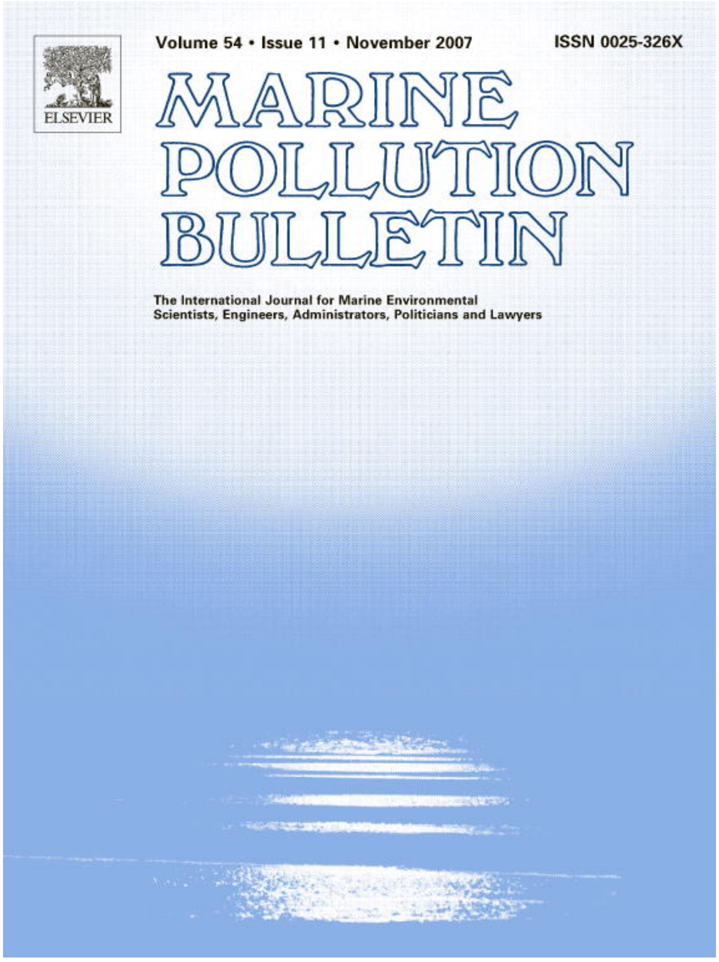
ELSEVIER
Volume 54 • Issue 11 • November 2007
ISSN 0025-326X
MARINE POLLUTION BULLETIN
The International Journal for Marine Environmental Scientists, Engineers, Administrators, Politicians and Lawyers

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Marine Pollution Bulletin 54 (2007) 1762–1776
MARINE POLLUTION BULLETIN
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Estuarine fish health assessment: Evidence of wastewater impacts based on nitrogen isotopes and histopathology

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Abstract

Sewage effluent is a powerful agent of ecological change in estuaries. While the effects of sewage pollution on water quality are usually well documented, biological responses of exposed organisms are not. We quantified health impacts in the form of pathological tissue changes across multiple organs in estuarine fish exposed to elevated levels of treated wastewater. Structural pathologies were compared in wild populations of four fish species from two subtropical estuaries on the east coast of Australia that differ substantially in the amount of direct wastewater loadings. Uptake of sewage-derived nitrogen by fish was traced with stable nitrogen isotopes. Pathologies were common in the liver, spleen, gill, kidney and muscle tissues, and included granulomas, melanomacrophage aggregates, and multiple deformities of the gill epithelia. Tissue deformities were more frequent in fish exposed directly to wastewater discharges. Mullet (*Valamugil georgii*) were most affected, with only a single specimen free of pathologies in the sewage-impacted estuary. Similarly, in those fish that had structural abnormalities, more deformities were generally found in individuals from sites receiving sewage. These spatial contrasts in impaired fish health correspond to significantly enriched δ^{15}N values in fish muscle as a consequence of fish assimilating sewage-N. Overall, the pattern of lower health and enriched δ^{15}N values in fish from sewage-impacted areas suggests that organism health is lowered by sewage inputs to estuaries. Measurements of organism health are required to understand the effects of sewage on estuarine ecosystems, and histopathology of fishes is a powerful tool to achieve this.

Keywords: Estuary; Fish; Pathology; Stable isotopes; Subtropical

1. Introduction

Estuaries are under escalating pressure from pollutants including human sewage (Kennish, 1992). Nitrogen dominates the nutrients in sewage, and nitrogen loads to coastal waters are increasing globally (Galloway et al., 2004). Management of sewage pollution usually targets nitrogen concentrations, mainly because of the multiple ecological impacts that excess nitrogen loads have in coastal waters (Anderson et al., 2002; Nixon and Buckley, 2002; Scavia and Bricker, 2006). Nitrogen inputs to estuaries can also come from numerous other sources, such as industrial wastes, agricultural and non-specific land run-off, stormwater discharges, and atmospheric deposition (Valiela et al., 2000). Such multiple pollution sources often pose complex management challenges which require the sources to be identified and their relative contributions to system-wide loads to be quantified (Valiela et al., 1997).

Sewage-derived nitrogen can be identified with stable nitrogen isotopes, based on isotopic differences between sewage and other nitrogen sources (Kendall, 1998). Discharges from sewage treatment plants and septic tanks typically have higher δ^{15}N values produced by isotopic fractionation during nitrogen transformations (e.g. ammonia volatilisation and denitrification) that enriches the

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doi:10.1016/j.marpolbul.2007.07.014

residual nitrogen pool with ^{15}N (Heaton, 1986; Valiela et al., 2000). Stable isotopes can trace the assimilation of sewage-N at all trophic levels from plants (Cole et al., 2004; Savage, 2005), to invertebrates (McKinney et al., 2002; Moore and Suthers, 2005; Bucci et al., 2007), and fish (Schlacher et al., 2005; Northington and Hershey, 2006).

Fish are sensitive and robust indicators to assess links between pollution and ecological condition in estuaries for a number of reasons: (1) fish provide direct biological responses compared with surrogate measures such as water quality that may not necessarily correspond with changes in the biota (Leamon et al., 2000); (2) fish have longer life spans and thus integrate pollution signals unlike spot measurements of water chemistry with high temporal variance (Schlacher et al., 2005); (3) fish display a diversity of trophic modes, life histories, and habitat preferences, enabling pollution assessments to encompass multiple levels of ecological organisation and function (Whitfield and Elliott, 2002); and (4) fish are of great recreational and economic importance which makes uptake of pollution assessments by managers and the general public much more likely (Whitfield and Elliott, 2002).

In applications where the fate and magnitude of wastewater pollution are the main question of interest, fish are, arguably, the preferred bio-indicators for detecting uptake of sewage-N in food webs for several reasons: (1) tissue turnover is slower in fish, and the assimilated nitrogen signal is therefore integrated over longer time periods (Gaston et al., 2004), (2) fish measure propagation of external nitrogen loads through the impacted food webs beyond basal producers and low-level consumers (McClelland et al., 1997), and (3) fish have a higher public appeal than most other bio-indicators and are a valuable recreational and commercial resource. These attributes increase the public acceptance of pollution assessment and management interventions (Whitfield and Elliott, 2002).

Whereas nitrogen is usually the principal focus of wastewater management, sewage is a complex chemical mixture of many toxicants (Pantsar-Kallio et al., 1999). Thus, biological impacts of sewage are wide-ranging; they can include pathological tissue alterations (Moore et al., 2003), endocrine disruptions (Gagné and Blaise, 2003; Jobling and Tyler, 2003), effects on population dynamics and production (Hindell and Quinn, 2000; deBruyn et al., 2002), and altered community structure and food web properties (McClelland and Valiela, 1998; Archambault et al., 2001; Morris and Keough, 2002; Bucci et al., 2007).

Fish are exposed to sewage-borne toxicants via direct contact with water and sediment, and ingestion of contaminated food and substrate (Mondon et al., 2001). Contaminants in both water and sediment can cause pathological alterations in exposed organisms (Myers and Hendricks, 1985). Pollutants reduce disease resistance because fish become stressed or are exposed to more pathogens that result in higher infection probabilities (Nowak, 1996). Pollutants, including sewage, can also elicit cytotoxic and other structural abnormalities and deformities in fish (Evans, 1987; Veethaak and Rheinalt, 1992). Examination of pathological changes to cells and tissue of fishes therefore serves as a powerful diagnostic tool of impaired organism health in estuarine food webs affected by anthropogenic pollutants (Mondon et al., 2001).

