

RESEARCH ARTICLE

Use of liver histological alterations and erythrocytic nuclear abnormalities of two native fish species in Kelani River, Sri Lanka as biomarkers for pollution impact assessments

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Abstract: Multi-biomarker approach is recognised as a complementary tool for environmental monitoring studies to better appraise how pollutants affect ecosystems. This study examined histological alterations in the liver tissues and nuclear abnormalities in the peripheral erythrocytes of two native fish species inhabiting the Kelani River (*Etrophus suratensis* and *Dawkinsia singhala*) as 'effect biomarkers' for assessing pollution impacts. Surface water and native fish were sampled from two polluted sites in the lower reach (Kaduwela and Mattakkuliya) and a less polluted site in the upper reach (Ruwanwella) of the river covering rainy and dry periods. Physico-chemical analyses of surface water confirmed an increasing trend of pollution towards the lower reach of the river. Significantly greater liver histopathologic condition indices and erythrocytic nuclear abnormality frequencies ($p < 0.05$) were found in the fish inhabiting lower reaches of the river compared to those in the upper catchment at Ruwanwella. Biomarker responses revealed that the fish populations inhabiting the polluted sites in the river are under stress especially due to hepatic damage and genotoxicity. The results suggest that endemic and nationally threatened fish species in the riverine ecosystem may be at risk due to the contaminant stress under long term exposure. This study supports utility of erythrocyte nuclear abnormality and liver histopathological biomarker responses of native fish as cost effective tools for the identification of potential biological hazards of river pollution.

Keywords: Biomarker, *Dawkinsia singhala*, *Etrophus suratensis*, Kelani River, pollution impact assessment.

INTRODUCTION

River pollution could produce unintentional irreversible damage to the resident biota and reduce the resource values of the rivers (Pan *et al.*, 2016). Kelani River, which is one of the largest water sheds in Sri Lanka, is increasingly being polluted due to urban, agricultural and industrial activities. The western region of the river, which passes through highly urbanised areas is becoming mostly polluted with industrial and urban waste. A recent study carried out to assess the surface water quality in Kelani River revealed that chemical oxygen demand levels in 90 % of the water samples were higher than the SLS drinking water quality standards (Mahagamage *et al.*, 2014). The importance of monitoring biological impacts associated with river pollution has been stressed in the proposed action plan for management and conservation of the Kelani River basin (Mallawatantri *et al.*, 2016).

Conventional river pollution monitoring approach focuses on a selected set of physico-chemical factors and pollutant levels. However, this approach does not completely provide information on the ecological conditions of the biota inhabiting the river (Colin *et al.*, 2016). For assessing biological impacts of aquatic pollution, fish biomarker assessment is a promising

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approach as the biomarkers may possibly reflect the interactive effects of the mixture of pollutants on impaired biological processes in the exposed organisms (van der Oost *et al.*, 2003). Biomarkers are defined as changes in biological (molecular, cellular, physiological or behavioural) responses, which can be related to exposure to or toxic effects of chemicals in the environment (Peakall, 1994). Multi-biomarker approach in fish is recognised as a complementary tool for environmental monitoring studies to better appraise how pollutants affect aquatic ecosystems (Pathiratne *et al.*, 2008; Ghisi *et al.*, 2016; Vieira *et al.*, 2017).

The liver which is a target organ of many toxicants, is one of the most intensively used organs in aquatic toxicological studies (Wolf & Wheeler, 2018). Liver histological assessments provide information pertinent to the health impacts associated with aquatic contaminants in various fish species (Wolf *et al.*, 2015; Kumar *et al.*, 2017). Genotoxicity studies are important in assessing the genetic integrity of fish exposed to various toxicants in their habitat (van der Oost *et al.*, 2003; Matos *et al.*, 2017; Sousa *et al.*, 2017). Erythrocyte nuclear abnormalities in fish indicate cytogenetic damage associated with the genotoxic effects of aquatic pollutants (Al-Sabti & Metcalfe, 1995; van der Oost *et al.*, 2003; Braham *et al.*, 2017). Genotoxic potential of a range of industrial effluents that are discharged into the Kelani River has been revealed recently based on laboratory exposure studies with a model fish, Nile tilapia, *Oreochromis niloticus* (Hemachandra & Pathiratne, 2016; 2017). More recently, Ruvinda and Pathiratne (2018) reported toxic impacts of selected effluent receiving canals in Kelani River using biomarker responses of Nile tilapia under laboratory exposure. However, scientific evidence is not available on the health of native organisms in Kelani River, which includes endemic and nationally threatened fish species. Use of the biomarker responses in native fish, which are exposed naturally to pollution loads in the riverine ecosystem over long periods is ideal for pollution impact assessments.

Etroplus suratensis (Family: Cichlidae) and *Dawkinsia singhala* (Family: Cyprinidae) are benthopelagic and omnivorous fish species found in tropical riverine ecosystems. From the conservation point of view, they are considered as 'least concern' by IUCN (2012). It is hypothesised that liver histological alterations and erythrocytic nuclear abnormalities of *E. suratensis* and *D. singhala*, inhabiting Kelani River could be used as warning signals to reflect pollution impacts associated with their habitat. The objective of this study was to assess pollution impacts of Kelani

River, by associating surface water quality with liver histopathological and erythrocyte nuclear abnormality responses in two native fish, *E. suratensis* and *D. singhala* inhabiting the river, with a view to assessing the use of these responses as effective biomarkers.

