv Ophthalmol 35:429–446

Swedmark M, Granmo Å (1981) Effects of mixtures of heavy metals and a surfacant on the development of cod (*Gadus morhua* L.). Rapports et procès-verbaux des Réunions, Conseil international pour l'exploration de mer 178:95–103

UNEP (2001) Eastern Africa atlas of coastal resources: Tanzania. United Nations Environment Programme, Nairobi

Von Westernhagen H (1988) Sublethal effects of pollutants on fish eggs and larvae. In: Hoar WS, Randall DJ (eds) The physiology of developing fish; Part A: Eggs and larvae. Fish physiology. Academic, Orlando, pp 253–346

Weis P, Weis JS (1977) Methylmercury teratogenesis in the killifish, *Fundulus heteroclitus*. Teratology 16:317–326

Wong CKC, Yeung HY, Cheung RYH, Yung KKL, Wong MH (2000) Ecotoxicological assessment of persistent organic and heavy metal contamination in Hong Kong coastal sediment. Arch Environ Contam Toxicol 38:486–493

Wurl O, Obbard JP (2005) Organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in Singapore's coastal marine sediments. Chemosphere 58:925–933