Ecological impacts of sewage are diverse, yet routine pollution assessments typically use a limited number of chemical water quality indicators and do not directly measure the response of organisms (EHMP, 2007). The main objective of this study was to test whether poor water quality caused chiefly by sewage loads causes impaired fish health. We assessed the biological responses of sewage inputs by measuring fish health in a subtropical estuary subjected to direct discharges of treated sewage. The extent of sewage inputs to the system is well understood through routine water quality monitoring (EHMP, 2007), modelling of loads from different sources (Beling and McAllister, 2004), and nitrogen isotope surveys of invertebrates and fish (Schlacher et al., 2001, 2005; Schlacher and Caruthers, 2002). Whether sewage inputs result in direct health effects in the organisms exposed to sewage is, however, unknown.

Fish health was measured by quantifying the incidence of pathological tissue changes in the main organs. The link between impaired organism health and water quality was tested by contrasting the prevalence of fish pathologies between the sewage-impacted estuary and an adjacent estuary that does not receive any direct inputs from sewage treatment plants.

2. Methods

2.1. The estuaries and sewage nitrogen

We sampled fish in two subtropical estuaries, the Maroochy and Noosa, located in SE-Queensland, Australia (Fig. 1). The estuaries have broadly similar physical characteristics: they are narrow (width 100–300 m), shallow (generally <3 m), and short (tidal head <30 km). The Maroochy Estuary is a narrowly incised channel, whereas several shallow coastal lakes, interconnected by narrow channels, form the Noosa Estuary (Fig. 1).

The upper catchment of the Noosa River comprises mainly natural forest in conservation areas, with a single, small human settlement. Conversely, large tracts of the Maroochy catchment have been cleared for agriculture and housing. Sugarcane is grown extensively on the floodplain of the Maroochy Estuary, whereas no significant agriculture exists along the Noosa Estuary. The lower sections of both estuaries have been largely modified, including armouring of shorelines, and construction of canal estates and urban settlements with numerous stormwater outlets. Recreational angling and boating are very popular in both estuaries, particularly in the lower sections near the tidal inlet.

Direct nitrogen loads from sewage treatment plants occur only in the Maroochy Estuary. Five sewage treatment plants discharge 47 t of nitrogen per year into the

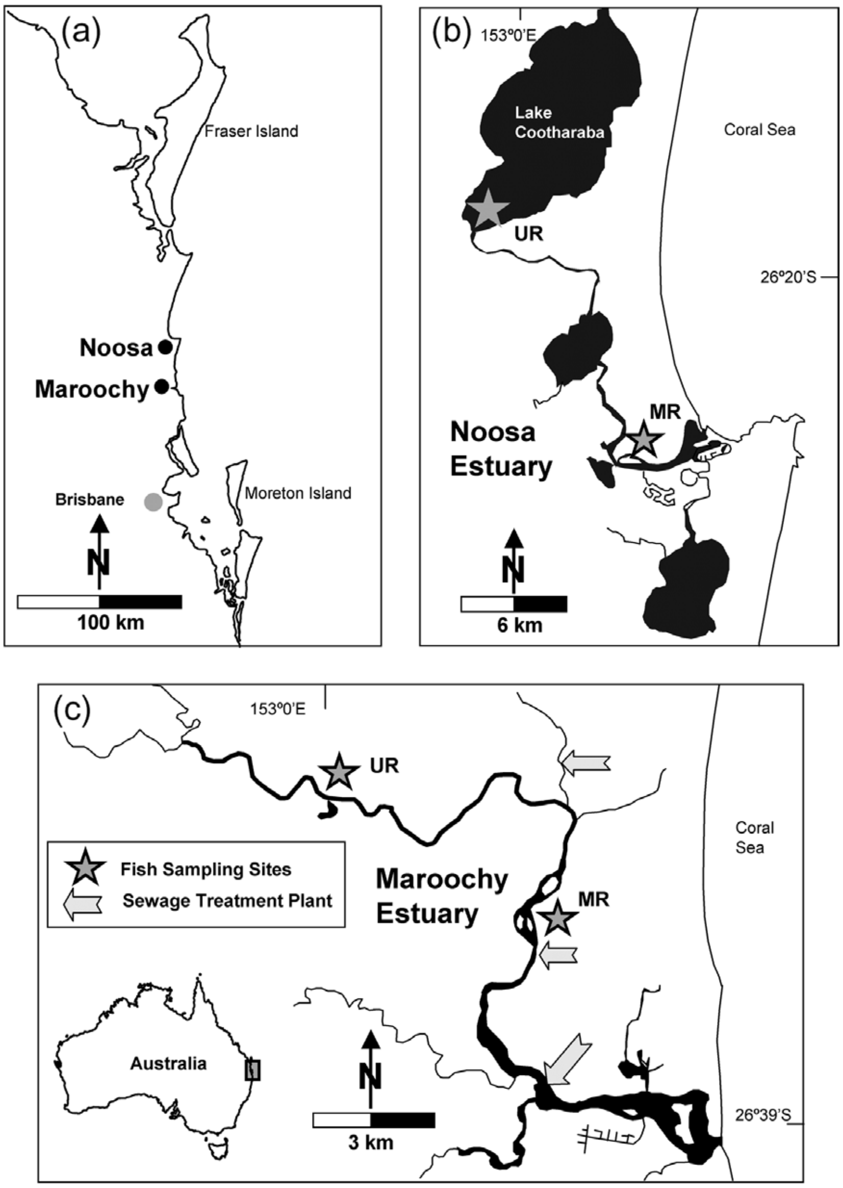

Fig. 1. Location of estuaries in south-east Queensland (a) and map of estuaries with fish sampling sites in the middle reaches (MR) and upper reaches (UR) of the Noosa Estuary (b) and Maroochy Estuary (c).

main channel of this estuary and contribute 65% of the total nitrogen load (Beling and McAllister, 2004). By contrast, no direct discharges from sewage treatment plants occur in the Noosa Estuary, where treated wastewater is diverted to a beach outfall 15 km to the south of the estuarine inlet. The Noosa Estuary therefore serves as the reference estuary in this study.

2.2. Sites and species coverage

Fish were collected from both the middle and upper reaches of each estuary (Table 1, Fig. 1). Water quality varies substantially along the length of each estuary. In the Maroochy, most treated sewage is discharged in the middle reaches of the estuary. We therefore predicted that impacts of sewage on fish health would be strongest in the middle reaches of this estuary. Additional sewage loads can, however, originate from diffuse sources (e.g. septic tanks) in the watershed, and tidal advection transports sewage upstream into the upper reaches. The net effect is a system-wide sewage enrichment of the Maroochy Estuary (Schlacher et al., 2001).

Fish were sampled with a small seine net (15×2 m, 19 mm mesh size) and a cast net (10 mm mesh size). Up to 100 net hauls were made in each reach. The captured fish were carefully removed from the nets, inspected for gross external morphological changes, and then promptly anaesthetised without recovery using clove oil to prevent post-mortem degeneration. For larger fish, the first gill arch was removed and fixed immediately in 10% buffered formalin, followed by the eye, liver, kidney, spleen, heart, gonad and muscle tissue with attached skin. For very small fish

Table 1
Fish lengths (median, range) by reach and estuary (standard lengths; mm)

Species	Estuary	Reach	*n*	Median	Range (min–max)
Ambassis jacksoniensis	Maroochy	Middle	23	49	41–56
(Port Jackson		Upper	23	41	34–56
Glassfish)		Total	46	46	34–56
$L_{max} = 70$	Noosa	Midle	19	40	35–48
		Upper	23	41	32–46
		Total	42	41	32–48
Gerres subfasciatus	Maroochy	Middle	23	48	43–100
(Silver Biddy)		Upper	22	75	59–118
$L_{max} = 200$		Total	45	62	43–118
	Noosa	Middle	22	86	48–138
		Upper	21	66	43–117
		Total	43	79	43–138
Sillago ciliata	Maroochy	Middle	27	87	64–137
(Summer Whiting)		Upper	21	101	83–241
$L_{max} = 510$		Total	48	87	64–241
	Noosa	Middle	19	134	108–174
		Upper	0	–	–
		Total	19	–	–
Valamugil georgii	Maroochy	Middle	28	88	53–103
(Fantail Mullet)		Upper	5	172	105–189
$L_{max} = 300$		Total	33	91	53–189
	Noosa	Middle	6	122	103–202
		Upper	2	117	117–117
		Total	8	119	103–202

Typical lengths at maturity (L_{max}) are shown in the species column (source: www.fishbase.org).