METHODOLOGY

Sampling sites

Three sampling sites (Figure 1) towards the western region of the Kelani River were selected primarily based on urban and industrial waste inputs. Site 1 (07° 2' 38.8" N, 80° 15' 6.4" E at Ruwanwella) is located towards the upper catchment which is a less urbanised area. Site 2 (06° 56' 34.2" N, 79° 59' 28.0" E at Kaduwela) and site 3 (06° 58' 53.2" N, 79° 52' 30.3" E at Mattakkuliya, Colombo) are located in the lower reaches of the river. Site 2 is a moderately urbanised and industrialised area. Site 3 passes through a highly urbanised and industrialised area.

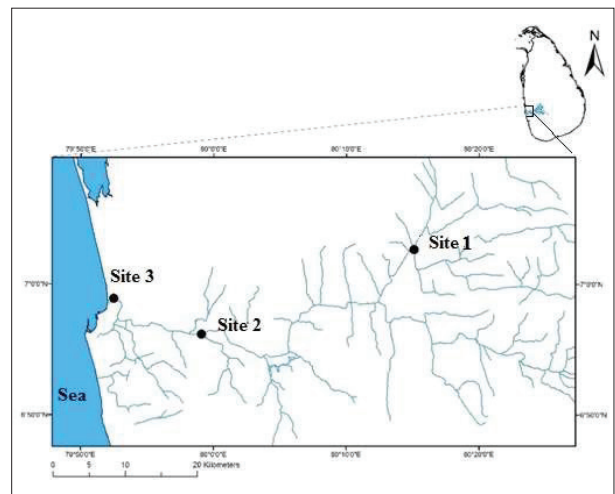


Figure 1: Location of sampling sites in the western region of Kelani River basin, Sri Lanka: Site 1 (Ruwanwella), Site 2 (Kaduwela) and Site 3 (Mattakkuliya, Colombo)

Sampling and analysis of river water

River water was sampled three times covering two dry periods (2014 February and 2015 February) and one rainy period (2015 July). In each sampling phase, river water from three randomly selected sub-sites at each site was collected and physico-chemically characterised. *In-situ* parameters *viz.* surface water temperature, pH,

conductivity, salinity and total dissolved solids were measured using water quality checker (MPS-556: Yellow Springs Instrument Company, USA). Dissolved oxygen, biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), total phosphate and nitrate nitrogen levels in surface water were determined in the laboratory as described in APHA (1998) using standard methods (5210 B for BOD₅; 5220 B for COD; 4500-P B.5 and 4500-P E for total phosphate; 4500 NO₃E for nitrate). Water samples (1 L) which were collected into acid washed polypropylene bottles, were preserved with ultrapure grade nitric acid (pH < 2) and stored at 4 °C until further analysis. The water samples were analysed within one month for commonly found four heavy metals (Cd, Cr, Cu and Pb) by Atomic Absorption Spectrophotometry (graphite furnace mode) (Analytik Jena: Model novAA400P) following APHA (1998), using standard methods (Method 3500 relevant to Cd, Cr, Cu and Pb). Standard solutions for calibrations were prepared using commercial standard reference solutions (Reagecon Diagnostics Limited, Ireland) for each metal separately. Deionised water was used as the blanks. For estimating detection limits, ten sample blanks were measured along with the samples for each metal. The limit of quantification (LOQ) was estimated by taking the sum of the mean and 10 standard deviations of sample blanks (Mikkelsen & Corton, 2004).

Fish collection

With the aid of fishermen, the fish were captured from three selected sites in the river using cast nets for biomarker studies. Capturing fish using the cast net was a difficult task due to the large volume of water and heavy water flow at the study sites. During the three sampling events, *E. suratensis* could be captured only from site 1 (n = 6 in the sampling event in 2014 dry period, n = 4 in the sampling event in 2015 dry period and n = 5 in the sampling event in 2015 rainy period) and site 3 (n = 6, 13 and 10 in the sampling events in 2014 dry period, 2015 dry period and 2015 rainy period, respectively). *D. singhala* could be captured only during two sampling events each, at site 1 (n = 6 and 10 in the 2015 dry period and rainy period, respectively) and site 2 (n = 3 and 12 in the 2015 dry period and rainy period, respectively). All fish captured during a specific sampling event were used for biomarker assessments. All applicable international ethical guidelines for the care and use of animals were followed during this study. The fish were transported to the laboratory in polythene bags filled with large volumes of water collected from the habitat from which they were captured. The water in the polythene bags was provided with sufficient oxygen during transport to reduce stress.

On the same day, the fish were anaesthetised with an overdose of benzocaine (70 mg L⁻¹) in the laboratory and body weight, total length and gender of each fish were recorded. Blood and liver tissue samples were taken from each fish for biomarker assessments. Condition factor of each fish was calculated using the formula, $100 \times \text{body weight (g)} / \text{length (cm)}^3$ (Bagenal & Tesch 1978, cited in van der Oost *et al.*, 2003).

Assessment of erythrocytic nuclear abnormality

Blood smears from each of the captured fish were prepared by severing the caudal peduncle and was processed for assessment of erythrocyte nuclear abnormalities. Blood smears were air-dried, fixed with methanol and stained with 5 % Giemsa stain. Excess stain was washed with 50 % methanol and nuclear abnormalities in at least 1000 mature erythrocytes per fish were determined under a binocular light microscope at 1000× magnification (Al-Sabti & Metcalfe, 1995). The blind scoring of nuclear abnormalities was performed on coded slides (one slide for each fish). The results are expressed as number of erythrocyte nuclear abnormalities per 1000 erythrocytes (‰).