(<50 mm) we removed and fixed the first gill arch and then fixed the whole body in 10% buffered formalin.

We selected four common species of fish representing a range of different feeding types. *Ambassis jacksoniensis* is purely pelagic and feeds on planktonic crustaceans (Hollingsworth and Connolly, 2006). *Gerres subfasciatus* also feeds predominantly on planktonic crustaceans but sometimes also consumes benthic invertebrates. *Sillago ciliata* has more of a benthic habit and eats benthic invertebrates (Burchmore et al., 1988). *Valamugil georgii*, for the size of individuals collected, is an almost exclusively benthic feeder, assimilating a variety of plant and animal types from the bulk sediment ingested (Morton et al., 1987). We focussed our collections on small species (*A. jacksoniensis*) and the juvenile stages of the larger species for two reasons: (1) they are more abundant and more reliably captured across different estuarine sites, an important practical requirement for environmental monitoring, and (2) they have more restricted movement and are therefore more likely to reflect site-specific differences in pollutant concentrations.

A total of 284 individuals were examined for histopathology (Table 1). We initially aimed for a sample size of 20 individuals per site for each species. This was achieved for *A. jacksoniensis* and *G. subfasciatus*, but *S. ciliata* and *V. georgii* were less abundant and could only be caught in lower numbers at some sites (*V. georgii*) or were not captured (i.e. *S. ciliata* in the upper Noosa Estuary; Table 1). Within a species, there was generally no marked and consistent difference in the size of fish analysed between estuaries and reaches (Table 1). For example, *A. jacksoniensis* ranged in length from 34 to 56 mm in the Maroochy and 32 to 48 mm in the Noosa Estuary, and *G. subfasciatus* was of similar length in the Maroochy (43–118 mm) and Noosa Estuary (43–138 mm).

Rainfall in the region is typically bimodal with a wet season during the austral summer (December–March), alternating with periods of low rainfall during the austral winter (June–September). Water chemistry in these estuaries is strongly influenced by rainfall and run-off from the catchments (Schlacher et al., 2001; EHMP, 2007). Nitrogen inputs into the estuary originating from the catchment versus those discharged from sewage treatment plants therefore vary seasonally. During the dry season, point sources (i.e. sewage treatment plant discharges) dominate the nitrogen budget, while higher catchment loads are typical during the wet season. We therefore sampled fish at the end of autumn (5–7 June, 2006) to avoid either seasonal extreme, and to capture conditions representative of average nitrogen inputs and water chemistry. Salinity values in the months before sampling were not markedly reduced (Fig. 2). There were several small rainfall events in the catchments in June, but all fish collections were completed before these events. Nitrogen concentrations are highly variable in both estuaries, but there were no abnormal spikes in nitrogen levels in the weeks prior to fish collections (Fig. 2).

2.3. Laboratory processing

For isotope analysis the dorsal lateral muscle was analysed, dissected from fresh specimens within 2 h of collection. Muscle tissue was rinsed twice in deionised water, decalcified (1 M HCl for 40 min), followed by four rinses in deionised water and drying at 65 °C. Stable isotope analysis was done in the Isotope Analytical Facilities of Griffith University on an automated Isoprime isotope-ratio mass spectrometer. Stable isotope ratios are expressed in ‰ units using the standard delta (δ) notation: $\delta^{15}N$ (‰) $= [(R_{sample}/R_{standard}) - 1] \times 1000$; where R is the $^{15}N/^{14}N$ ratio, and laboratory standards were calibrated against the IAEA international standard of the $^{15}N/^{14}N$ ratio in air.

Histology samples were dehydrated in ascending grades of alcohol (70–100%), cleared in xylene and embedded in paraffin. Tissue was sectioned using a MICROM Heidelberg microtome at 4 µm. Standard haematoxylin and eosin (H&E) stained sections were prepared from each tissue block (Bancroft and Cook, 1994) and examined blind (i.e. the sample identifier was not known to the examiner), under a ZEISS Axiovert 40CFL stereomicroscope at 50–400× magnification.

3. Results

3.1. Nitrogen isotopes

Fish from the Maroochy Estuary had significantly higher $\delta^{15}N$ values. This isotopic enrichment reflects

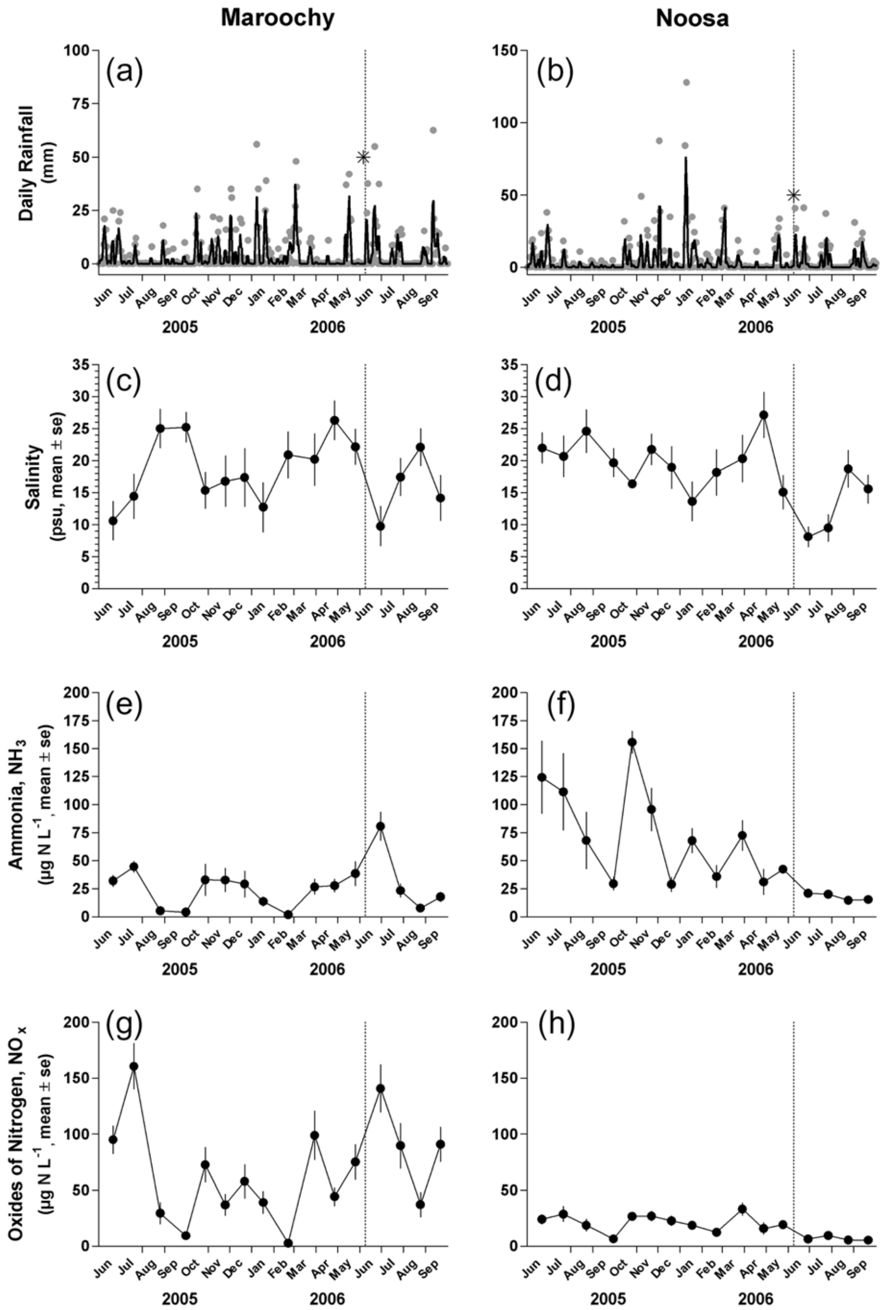

Fig. 2. Variation in rainfall (a and b), salinity (c and d), ammonia (e and f), and nitrate and nitrite (g and h) in the Maroochy Estuary (left panels) and Noosa Estuary (right panels). Plotted data are means of samples collected at 10 (Maroochy) and 11 (Noosa) sites located along the main axis of the estuary from the mouth to the tidal limit, sampled at monthly intervals. Data source for water quality is the Ecological Health Monitoring Program (EHMP – http:/www.ehmp.org/). Dotted lines denote times of fish collections for this study.

increased assimilation of sewage-N delivered via the sewage discharges into the estuary (Table 2, Fig. 3). The major source of variation in nitrogen isotope ratios was at the scale of inter-estuarine contrasts: all species showed significantly elevated δ^{15}N values in the Maroochy (ANOVAs for all species, $P < 0.001$ for estuary effect), by between 3.80‰ and 5.73‰. Compared with the strong effect of inter-estuarine differences on nitrogen isotope ratios, differences between reaches were relatively minor; a slight enrichment in the middle reaches was evident in all species except *V. georgii* (Table 2).

The isotope ratios are remarkably consistent with values obtained five years earlier for the same species (Table 2). This concurrence of inter-estuarine isotopic differences in fish indicates that nitrogen loads from sewage sources are a continuous pollution pressure in the Maroochy Estuary.

3.2. Fish pathology

3.2.1. Types of histopathologies observed

Fish had histopathological alterations in all organs except the eye. Gills exhibited lamellar fusion with associ-

Table 2
Comparison of nitrogen stable isotope ratios in fish muscle between the Maroochy and Noosa estuaries

	Maroochy $\delta^{15}N$ (‰) Mean ± (SE)	Noosa $\delta^{15}N$ (‰) Mean ± (SE)	Δ Maroochy–Noosa 2006[a]	2000/2001[b]
Ambassis jacksoniensis				
All reaches	15.34 ± (0.24)	9.61 ± (0.29)	Δ + 5.73	(Δ + 5.51)
Middle reaches	15.69 ± (0.35)	11.36 ± (0.27)	Δ + 4.33	(Δ + 6.60)
Upper reaches	14.99 ± (0.32)	9.26 ± (0.19)	Δ + 5.73	–
Gerres subfasciatus				
All reaches	16.10 ± (0.22)	11.12 ± (0.13)	Δ + 4.97	(Δ + 5.10)
Middle reaches	16.80 ± (0.24)	11.42 ± (0.15)	Δ + 5.38	(Δ + 5.24)
Upper reaches	15.39 ± (0.20)	10.82 ± (0.17)	Δ + 4.57	(Δ + 4.79)
Sillago ciliata				
All reaches	16.28 ± (0.16)	11.43 ± (0.11)	Δ + 4.85	(Δ + 5.39)
Middle reaches	16.72 ± (0.10)	11.43 ± (0.11)	Δ + 5.29	(Δ + 6.02)
Upper reaches	15.84 ± (0.23)	–	–	(Δ + 6.31)
Valamugil georgii				
All reaches	12.71 ± (0.33)	8.91 ± (0.15)	Δ + 3.80	–
Middle reaches	12.39 ± (0.41)	9.04 ± (0.16)	Δ + 3.35	–
Upper reaches	13.34 ± (0.48)	8.52 ± (0.27)	Δ + 4.82	–

Contrasts (Δ) refer to differences between means, with positive values denoting higher isotope ratios in fish from the Maroochy Estuary.
[a] This study.
[b] Schlacher et al. (2005).

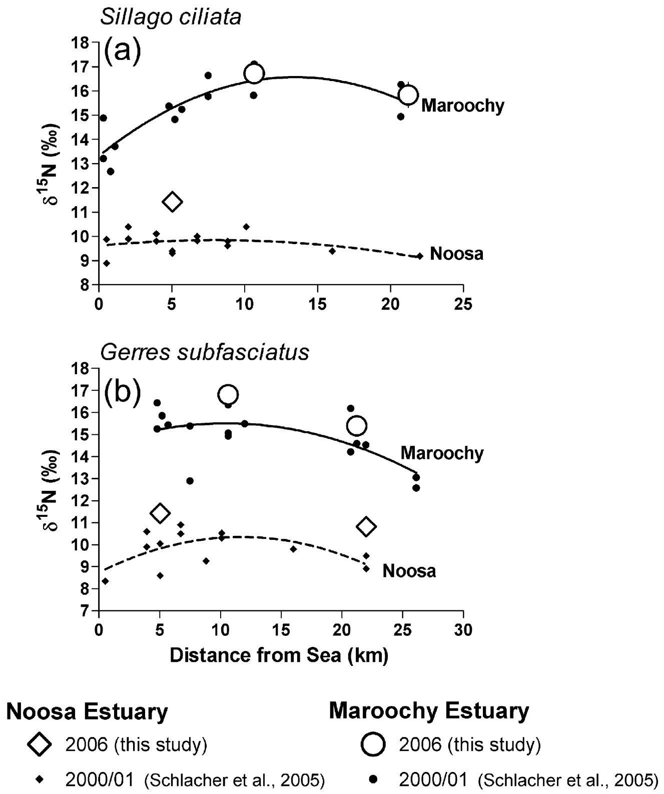

Fig. 3. Variation in nitrogen isotope ratios along the main axis of the estuary in two species of fish: (a) Summer Whiting, and (b) Silver Biddy. Small symbols are data from 2000 to 2001 (Schlacher et al., 2005) compared with data obtained in 2006 during the present study denoted by large symbols for each estuary (error bars for 2006 data are smaller than symbols).

ated epithelial hyperplasia or proliferation of hypertrophic cells, resulting in a corresponding increase in the size of the gill tissue (Fig. 4). Oedema with epithelial lifting, telangiectasia, granuloma and epitheliocystis of the gill epithelium was present in a number of individuals. External parasites were mostly limited to the gills, with no evidence of infestation of the skin or eyes. Focal inflammatory response with minimal epidermal loss was present in the skin tissue. With the exception of one individual (*V. georgii*) that showed evidence of anal fin erosion, fish appeared externally healthy without gross morphological alterations such as skin lesions.

The heart and skeletal muscle exhibited granulomas, inflammation and coagulative necrosis. Fibrosis and melanomacrophage aggregate proliferation were present only in the skeletal muscle. In some cases, the granuloma in both the heart and skeletal muscle was a clear immune response to endoparasites, but in many instances the cause of the granulomatous response was unclear. Loss of skeletal muscle with adipose tissue was extensive in some individuals.

Both the kidney and spleen exhibited a proliferation of melanomacrophage aggregates, granulomas and decoalising fat necrosis (Fig. 4). Vacuolation of kidney tubules resulting in breakdown of cellular structure, and hyaline droplet accumulation of protein in cells that caused tubule degeneration were also evident. The liver showed lipid accumulation (potentially lipoidosis), inflammation, cysts, coagulative necrosis, decoalising fat necrosis, melanomacrophage aggregate proliferation and granulomas.

Testes of male fish ranged from early stages of testicular development containing spermatocysts with spermatocytes through to fully developed testes containing spermatocytes at all stages of development. Ovotestis (intersex) was not evident in any male fish. In female fish, several stages of follicular development were evident, with two individuals exhibiting slightly abnormal follicle development.

Histological alterations varied between the fish species but, overall, the most prevalent alterations were recorded in the liver, spleen, gill, kidney and muscle tissues. The most common lesions in fish from both the Maroochy and Noosa estuaries were granulomas present in the liver, spleen, kidney, cardiac and skeletal muscle, and proliferation of melanomacrophage aggregates in the liver, spleen and kidney. An inflammatory response associated with granulomatous tissue was often associated with necrotic patches surrounding the granuloma, in addition to the development of caseous (necrotic) centres within some of the granulomas. Endoparasites eliciting granuloma