Assessment of liver histological structure

Small pieces of liver tissue of each captured fish were fixed by placing in 10 % neutral buffered formalin for histopathology. Preserved liver tissues were processed using the standard paraffin embedding technique. The sections were cut at 5 µm thickness and stained with haematoxylin and eosin using standard protocol. The stained liver sections were examined for histopathological alterations following the guide given by Wolf *et al.* (2015). Frequency of histological alterations was expressed by dividing the number of fish on which the change was found by the number of fish analysed.

For assessment of aquatic pollution impacts, the 'liver histopathologic condition indices' based on 'specific reaction indices' were also estimated per individual fish following the standardised histopathological assessment method described by Bernet *et al.* (1999). In this method, biological significance of the histological changes of fish is assessed based on two factors: (i) rating the extension of a pathological change with a score value and (ii) defining the pathological importance of this alteration as an importance factor (Bernet *et al.*, 1999). As progressive changes and tumours were not observed in the livers of the examined fish, identified histopathological alterations in the liver of each fish were categorised into three reaction patterns, namely,

circulatory disturbances, regressive alterations and inflammatory responses. Pathological conditions of blood flow are classified within circulatory disturbances while regressive alterations are defined as processes, which lead to reduction in function or loss of an organ. Inflammatory responses are frequently coupled with processes belonging to other reaction patterns (Bernet *et al.*, 1999).

An importance factor (W) ranging from 1 to 3, which reflects the ability of the histological alteration to become reversible after the removal of the stressor (1: easily reversible; 2: reversible in most cases; 3: generally irreversible) was attributed to each histopathological alteration as described by Bernet *et al.* (1999) and Kostic *et al.* (2017). The identified histological alterations under the circulatory disturbances reaction pattern category, and the W values given for each alteration were as follows: blood congestion in the sinusoids (W = 1) and stasis in small veins (W = 1). For regressive alterations reaction pattern category, the identified histological changes and corresponding W values were as follows: architectural changes such as tissue structure, cell shape and arrangements (W = 1), plasma alterations such as hyaline/colloid droplets/deposits (W = 1), vacuolar degeneration (W = 2), nuclear alterations such as pycnotic nuclei (W = 2) and necrosis (W = 3). For the regressive alterations relevant W values were assigned separately for hepatic tissue, intrahepatic pancreatic tissue and bile duct. The W values assigned for the inflammatory response reaction pattern category are as follows: activation of reticuloendothelial system/presence of melanomacrophage centres (W = 1) and leucocyte infiltration (W = 2). In an individual fish liver, every histopathological alteration relevant to a particular reaction pattern category was assessed using a score value (*a*) ranging from 0 to 6, depending on the degree and the extent of the alteration, 0: unchanged; 2: mild occurrence; 4: moderate occurrence; and 6: severe occurrence. Intermediate values are also given depending on the alteration (Bernet *et al.*, 1999).

For each fish, one coded slide with liver sections was used for analysis. Ten randomly selected areas (based on the systematic up and down movement of the slide) of a well stained liver section were analysed under a light microscope. For each reaction pattern category, reaction index (RI) was calculated for each fish using the following equation:

$$RI = \sum_i^n W_i * a_i$$

where, *RI* = reaction index for a specific reaction pattern category, W_i = importance factor for a histopathologic alteration (*i*) relevant to the reaction pattern category, a_i = score value for the histopathologic alteration (*i*) relevant to the reaction pattern category and *n* = total number of histopathologic alterations analysed in the respective reaction pattern category.

After calculating reaction indices separately for the three categories of reaction patterns identified in the fish liver section, the total liver histopathologic condition index was estimated by the sum of the histopathologic reaction indices for the observed reaction patterns of the liver per individual fish.

$$HI_{Liver} = RI_{CD} + RI_{RA} + RI_{IR}$$

where, HI_{Liver} = liver histopathologic condition index for an individual fish; RI_{CD} = liver reaction pattern index for circulatory disturbance category, RI_{RA} = liver reaction pattern index for regressive alterations category and RI_{IR} = liver reaction pattern index for inflammatory response category. A greater value of the HI_{Liver} reflects a more severely affected individual fish.

Statistical analysis

Spatial variations of physico-chemical characteristics in water and condition factor of the fish were statistically analysed separately using analysis of variance (ANOVA) test after verifying assumptions of ANOVA. If there were significant differences, Tukey's pair-wise comparison test was used for comparison of means. The proportional nuclear abnormality data were subjected to ArcSin-square root transformations prior to analysis (Zar, 1998). Liver histopathologic condition indices were analysed using non-parametric Kruskal-Wallis test and/or Mann-Whitney U test as appropriate. Potential associations between specific biomarker data and selected physico-chemical variables (BOD_5 , COD and heavy metals) were tested by Pearson's correlation test. In all cases, accepted level of significance was $p < 0.05$ (Zar, 1998).