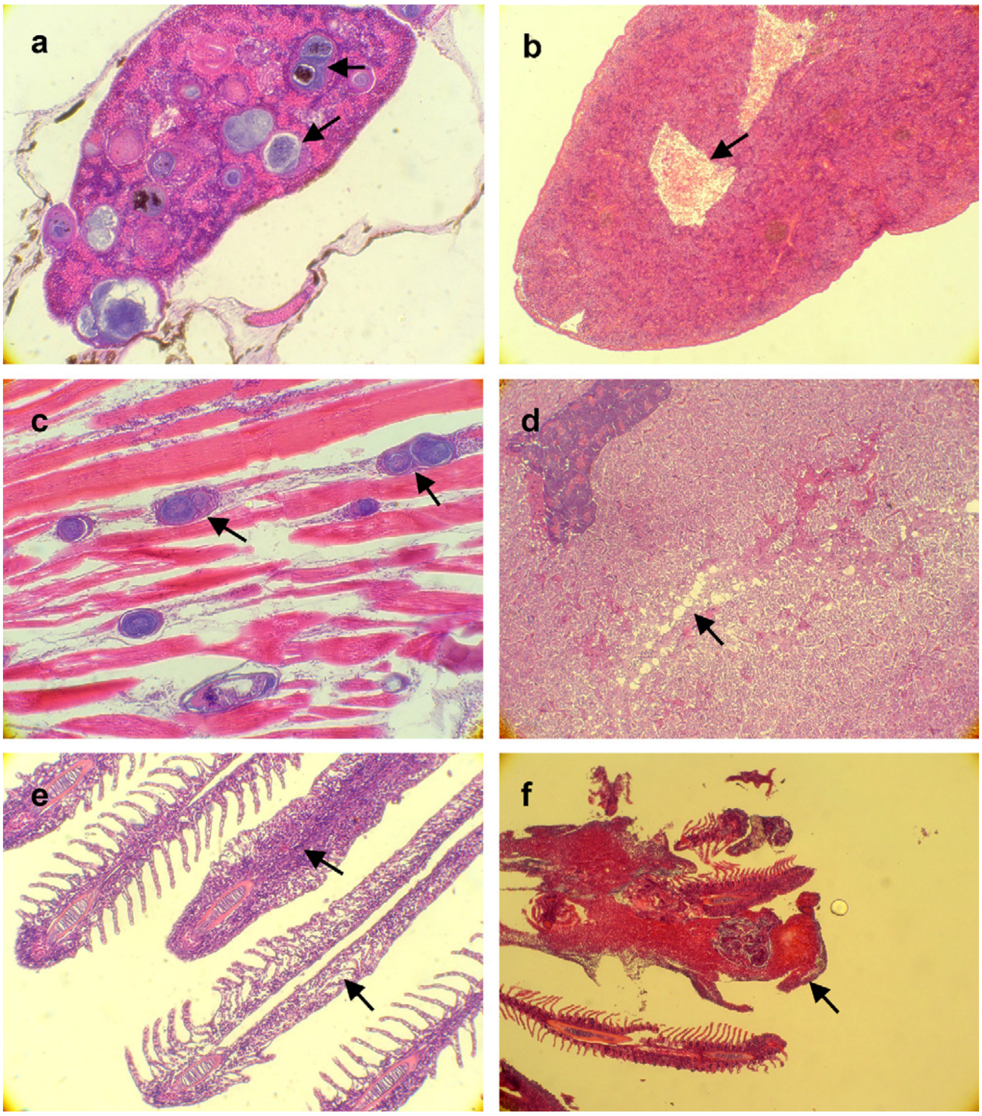

Fig. 4. Representative fish histopathologies: (a) multiple granulomatose lesions indicating extensive and prolonged pathology (*Ambassis jacksoniensis*, ×200); (b) coagulative necrosis and melanomacrophage aggregates in the spleen (*Valamugil georgii*, ×100); (c) multiple granulomata in skeletal muscle with associated inflammatory response (*Ambassis jacksoniensis*, ×200); (d) decoalising fat necrosis in liver tissue (*Sillago ciliata*, ×200); (e) lamellar fusion with associated epithelial hyperplasia (*Gerres subfaciatus*, ×200); (f) metaplasia of the distal gill lamellae (*Valamugil georgii*, ×100).

formation were more prevalent in fish from the Maroochy Estuary. Lamellar fusion in the gills associated with hyperplasia and the presence of granulomas and epitheliocystis was found in fish from both estuaries.

3.2.2. Prevalence of histopathologies

Of the 284 individuals examined, 194 (68%) showed pathologies. That is, only 32% of fish were healthy, without any signs of pathological tissue alterations. The proportion of healthy fish, however, varied markedly between estuaries (Table 3), with a significantly higher proportion of fish free of pathologies in the Noosa (39%) than in the Maroochy Estuary (27%). The prevalence of pathologies also varied among species (Table 3). The most heavily impaired species was *V. georgii*, with only a single specimen (out of 33) found to be free of pathologies in the Maroochy Estuary. By contrast, *A. jacksoniensis* had the lowest rates of pathologies with, for example, as high as 57% of individuals of this species free of pathologies in the Noosa Estuary (Table 3).

In those fish that showed any sign of pathology, individuals had on average between 1.6 and 3.3 tissue alterations (maximum 9). Significantly more alterations were recorded in the Maroochy Estuary (ANOVA, estuary effect: $F_{(1,186)} = 4.40$, $P = 0.037$). The largest contrast between estuaries in the mean number of pathologies per fish was recorded for *A. jacksoniensis* (Table 3), for which the number of pathologies was elevated by 65% in the Maroochy Estuary compared with the Noosa Estuary (mean 2.29 and 1.39, respectively). Similarly, *V. georgii* recorded on average a 43% higher number of pathologies per individual in the Maroochy Estuary (Table 3). The mean number of

Table 3
Comparison of fish pathology prevalence between fishes from the Maroochy and Noosa estuaries

Species	Estuary	*n*	Individuals free of pathologies (%)	No of pathological alterations per affected individual		No of organs affected	
				Mean ± (SE)	Max	Mean ± (SE)	Max
All species	Maroochy	172	27	2.43 ± (0.16)	9	1.76 ± (0.08)	5
	Noosa	112	39	1.90 ± (0.13)	5	1.53 ± (0.09)	4
Ambassis jacksoniensis	Maroochy	46	39	2.29 ± (0.37)	8	1.64 ± (0.16)	4
	Noosa	42	57	1.39 ± (0.14)	3	1.17 ± (0.09)	2
Gerres subfasciatus	Maroochy	45	27	1.97 ± (0.22)	5	1.48 ± (0.12)	3
	Noosa	43	37	2.33 ± (0.24)	5	1.81 ± (0.18)	4
Sillago ciliata	Maroochy	48	31	2.12 ± (0.30)	9	1.67 ± (0.18)	5
	Noosa	19	11	1.59 ± (0.19)	3	1.47 ± (0.17)	3
Valamugil georgii	Maroochy	33	3	3.34 ± (0.31)	7	2.25 ± (0.19)	4
	Noosa	8	23	2.33 ± (0.56)	4	1.50 ± (0.22)	2

pathologically-altered organs was also higher in fish from the Maroochy (1.76) than the Noosa Estuary (1.53; ANOVA, estuary effect: $F_{(1,186)} = 3.65$, $P = 0.057$). The increase in the mean number of organs affected in fish from the Maroochy was highest for *V. georgii* (+50%), followed by *A. jacksoniensis* (+40%).

Multivariate analysis of the entire suite of histopathology indicators showed that the main difference in fish health was between estuaries, with a secondary effect of reaches within estuaries. The contrast between estuaries was especially pronounced for *V. georgii* and *S. ciliata* (Fig. 5), but was weaker for *G. subfasciatus* (Fig. 5). *A.*