RESULTS AND DISCUSSION

Physico-chemical parameters of surface waters of the three selected sites of Kelani River, site 1 (upper catchment, Ruwanwella), sites 2 and 3 (lower reach, Kaduwela and Mattakkuliya) are given in Table 1. No significant spatial variation was detected for water temperature and pH levels during the three sampling events. Salinity and TDS levels in the water were

Table 1: Physico-chemical characteristics of surface water of the fish sampling sites of Kelani River

Parameter	2014 Dry period			2015 Dry period			2015 Rainy period		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Temperature (°C)	27.4 ± 0.6 ^a	29.7 ± 0.7 ^a	27.1 ± 0.1 ^a	25.9 ± 0.7 ^a	27.2 ± 0.1 ^a	26.3 ± 0.2 ^a	28.3 ± 0.2 ^a	29.6 ± 0.2 ^a	27.8 ± 0.2 ^a
pH	8.2 ± 1 ^a	8.1 ± 1 ^a	7.7 ± 0.1 ^a	7.5 ± 0.2 ^a	7.1 ± 0.1 ^a	7.2 ± 0.1 ^a	7.1 ± 0.1 ^a	6.7 ± 0.1 ^a	7.3 ± 0.2 ^a
Salinity (g L ⁻¹)	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	7.3 ± 1.3^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	6.5 ± 0.6^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.2 ± 0.1^b
TDS (mg L ⁻¹)	29 ± 3 ^a	39 ± 3 ^a	8316 ± 1380^b	26 ± 6 ^a	22 ± 1 ^a	6257 ± 553^b	20 ± 2 ^a	25 ± 1 ^a	1539 ± 167^b
DO (mg L ⁻¹)	7.8 ± 0.4 ^a	7.2 ± 0.1 ^a	5.3 ± 0.7 ^a	7.9 ± 0.4 ^a	6.7 ± 0.1 ^a	3.5 ± 0.9^b	7.8 ± 0.1 ^a	7.0 ± 0.3 ^a	4.9 ± 0.4 ^a
BOD ₅ (mg L ⁻¹)	0.8 ± 0.1 ^a	1.7 ± 1.2 ^b	4.7 ± 0.5^b	1.2 ± 0.0 ^a	1.2 ± 0.2 ^a	1.8 ± 0.8 ^a	1.4 ± 0.2 ^a	1.8 ± 0.4 ^a	2.8 ± 0.6 ^a
COD (mg L ⁻¹)	1 ± 0 ^a	21 ± 2^b	208 ± 5^c	8 ± 3 ^a	20 ± 1^b	109 ± 37^c	10 ± 3 ^a	42 ± 1^b	166 ± 1^c
PO ₄ ³⁻ (mg L ⁻¹)	0.08 ± 0.01 ^a	0.05 ± 0.02 ^a	0.12 ± 0.04 ^a	0.0 ± 0.0 ^a	0.13 ± 0.04^b	0.23 ± 0.07^b	0.23 ± 0.11 ^a	0.19 ± 0.02 ^a	0.48 ± 0.05^b
NO ₃ ⁻ (mg L ⁻¹)	0.20 ± 0.16 ^a	0.28 ± 0.04 ^a	0.51 ± 0.06 ^a	0.0 ± 0.0 ^a	0.27 ± 0.06^b	0.47 ± 0.13^b	0.66 ± 0.07 ^a	0.76 ± 0.06 ^a	0.66 ± 0.03 ^a
Cd (µg L ⁻¹)	< 1	3 ± 1 ^a	44 ± 8^b	8 ± 9 ^a	34 ± 14^b	53 ± 5^b	< 1	< 1	< 1
Cr (µg L ⁻¹)	11 ± 1 ^a	7 ± 1 ^a	5 ± 1 ^a	< 0.2	< 0.2	< 0.2	2 ± 0 ^a	1 ± 0 ^a	3 ± 1 ^a
Cu (µg L ⁻¹)	3 ± 04 ^a	22 ± 2^b	23 ± 6^b	< 3	< 3	8 ± 2	7 ± 3 ^a	9 ± 1 ^a	11 ± 3 ^a
Pb (µg L ⁻¹)	< 6	< 6	501 ± 88	< 6	< 6	325 ± 25	< 6	< 6	< 6

TDS: total dissolved solid; DO: dissolved oxygen; BOD₅: biochemical oxygen demand; COD: chemical oxygen demand; PO₄³⁻: total phosphate; NO₃⁻: nitrate nitrogen. Limit of quantification for Cd, Cr, Cu, and Pb are 1, 0.2, 3, and 6 µg L⁻¹, respectively. Data are presented as mean ± SEM (n = 3 sub sampling sites). For a specific sampling period, site specific data in a row denoted by different superscript letters are significantly different from each other (p < 0.05). Bold numerals indicate significant difference compared to the Site 1 (upper catchment)

consistently higher at site 3 compared to the other sites (p < 0.05). Elevated BOD₅ levels during the 2014 dry period and decreased DO levels during the 2015 dry period were recorded from site 3 compared to the other sites (p < 0.05). Irrespective of the sampling events, COD

levels in the surface water showed an increase from site 1 to site 3. Furthermore, total phosphate levels and nitrate nitrogen levels in the water were significantly higher at sites 2 and 3 in the 2015 dry period compared to those at site 1. Total phosphate level in the water was higher at

site 3 than at site 1 in the 2015 rainy period. Heavy metal concentrations recorded in the surface water of the three sampling sites (Table 1) indicate significantly higher Cd, Cu, and Pb levels at site 3 and Cu level at site 2 during the 2014 dry period compared to those at site 1. During the 2015 dry period, Cd and Pb levels at site 3 and Cd level at site 2 were significantly higher than those at site 1 ($p < 0.05$). No significant spatial variations in the measured heavy metal levels were found in the 2015 rainy period. In general, overall pollution pattern followed the increasing order: Ruwanwella site < Kaduwela site < Mattakkuliya site. Physico-chemical characterisation confirmed that the pollution of Kelani River shows an increasing trend towards the lower reach. Mahagama *et al.* (2014) monitored the gross pollution status in surface water of Kelani River during October 2012 to March 2013 based on microbiological and physico-chemical parameters and found that 80 % sampling points in the head and transitional zones and 90 % locations in the meandering zone of the river basin were contaminated with total coliform and faecal coliform bacteria. Further, the COD level in 90 % of the water samples was also higher than 10 mgL^{-1} whereas Cd, Pb, Cr and Cu concentrations in surface water (in μgL^{-1}) were reported to be within the range 0.1183–0.775, 0.078–5.34, 0.00156–3.50 and 3.11–14.44, respectively (Mahagama *et al.*, 2014). However, sampling site specific physico-chemical data are not provided in their study for direct comparison with the results obtained in the present study.

The presence of heavy metals in surface waters represent a significant source of environmental contamination, since metals are potentially genotoxic and carcinogenic (Matos *et al.*, 2017). To the best of our knowledge, guideline values or threshold limits of heavy metals for protection of aquatic life are not yet established in Sri Lanka. Based on internationally established criteria/standards, benchmark values (in μgL^{-1}) proposed by UNEP (2016) for Cd, Cr (iii), Cr (vi), Cu and Pb for Category I (high integrity) freshwater ecosystems are 0.08, 10, 1, 1 and 2, respectively, whereas corresponding metal levels for Category 4 (extreme impairment) ecosystems are 1, 75, 40, 2.5, and 5, respectively. The levels of Cd, Cu and Pb in the water (Table 1) at site 3 (Mattakkuliya) exceeded the benchmark for the respective metals for Category 4 ecosystems. The levels of Cd and Cu in the water at site 2 (Kaduwela) and site 1 (Ruwanwella) during some sampling events also exceeded the benchmark for the respective metals for Category 4 ecosystems. These results indicate that the part of Kelani River passing through Mattakkuliya area are at 'extreme impairment conditions'. For freshwater life protection, Criterion Continuous Concentration (CCC) established by the United States Environment Protection Agency for Cd, Cr(iii), Cr(vi), Cu and Pb (in μgL^{-1}) are 0.72, 74, 11, 2.2 and 3.2, respectively (USEPA 2005; 2019). Comparison of measured metal levels at the three sites (Table 1) of Kelani River with the CCC for respective metals indicate that the levels of more than one metal in most cases exceeded the respective

Table 2: Profile of *E. suratensis* and *D. singhala* used for biomarker assessments

Fish, sampling season and site	Number of fish	Males:females	Total length (cm)	Condition factor (gcm^{-3})
<i>Etroplus suratensis</i>				
2014 dry period Site 1	6	1:5	17.2 ± 1.7^a	2.40 ± 0.10^a
Site 3	6	4:2	17.2 ± 2.3^a	2.51 ± 0.19^a
2015 dry period Site 1	4	1:3	17.5 ± 0.7^a	2.18 ± 0.07^a
Site 3	13	9:4	18.7 ± 2.1^a	2.37 ± 0.05^a
2015 rainy period Site 1	5	3:2	21.8 ± 1.7^a	2.27 ± 0.08^a
Site 3	10	6:4	20.1 ± 2.4^a	2.45 ± 0.16^a
<i>Dawkinsia singhala</i>				
2015 dry period Site 1	6	2:4	12.3 ± 1.7^a	1.82 ± 0.07^a
Site 2	3	1:2	10.9 ± 1.4^a	2.06 ± 0.09^a
2015 rainy period Site 1	10	3:7	10.3 ± 1.3^a	1.65 ± 0.09^a
Site 2	12	2:10	9.8 ± 0.8^a	1.79 ± 0.09^a

Data are presented as mean \pm SEM. For a particular fish species, the data in a column denoted by the same superscript indicate no significant difference from each other ($p > 0.05$).

CCC for aquatic life protection. In all sampling events, Cd, Cu and Pb levels in the water at site 3 exceeded the CCC for respective metals indicating high health risks for the species residing in this site of the river.

Native fish are considered as key elements for assessing the quality of aquatic ecosystems due to their high ecological relevance in the aquatic environment (Colin *et al.*, 2016). In this study, the two native fishes of Kelani River (*E. suratensis* and *D. singhala*) used for biomarker assessments included both genders (Table 2). No significant temporal or spatial differences were found with respect to the Condition Factors of the captured fish ($p > 0.05$). In the three sampling events, *E. suratensis* could be captured only from site 1 and site 3. *D. singhala* was available only in two sampling events at site 1 and site 2. It is assumed that the individuals of two species of fish are restricted to the area of the sampling sites, since it is highly unlikely that they can move from the

lower reach (Kaduwela site or Mattakkuliya site) to the Ruwanwella site in the upper reach, which is located nearly 50 km from site 2 and over 75 km from site 3. Therefore, it is expected that with respect to a particular fish species, biomarker responses in the fish population at site 1 (reference site) would be different from the fish population in the lower reach. Distribution patterns of the two species in different sites of the river may also be associated with the species-specific habitat preferences.

Histopathological biomarkers are valuable for indication of the general health of fish residing in polluted water bodies (Bernet *et al.*, 1999; Ballesteros *et al.*, 2017; Kumar *et al.*, 2017). The liver is a vital organ of fish and is a target organ of many toxicants (Wolf & Wheeler, 2018). The liver of *E. suratensis* specimens captured from site 3 exhibited prominent histopathological changes (Figure 2), especially congestion of sinusoids (Figure 2a1), cells with pyknotic nuclei (Figure 2a2), leukocyte infiltration areas (Figure 2a3), frequent melanomacrophage centres (Figure 2a3), extensive vacuolations and focal necrotic areas (Figure 2a4) compared to those of fish collected from the Site 1. Irrespective of the sampling period, percentage of occurrence of each of these prominent histological changes in *E. suratensis* specimens collected from site 3 is greater than those from site 1 (Table 3). Liver of *D. singhala* sampled from site 2 (Figure 2) also showed prominent sinusoid congestion (Figure 2b1), vacuolations (Figure 2b1) and focal necrosis (Figure 2b1), compared to the fish from Site 1. As indicated in Table 3, percentage occurrence of these prominent histological changes in *D. singhala* specimens collected from site 2 is greater than those from site 1.