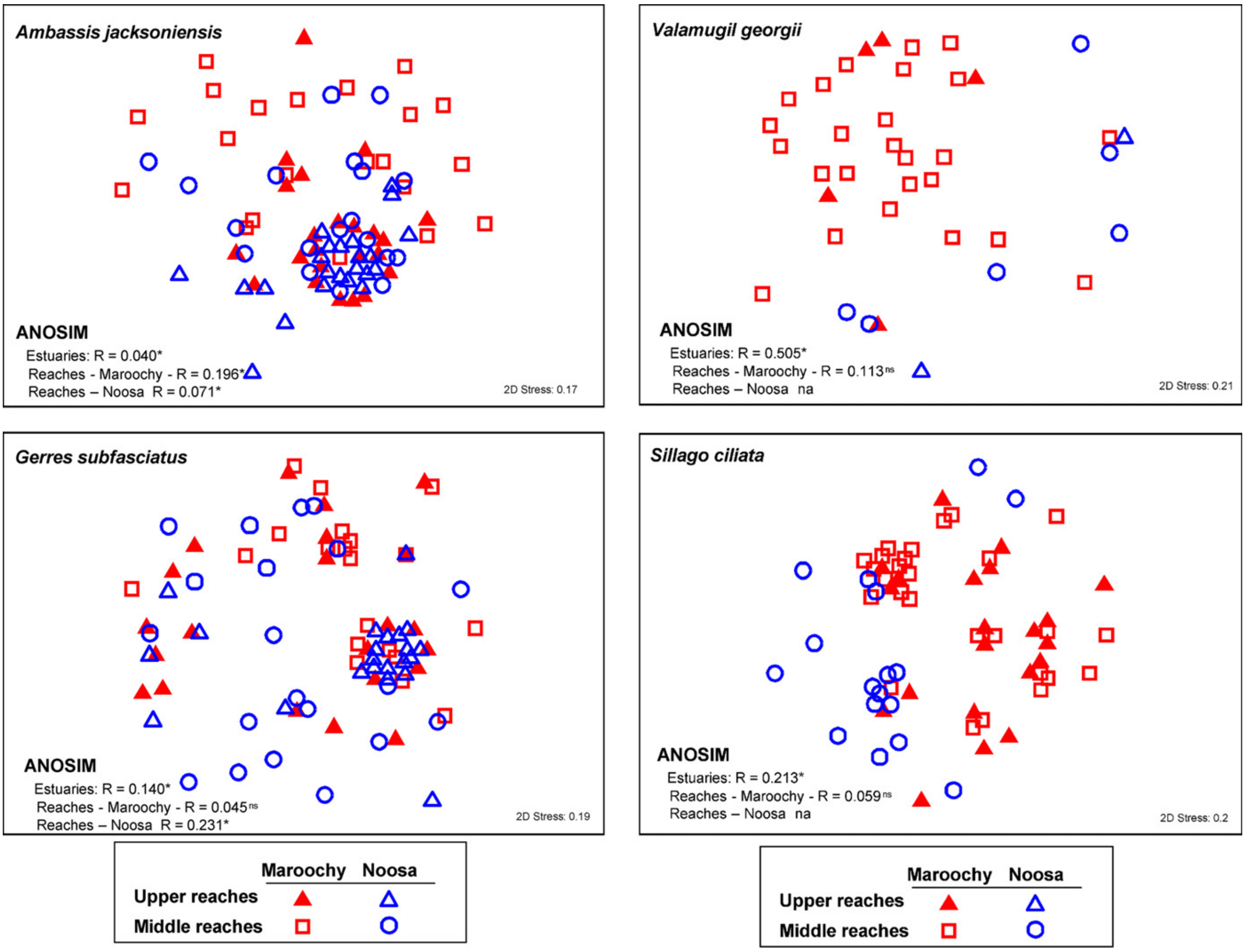

Fig. 5. Ordinations (non-metric multidimensional scaling) of fishes based on the presence of histological pathologies. Each symbol represents a single individual, with the resemblance between individuals based on Bray–Curtis dissimilarity coefficients calculated from presence/absence data over the full suite of 22 pathologies examined.

Table 4
Prevalence of pathological tissue changes in *Ambassis jacksoniensis* and *Gerres subfasciatus* from the middle (Mid) and upper (Up) reaches of the Maroochy and Noosa Estuaries (tabulated values are the percentage of individuals affected)

	Ambassis jacksoniensis						*Gerres subfasciatus*					
	Maroochy			Noosa			Maroochy			Noosa		
	Mid	Up	Tot	Mid	Up	Tot	Mid	Up	Tot	Mid	Up	Tot
Sample size (# of fish)	23	23	46	19	23	42	23	22	45	21	22	43
Gill												
Lamella fusion	4	4	4	4	4	4	4	32	18	10	18	14
Cell hyperplasia	0	4	2	0	0	0	0	5	2	5	0	2
Telangiectasis–epitheliocystis	17	0	9	17	0	9	0	5	2	0	5	2
Epithelial lifting	0	0	0	0	0	0	4	18	11	10	0	5
Granuloma	0	0	0	0	4	2	0	0	0	5	0	2
Cellular proliferation	4	4	4	9	0	4	9	27	18	19	18	19
UFO	0	0	0	0	4	2	0	5	2	10	0	5
Total	22	4	13	17	9	13	9	41	24	33	23	28
Liver												
Lipid retention	17	0	9	0	0	0	17	18	18	10	9	9
Necrosis	13	4	9	4	0	2	39	27	33	33	0	16
Granuloma	4	0	2	0	4	2	4	5	4	5	0	2
Melanomacrophage aggregates	9	0	4	0	0	0	0	0	0	10	5	7
Total	17	4	11	4	4	4	57	41	49	43	14	28
Kidney												
Tubule vacuolation	26	9	17	17	0	9	4	9	7	19	5	12
Melanomacrophage aggregates	26	4	15	4	9	7	0	0	0	5	9	7
Granuloma	4	0	2	0	0	0	0	0	0	0	0	0
Total	43	9	26	22	9	15	4	9	7	19	14	16
Spleen												
Melanomacrophage aggregates	9	4	7	0	9	4	0	18	9	48	9	28
Necrosis	4	0	2	4	0	2	0	0	0	5	0	2
Granuloma	4	0	2	0	4	2	0	0	0	0	0	0
Total	9	4	7	4	13	9	0	18	9	48	9	28
Muscle												
Muscle granuloma	26	17	22	4	0	2	5	0	2	5	5	5
Muscle necrosis	26	0	13	0	0	0	17	9	13	0	0	0
Total	39	17	28	4	0	2	22	9	16	5	5	5
Heart – granuloma (muscle)	0	0	0	0	0	0	0	0	0	19	0	9
Skin – inflammation	22	4	13	0	4	2	9	0	4	0	0	0

jacksoniensis showed a different pattern of variation, with fish from the middle reaches of the Maroochy showing a distinctly different spectrum of pathologies to fish from all other areas (Fig. 5).

The patterns based on the entire suite of histopathological indicators were largely driven by conspicuous differences in the incidence rates of pathology in certain organs. Liver pathologies were significantly more frequent in fish from the Maroochy for three species (Tables 4 and 5, Fig. 6). The incidence of pathology was also higher in the Maroochy for muscle and skin of *A. jacksoniensis*. Conversely, the rate of pathology was higher in fish from the Noosa Estuary for spleens of two species (*S. ciliata, G. subfasciatus*) and hearts of *S. ciliata*. The site effect detected for *A. jacksoniensis* using the full suite of indicators was mainly due to much higher rates of pathologies in the livers, muscle and skin of fish from the middle reaches of the Maroochy Estuary (Fig. 7). Lipid retention in the liver was more frequent in all species from the Maroochy than the Noosa Estuary, with *V. georgii* recording the highest prevalence of lipid retention overall (Table 5). Similarly, the prevalence of necrosis in the liver tissue was higher in all species from the Maroochy Estuary compared to the Noosa. Again, liver necrosis was highest in *V. georgii*, and individuals of this species from the Maroochy Estuary also consistently recorded the highest prevalence of granuloma and melanomacrophage aggregates in the liver.

Table 5
Prevalence of pathological tissue changes in *Valamugil georgii* and *Sillago ciliata* from the middle (Mid) and upper (Up) reaches of the Maroochy and Noosa Estuaries (tabulated values are the percentage of individuals affected)

	Valamugil georgii						*Sillago ciliata*					
	Maroochy			Noosa			Maroochy			Noosa		
	Mid	Up	Tot	Mid	Up	Tot	Mid	Up	Tot	Mid	Up	Tot
Sample size (# of fish)	28	5	33	6	2	8	27	21	48	19	0	19
Gill												
Lamella fusion	7	20	9	33	50	38	4	5	4	5		5
Cell hyerplasia	11	20	12	17	0	13	4	10	6	0		0
Telangiectasis–epitheliocystis	14	20	15	17	0	13	7	29	17	11		11
Epithelial lifting	11	0	9	0	0	0	4	0	2	0		0
Granuloma	4	0	3	0	0	0	0	0	0	0		0
Cellular proliferation	18	40	21	17	50	25	15	10	13	11		11
UFO	14	0	12	17	0	13	4	5	4	0		0
Total	43	40	42	33	50	38	22	38	29	16		16
Liver												
Lipid retention	32	60	36	0	0	0	15	33	23	5		5
Necrosis	79	80	79	0	0	0	37	43	40	0		0
Granuloma	32	0	27	0	0	0	4	0	2	5		5
Melanomacrophage aggregates	0	40	6	17	0	13	0	0	0	11		11
Total	86	80	85	17	0	13	44	62	52	21		21
Kidney												
Tubule vacuolation	9	0	7	0	0	0	0	0	0	0		0
Melanomacrophage aggregates	0	0	0	0	0	0	0	0	0	0		0
Granuloma	0	0	0	0	0	0	0	0	0	0		0
Total	7	0	6	0	0	0	0	0	0	0		0
Spleen												
Melanomacrophage aggregates	57	40	55	50	0	38	11	29	19	53		53
Necrosis	4	20	6	17	0	13	0	0	0	0		0
Granuloma	0	0	0	0	0	0	0	0	0	0		0
Total	57	60	58	67	0	50	11	29	19	53		53
Muscle												
Muscle granuloma	0	20	3	0	0	0	4	0	2	5		5
Muscle necrosis	14	20	15	0	0	0	7	5	6	5		5
Total	14	40	18	0	0	0	7	5	6	11		11
Heart – granuloma (muscle)	4	20	6	17	0	13	4	5	4	32		32
Skin – inflammation	4	0	3	0	0	0	4	0	2	0		0