In the present study, liver histopathologic condition indices were estimated per individual fish based on quantified reaction indices as described by Bernet *et al.* (1999). This histopathological assessment method has been used in recent studies for assessing organ damage and ill health effects in fish associated with pollutant exposure (Ballesteros *et al.*, 2017; Kostić *et al.*, 2017; Pérez *et al.*, 2018). Table 4 presents histopathologic condition indices of liver tissues of *E. suratensis* and *D. singhala* collected from Kelani River along with reaction indices for the three quantified categories of histopathological changes. In comparison to fish captured from site 1, liver histopathologic condition indices were significantly higher ($p < 0.05$) in *E. suratensis* from site 3 (1.9 to 2.8 fold) and *D. singhala* from site 2 (1.6 to 2.7 fold). A greater value of liver histopathologic condition index reflects a more severely affected individual fish (Bernet *et al.*, 1999). The higher liver histopathologic condition indices in both fish species inhabiting lower

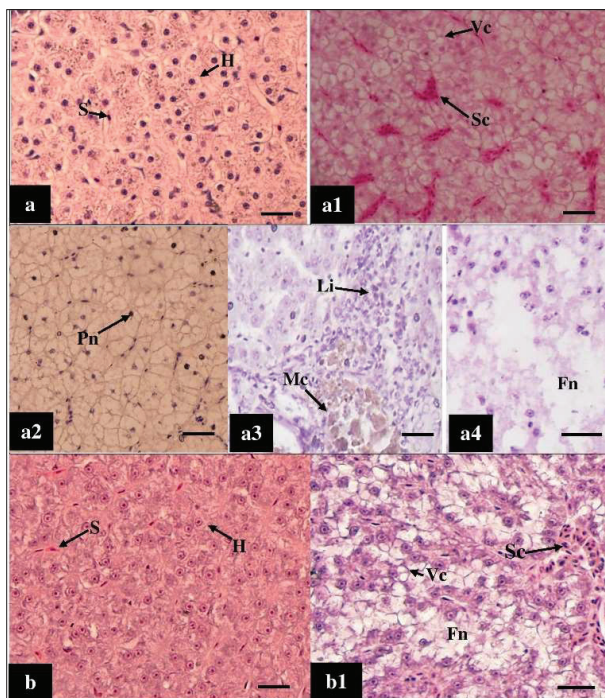


Figure 2: Liver structure of (a) *E. suratensis* and (b) *D. singhala* from the site 1 of Kelani River showing normal hepatocytes (H) and sinusoid (S). Prominent histological changes observed in liver of *E. suratensis* specimens (a1, a2, a3, and a4) from site 3 and liver of *D. singhala* (b1) from site 2 of Kelani River. Sinusoid congestion (Sc); vacuolation (Vc); hepatocytes with small pyknotic nuclei (Pn); leukocytes infiltration (Li); melanomacrophage centres (Mc); focal necrosis in hepatic tissue (Fn). Scale bar represents 10 μm .

reaches of the river compared to those in fish species inhabiting the upper reach irrespective of the sampling period reflect the impacts of pollution in fish in the lower reaches of the river. Of the three quantified reaction indices (Table 4), reaction indices for regressive alterations mainly contributed to the liver histopathologic condition indices in the sampled fish. Regressive alterations

reaction pattern has been considered as a process which results in a functional reduction or loss of an organ (Bernet *et al.*, 1999). Such regressive damage has been reported as a response to exposure to various organic and inorganic toxicants, regardless of their chemical nature (Ballesteros *et al.*, 2017; Kostić *et al.*, 2017; Pérez *et al.*, 2018).

Table 3: Frequencies of prominent histological alterations in liver tissues of *E. suratensis* and *D. singhala* collected from Kelani River

Fish, sampling season and site	Sinusoid congestion	Vacuolation	Pycnosis	Necrosis	Leucocyte infiltration	Melano-macrophage centres
<i>Etroplus suratensis</i>						
2014 dry period Site 1	33 % (2/6)	83 % (5/6)	50 % (3/6)	0 % (0/6)	0 % (0/6)	0 % (0/6)
Site 3	100 % (6/6)	100 % (6/6)	83 % (5/6)	100 % (6/6)	33 % (2/6)	83 % (5/6)
2015 dry period Site 1	25 % (1/4)	50 % (2/4)	50 % (2/4)	25 % (1/4)	0 % (0/4)	0 % (0/4)
Site 3	100 % (13/13)	100 % (13/13)	100 % (13/13)	92 % (12/13)	23 % (3/13)	100 % (13/13)
2015 rainy period Site 1	40 % (2/5)	60 % (3/5)	40 % (2/5)	20 % (1/5)	0 % (0/5)	20 % (1/5)
Site 3	100 % (10/10)	90 % (9/10)	80 % (8/10)	80 % (8/10)	30 % (3/10)	90 % (9/10)
<i>Dawkinsia singhala</i>						
2015 dry period Site 1	33 % (2/6)	33 % (2/6)	33 % (2/6)	17 % (1/6)	0 % (0/6)	0 % (0/6)
Site 2	66 % (2/3)	100 % (3/3)	66 % (2/3)	66 % (2/3)	0 % (0/3)	0 % (0/3)
2015 rainy period Site 1	30 % (3/10)	40 % (4/10)	20 % (2/10)	0 % (0/10)	0 % (0/10)	10 % (1/10)
Site 2	50 % (6/12)	100 % (12/12)	58 % (7/12)	50 % (6/12)	0 % (0/12)	17 % (2/12)

Frequencies of histological alterations are expressed as percentages. Ratios in parentheses indicate the number of fish on which the change was found out of the total number of fish analysed in each sampling site during the specific sampling period.

Table 4: Reaction indices (RI) for quantified categories of histopathological changes and histopathologic condition indices of liver tissues of *E. suratensis* and *D. singhala* collected from Kelani River

Fish, sampling season and site	Number of fish	RI for circulatory disturbances	RI for regressive alterations	RI for inflammatory response	Liver histopathologic condition index
<i>Etroplus suratensis</i>					
2014 dry period Site 1	6	1.5 ± 0.4	39 ± 7	0	41 ± 7 ^a
Site 3	6	10.3 ± 1.3	61 ± 6	4.7 ± 1.8	76 ± 7 ^b
2015 dry period Site 1	4	1.0 ± 0.4	27 ± 3	0	28 ± 3 ^a
Site 3	13	7.9 ± 1.4	66 ± 4	4.4 ± 0.9	78 ± 6 ^b
2015 rainy period Site 1	5	1.2 ± 0.3	30 ± 6	0.2 ± 0.2	31 ± 5 ^a
Site 3	10	6.9 ± 0.9	57 ± 7	2.6 ± 0.7	67 ± 7 ^b
<i>Dawkinsia singhala</i>					
2015 dry period Site 1	6	2.3 ± 0.9	31 ± 3	0	33 ± 4 ^a
Site 2	3	4.3 ± 1.8	48 ± 7	0	52 ± 6 ^b
2015 rainy period Site 1	10	1.4 ± 0.3	22 ± 2	0.1 ± 0.1	23 ± 3 ^a
Site 2	12	1.8 ± 0.3	59 ± 4	0.6 ± 0.2	61 ± 4 ^b

Data for reaction indices and histopathologic condition indices of the number of fish indicated above for each group are presented as mean ± SEM. For a particular fish species, liver histopathologic condition indices denoted by different superscript letters indicate statistical differences among sampling sites and sampling periods ($p < 0.05$)

The correlation test was conducted only for *E. suratensis* due to the limitations in the sampling events for *D. singhala*. Pearson correlation indicated that the liver histopathologic condition index of *E. suratensis* positively correlated with BOD₅, COD, Cd, Cu and Pb levels in the river water ($p < 0.05$). The results indicate that chemical contaminants could induce liver lesions in fish inhabiting polluted sites. As the fish liver is a vital organ, such structural alterations could lead to serious health implications in the fish under long term exposure to the pollutants. Induction of liver histological alterations in these fish may be considered as early warning signals for fish populations residing in these sites. Similar to our study, Kumar *et al.* (2017) found significant alterations in the liver histological structure of the fish *Oreochromis mossambicus* collected from contaminated sites along the Bhima River, India. Liver histopathological alterations seen in the two species of fish from Kelani River demonstrate potential health impacts to native fish populations in the riverine ecosystem.

Induction of erythrocytic micronuclei and nuclear abnormalities in various fish species have been linked to the exposure of chemicals such as heavy metals, pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls (Braham *et al.*, 2017). Micronuclei can be formed as a result of chromosomal breaks or mitotic spindle dysfunctions (Al-Sabti & Metcalfe, 1995). Although the exact mechanisms of induction of erythrocytic nuclear abnormalities are less understood, they have been used as indicators of exposure to various genotoxic and mutagenic contaminants (Ghisi *et al.*, 2016; Braham *et al.*, 2017; Vieira *et al.*, 2017). In the present study, nuclear abnormalities detected in the erythrocytes of the fish (Figure 3) were nuclear buds (Figures 3a2 and 3b1), notched nuclei (Figures 3a1, 3a2 and 3b3), vacuolated nuclei (Figure 3b1) and micronuclei (Figure 3b2). Percentage occurrence of each nuclear abnormality condition is presented in Table 5. Nuclear buds and notched nuclei were the most conspicuous nuclear abnormalities seen in the erythrocytes of the two fish species (Table 5). Vacuolated nuclei were found in the erythrocytes of *D. singhala* collected from site 1 ($0.9 \pm 0.3 \%$) and site 2 ($2.5 \pm 0.6 \%$) during the rainy period. In addition, micronuclei in the erythrocytes were detected only in one *D. singhala* collected from site 2 during the rainy period (Figure 3b2). Significant temporal variations in the occurrence of total nuclear abnormalities were found in *E. suratensis* sampled from both sites and *D. singhala* sampled from site 2 (Table 5). In all cases, the occurrence of nuclear abnormalities was significantly higher in *D. singhala* collected from site 2 (1.8 to 17 fold) and *E. suratensis* from site 3 (1.5 to 5

fold) compared to those in site 1. The results indicate that potential genotoxic impacts are much greater in the lower reaches of the river compared to the upper catchment (site 1). The frequency of nuclear abnormalities in the erythrocytes of *E. suratensis* from sites 1 and 3 showed significant increases during the dry period in 2015 (8 and 3.8 fold, respectively) compared to those in 2014. Erythrocytes of *D. singhala* sampled from site 2 showed increase (6 fold) in nuclear abnormalities in the dry period compared to the wet period in 2015. The temporal variability in nuclear lesions may be associated with the variations in genotoxic pollutant inputs to the lotic habitats, in addition to pollutant concentration effect in the dry period due to the evapotranspiration and pollutant dilution effect in the rainy period. Pearson correlation indicated that total erythrocytic nuclear abnormalities in *E. suratensis* was positively correlated with BOD₅, COD, Cu and Pb levels in the river water ($p < 0.05$). The results indicate that chemical contaminants could induce genetic damage in fish inhabiting polluted sites. Persistent genotoxic stress on fish populations inhabiting polluted sites in the river may eventually lead to an accumulation of mutations affecting their general health, survival and reproduction. Increase in the occurrence of erythrocytic nuclear abnormalities in two species of fish captured from Kelani River demonstrates the potential genotoxic impacts on native fish populations in the riverine ecosystem.