4. Discussion

4.1. Effects of sewage on fish

This study provides evidence that fish health is poorer in the Maroochy than in the Noosa Estuary. While the prevalence of pathologies in fish varied among species and from site to site, the comparison of fish from the Maroochy and Noosa estuaries demonstrated a much higher incidence of pathologies in the Maroochy Estuary overall. This is consistent with a demonstration of higher pathology rates of fish exposed to sewage in oceanic waters (Nowak, 1996). The effect of sewage in the present study was detectable using an analysis of the entire suite of pathologies. More detailed analysis of pathologies manifested in particular organs confirmed the major difference between the estuaries, and also shed light on the health susceptibility of different fish species to pollutants in the estuaries. Mullet, for example, had high rates of pathologies across all sites, but with much higher rates at both the middle and upper reaches of the Maroochy Estuary. The effects on this species were evident predominantly in organs related to feeding (e.g. liver), reflecting the primary pathway for toxicant transfer to this species via assimilation of microscopic organisms from the sediment.

A secondary difference was detected within the Maroochy Estuary, where a higher incidence of pathology was recorded in the middle than in the upper reaches. This

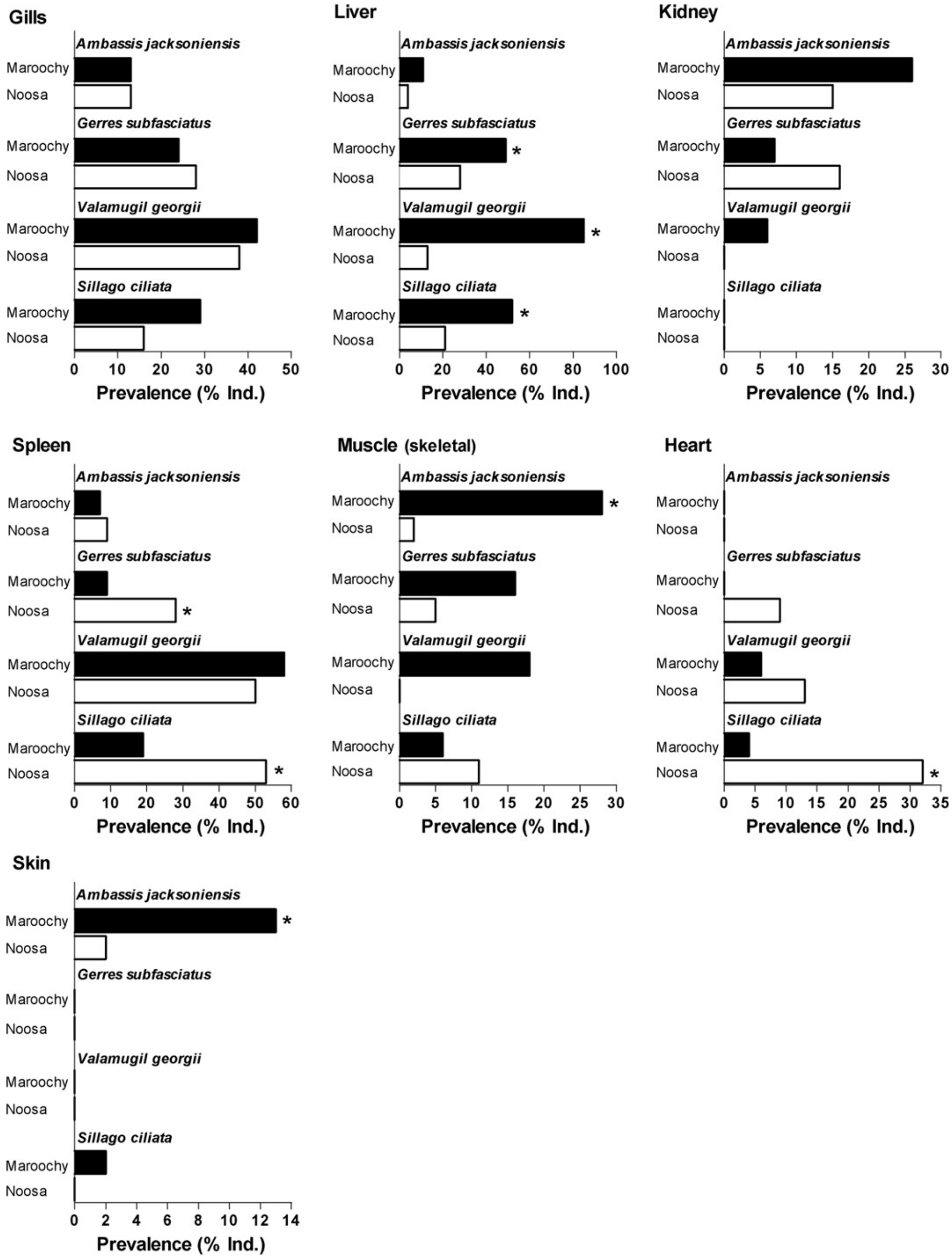

Fig. 6. Pathological prevalence in major organs in fish from the Maroochy (filled bars) and Noosa Estuary (open bars; asterisks denote significantly, $P < 0.05$, higher rates).

pattern was evident in several species, but was shown most clearly by glassfish, which suffered high rates of pathologies in several organs (liver, muscle tissue and skin) in the middle reaches.

Nitrogen stable isotope results, in conjunction with previous surveys, demonstrated a long-term pattern of assimilation of sewage-N by fish sourced from inputs of treated wastewater effluent into the middle reaches of the Maroochy Estuary. The pattern of pathologies in fish matched closely the extent of sewage pollution which, whilst most obvious in the middle reaches, is also dispersed by tidal currents to some extent upstream.

4.2. Histopathology as an indicator of fish health

Pathological alterations in fish are the net result of adverse biochemical and physiological changes within the organism. Histopathologies are clear symptoms of in situ exposure to pollution in the form of structural alterations (Hinton and Lauren, 1990). Such pathological changes in

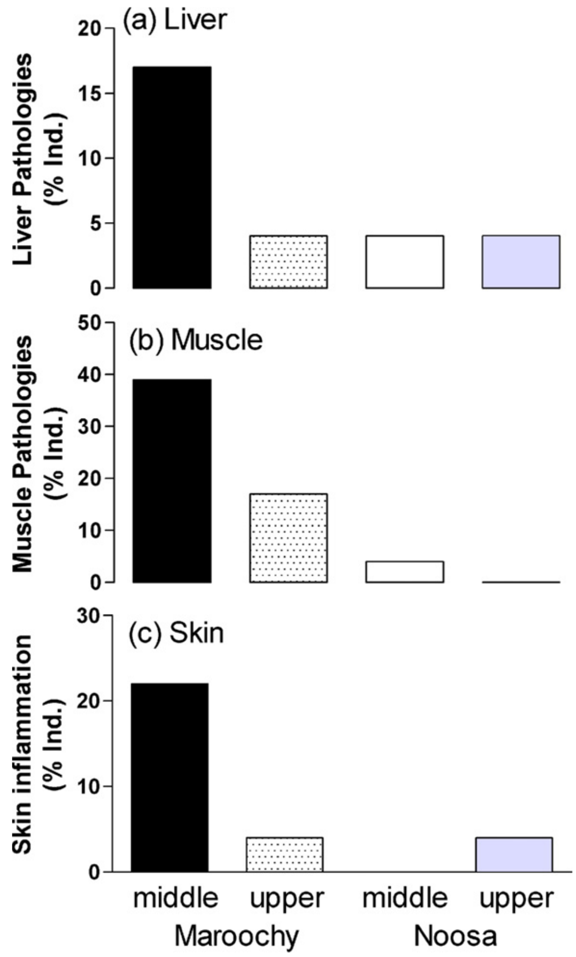

Fig. 7. Site-to-site differences in the prevalence of liver (a), skeletal muscle (b), and skin pathologies (c) in *Ambassis jacksoniensis*.

histological structure can substantially impair the function of tissues and organs in fish (Couch and Fournie, 1993), and therefore present a measure of an organism's health status. Histology can also detect health effects that are not readily discernable with gross visual inspection and provide early warning signals for secondary diseases (Couillard et al., 1988).

A practical advantage of using fish histopathology in environmental assessments is that multiple organs can be examined; this increases the sensitivity at which pollution impacts can be detected. Whilst some histological changes represent a ''non-specific'' stress response, certain structural abnormalities have been attributed to specific toxicants (Hylland et al., 2003).

Specific classes of toxicants have been linked to a number of histological alterations in gills. Fish exposed to sewage have more frequent and severe epitheliocystis (Nowak and LaPatra, 2006). Many toxicants (e.g. hydrocarbons, organochlorines, ammonia, and tributyl tin) cause a wide range of gill pathologies that include telangiectasis and lamellar fusion, epithelial hyperplasia, hypertrophy of chloride and mucus cells, and hyperplasia, as well as higher infestation rates with ectoparasites (DiMichelle and Taylor, 1978; Skinner and Kandrashoff, 1988; Glazebrook, 1990; Nowak, 1992; Mondon et al., 2001).

The liver is particularly susceptible to damage from a variety of toxicants. Because it is a major storage site of lipids in fish, liver metabolism is a potential target for the toxic action of chemicals (Hinton et al., 2001). Fish in urban and industrialised areas are frequently affected by liver and kidney damage, particularly those exposed to contaminated sediments and prey organisms (Mondon et al., 2001; Hansen et al., 2004).

Benthic fish exposed to complex metal and organic contaminants in the sediments are susceptible to skin disorders such as epidermal loss (Mondon et al., 2001), leaving fish vulnerable to ulceration and loss of osmoregulation capability. Additionally, the link between polluted sediment and the presence of cysts in bottom dwelling fish has been established across a number of studies (Nowak, 1996).

We found histopathological alterations across a number of organs in each fish species, indicating that fish health is impaired in these estuaries. These pathological responses of fish can be linked to sewage pollution based on the concordance between the higher number of pathologies and elevated $\delta^{15}N$ levels in fish from the estuary subjected to direct sewage inputs. Histological alterations were particularly prevalent in the bottom-feeding mullet *V. georgii*, corroborating several studies that report more pathologies in bottom-feeding species (Skinner and Kandrashoff, 1988) due to prolonged contact with contaminated sediment and benthic prey items (Mondon et al., 2001).

Fish that have histological alterations are clearly unhealthy. Structural abnormalities can result in the suppression or inhibition of physiological function, irrespective of whether the pathologies are caused by chemical, physical or secondary parasitic irritation. In this study, fish exhibited multifocal pathologies in the gills, liver, kidney, heart, spleen and skeletal muscle tissue, indicating a sublethal change and potential reduction in the functional efficiency of these organs. The wide range of sublethal changes detected across multiple organs and tissue types indicates that multiple contaminants or agents capable of causing tissue alterations are likely to be present as pollutants.

Variability in pathological tissue changes among and within species may partly be caused by differences in the actual levels of contaminants in fish tissues. We measured stable nitrogen isotopes as a surrogate for the potential bioavailability of other sewage-borne contaminants and found a good match between isotopic enrichment and the prevalence of histopathologies. Data on the concentrations of pollutants from sewage and other sources in the estuaries would be helpful to locate specific effects, but such toxicant data are generally not available for the study region.

High levels of contaminants can lead to acute and drastic responses in fish that are readily visible as 'fish kills' in extreme situations. In the present situation, contamination appears to be chronic and sufficiently high to cause sublethal alterations in the fish. We could not have detected such effects without the use of histopathological markers, stressing the importance of employing suitable bio-indicators before irreversible and drastic ecological impacts occur.

This study measured fish health at a single time. Long-term or seasonal comparative data are not available for any estuary in the region. The histopathological data nevertheless indicate a correlation between impaired health and increased uptake of sewage matter (i.e. elevated stable nitrogen isotopes) in estuarine fish.

4.3. Fish as indicators of estuarine health

The application of fishes as biological indicators of ecological condition in estuaries is based on the sensitivity of fishes to changes in water quality, habitat availability and quality, and the intensity of fishery exploitation (Karr, 1981; Leamon et al., 2000; Whitfield and Elliott, 2002; Harrison and Whitfield, 2004; Breine et al., 2007). For example, the cascading effects of harvesting on estuarine fish communities are well known (Whitfield and Elliott, 2002). Population and community dynamics of fishes are, however, strongly driven by fish health that must be adequate to fulfil key functions such as reproduction, recruitment, and growth. Thus, impaired fish health is likely to result in ecological effects beyond the individual organisms.

Our data demonstrate that fish health is impaired in the Maroochy Estuary, and provide the link between poor fish health and increased assimilation of sewage-derived nitrogen. It is likely that changes in individual health manifest themselves at higher levels of ecological organisation, leading to reduced abundance, production and recruitment of fish in the Maroochy Estuary. Recreational fishing is very popular in both estuaries (O'Neill, 2000), and reductions in individual fish health that lead to declines in fish stocks would have considerable socio-economic costs. The overall impact of poor fish health on fish communities has not been measured, and future fish health monitoring would benefit from complementary stock assessments to determine the community-wide response of fish to human perturbations (Harrison and Whitfield, 2004; Breine et al., 2007).

The use of fish as biological indicators of estuarine conditions has several key advantages (reviewed by Whitfield and Elliott, 2002). First, because physical and chemical attributes of the water column (water quality) may not necessarily be an adequate surrogate measure for ecological responses (Karr and Dudley, 1981; Oberdorff and Hughes, 1992; Leamon et al., 2000), direct measurements of aquatic biota such as fish can provide a more sensitive assessment of the link between human activities and their ecological consequences (Whitfield and Elliott, 2002).

Second, many fish are relatively long-lived with slow tissue turnover rates, and can thus integrate pollution signals over longer periods (Hesslein et al., 1993; Schlacher et al., 2005). By contrast, standard physico-chemical variables of the water column, and measurements on small plants and invertebrates, typically show considerably higher temporal variance when used as biological indicators (Cabana and Rasmussen, 1996).

Third, fish encompass a diversity of trophic roles and can therefore signal pollution at different levels of trophic organisation. Detection of elevated $\delta^{15}N$ (this study) or other pollution signals in fish demonstrates movement of pollutants through the estuarine food web, whereas plants can generally indicate only lower-order effects (Costanzo et al., 2001; Cole et al., 2004).

Fourth, fish provide multiple lines of evidence for pollution assessments – an important aspect in politically sensitive applications such as this one. These multiple lines of evidence can be derived through a combination of (a) using several fish species (or entire communities), and (b) measuring a variety of end-points in each specimen; these end-points can encompass a wide range of biomarkers of pollution (reviewed by van der Oost et al., 2003). In the present study, we successfully demonstrated the simultaneous application of stable nitrogen ratios to test for the assimilation of sewage-N and linked these with impairments of fish health.

Finally, the ultimate goal of any environmental assessment is the development and implementation of an effective management response that mitigates the anthropogenic impacts. This is most successful when supported by the public. Given the high public profile that fish enjoy, the general public is much more likely to respond positively to environmental initiatives based on fish data than those derived from other markers of pollution. Fish also provide an important recreational and commercial resource, and declines in fish stocks have direct socio-economic costs.

5. Conclusions

Estuarine water quality is receiving an ever-increasing focus from all levels of government as a significant coastal management issue. Monitoring does already provide a large data set on the physico-chemical conditions of estuaries. However, the extent to which those physico-chemical aspects translate into effects on the health of animals of interest to the general public, or on overall ecosystem health, has not been addressed. Our results support the level of concern being shown over point-source input of treated sewage into estuaries. The consequences of poor fish health for human consumption of fish and for higher level ecosystem processes have not been measured, and these matters should be addressed in future studies. It is clear, nonetheless, that fish are useful as higher level indicators of estuarine health, and that a subset of the fish species and histological indicators used in this study could usefully be included in routine estuarine monitoring.

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