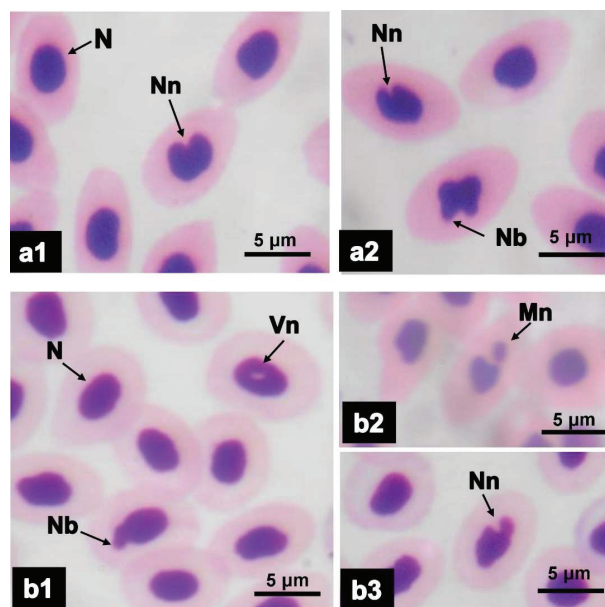


Figure 3: Erythrocyte nuclear abnormalities seen in *E. suratensis* (a1, a2) and *D. singhala* (b1, b2, b3) captured from Kelani River: normal nucleus (N); nuclear bud (Nb); notched nucleus (Nn); vacuolated nucleus (Vn); micronucleus (Mn)

Table 5: Frequencies of erythrocyte nuclear abnormalities in 1000 mature erythrocytes of *E. suratensis* and *D. singhala* collected from Kelani River

Fish, sampling season and site	Number of fish	Nuclear buds ‰	Notched nuclei ‰	Vacuolated nuclei ‰	Total nuclear abnormalities ‰
<i>Etroplus suratensis</i>					
2014 dry period Site 1	6	0.3 ± 0.1	3.1 ± 0.9	0	3.4 ± 0.9 ^a
Site 3	6	1.8 ± 0.7	15.2 ± 2.9	0	17.1 ± 3.2 ^b
2015 dry period Site 1	4	5.8 ± 1.2	21.0 ± 5.4	0	26.8 ± 5.1 ^{bc}
Site 3	13	20.7 ± 2.0	44.6 ± 3.7	0	65.3 ± 5.7 ^d
2015 wet period Site 1	5	5.8 ± 1.2	30.6 ± 5.1	0	36.4 ± 5.4 ^c
Site 3	10	14.0 ± 0.9	39.3 ± 3.2	0	53.3 ± 2.9 ^d
<i>Dawkinsia singhala</i>					
2015 dry period Site 1	6	0.8 ± 0.4	3.1 ± 0.7	0	3.9 ± 0.8 ^a
Site 2	3	18.3 ± 1.7	49.0 ± 5.1	0	67.3 ± 4.6 ^c
2015 wet period Site 1	10	0.2 ± 0.1	4.8 ± 0.6	0.9 ± 0.3	6.0 ± 0.7 ^a
Site 2	12	2.8 ± 0.3	5.6 ± 0.8	2.5 ± 0.6	10.9 ± 0.5 ^b

Frequencies of erythrocyte nuclear abnormalities in the number of fish indicated above for each group are presented as mean ± SEM. For a particular fish species, frequencies of total nuclear abnormalities denoted by different superscript letters indicate statistical differences among sampling sites and sampling periods ($p < 0.05$)

This study demonstrates the potential utility of liver histopathological alterations and nuclear abnormalities in erythrocytes of native fish as ‘effect biomarkers’ for assessing pollution impacts in riverine ecosystems. This study showed the appropriateness of the examined biomarker responses of the two native fish species to differentiate a contaminated site from a reference site. *E. suratensis* and *D. singhala* may be used as environmental sentinels in monitoring pollution impacts in inland water bodies in Sri Lanka. However, considering the small sample size of fish of two species analysed in this study, more extensive studies using larger samples of fish from other polluted sites are warranted to confirm the suitability of using these two species as environmental sentinels for monitoring pollution impacts in riverine ecosystems.

CONCLUSION

Physico-chemical analyses of surface water confirmed an increasing trend of pollution towards the lower reach of the Kelani River. In the biomarker assessments, liver histological alterations and erythrocyte nuclear abnormalities were observed at all sites with variable rates among sites and fish species indicating potential ill-health conditions of the native fish populations. Elevated liver histopathologic condition indices and erythrocyte

nuclear abnormalities in the fish captured from lower reaches of the river with high anthropogenic influences compared to the fish in upper catchment indicate greater hepatotoxic and genotoxic effects of the contaminants present in the lower reach. The results suggest that endemic and nationally threatened fish species in the riverine ecosystem may also be at risk due to the contaminant stress under long term exposure.

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