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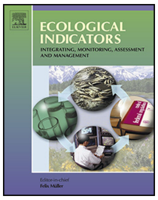

Original articles

Insights into the drivers of histopathological changes and potential as bio-indicator of riverine health of an aquatic apex predator from a premier conservation area: A multiple lines of evidence and multivariate statistics approach

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ABSTRACT

There is a need for sensitive bio-monitoring and indicator tools in toxicant impact assessment to show the effect on fish health. Histopathological assessment of fish tissue is a bio-monitoring tool allowing for early warning signs of disease and detection of long term injury in cells, tissues or organs. The aims of this study were firstly to determine and compare the health status of the tigerfish (*Hydrocynus vittatus*) from two lowland rivers in Kruger National Park (KNP), secondly to attempt to explain the histological changes observed, through the application of a suite of multivariate statistics to relate changes to biotic levels of selected metals and organochlorine pesticides (OCPs) and thirdly to determine the suitability of *H. vittatus* as a bio-indicator of riverine health. Tigerfish were caught using rod and reel from the Olifants (n = 37) and Luvuvhu Rivers (n = 34) between 2009 and 2011. The histology-based fish health assessment (liver, kidney, gills and gonads) indicated that fish were in relatively good health. Even though fish were considered to be healthy, the general fish health in both rivers improved over time, corresponding to an overall decrease in river pollution, most notably in terms of metals. The incorporation of multiple lines of evidence in tigerfish, including histopathological changes and organ and whole organism indices, proved to be a valuable tool in using a bio-indicator approach toward river monitoring. These histopathological changes serve as an early warning system to more serious health concerns arising if the pollution in the rivers of the KNP is not dealt with. The use of a suite of uni- and multivariate statistics proved helpful in determining the links between fish health and river contamination and further proved to be a valuable tool in assessing spatial and temporal differences in river pollution and the effects thereof on the selected bioindicator.

1. Introduction

The Kruger National Park (KNP) is South Africa's foremost conservation area and is situated along the eastern border with Mozambique. The managing organisation, South African National Parks (SANParks) is mandated by legislation to maintain crucial ecological processes and life support systems, whilst concurrently safeguarding genetic diversity and the sustainable utilisation of species and ecosystems (Moore et al., 1991; Venter and Deacon 1992). This task is made even more arduous in terms of aquatic ecosystems given that all six of the major rivers flowing through the KNP, namely; the Luvuvhu, Letaba, Olifants, Sabie, Crocodile and Shingwedzi Rivers, arise from outside the KNPs western border. Numerous human impacts such as flow regulation, water abstraction and various anthropogenic inputs occur to various degrees within the catchments of the aforementioned rivers, compounding the matter of aquatic ecosystem conservation in the KNP even further (Gerber et al., 2015a, 2015b, 2016a, 2016b). Recently much attention has been focused on the aquatic systems of the KNP, as a result of large numbers of Nile crocodile (*Crocodylus niloticus*) deaths within its borders in specifically the Olifants River during the winters of 2008 and 2009. These deaths were attributed

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to pansteatitis, with no answer as to the cause of the pansteatitis (Huchzermeyer et al., 2011; du Preez et al., 2016). As such the section of the Olifants River within the KNP was selected as one of the study areas of the present study, along with the Luvuvhu River. Notably the Olifants River has long been regarded as South Africa's most used and abused system with pollutant impacts seen in the KNP (Gerber et al., 2015a, 2015b, 2016a), while the Luvuvhu River section within the KNP was regarded to be in a natural state (Angliss et al., 2001). Current research, however, suggests otherwise and indicates that the Luvuvhu River is similarly impacted by selected metals, but most notably much more affected by various organochlorine pesticides (OCPs), including 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT). (Barnhoorn et al., 2009; Bornman et al., 2010; Smit et al., 2013; Gerber et al., 2015b, 2016a, 2016b).

Both metals and OCPs have previously been shown to be linked to histological changes in fish from various aquatic systems (Bernet et al., 1999; Marchand et al., 2012; McHugh et al., 2011; Wepener et al., 2011). Histopathological changes are biomarkers of effect as well as exposure to environmental stressors, revealing changes in physiological and or biochemical function (Hinton et al., 1992). These changes also incorporate biotic factors, therefore are able to provide a more holistic view of fish health (Handy et al., 2002). It has been shown that a histological assessment can show changes before it manifests macroscopically and can therefore be used as an early warning system for pathological changes in the fish assessed (Marchand et al., 2008). Histopathology-based bioassessment studies have found liver (Marchand et al., 2008; van Dyk et al., 2012), gill (van Dyk et al., 2009b) and gonadal changes (Wagenaar et al., 2012) of fish in a polluted urban nature reserve. By using the protocol of Bernet et al. (1999) adapted by van Dyk et al. (2009a) histological changes noted can be quantified to allow for meaningful comparison between species, sites, as well as between organs. A necropsy of fish by observing and noting internal and external macroscopic changes would give an indication of the environmental stress of a fish population (Adams et al., 1993).

The advantages of the use of fish as bio-indicators of aquatic health is well discussed by Whitfield and Elliot (2002) and Schlacher et al. (2007) and include several key aspects. Where, firstly the link between anthropogenic activities and their resultant ecological impacts may be elucidated through more sensitive approaches such as direct measurements of aquatic organisms, for example fish. Especially since determining the water quality through measuring the physical and chemical properties of the water column may not be an adequate substitute for ecological responses. Further, fish have slow tissue turnover rates as they are long lived and can therefore integrate pollution over long periods. Thus providing a more time integrated view of what is happening in the system, compared to water quality, which only provides a snapshot of what is occurring within the system. Thirdly, fish occupy a wide variety of habitats and trophic positions and can provide an indication of pollution at different levels of trophic organisation. The fourth important aspect is that fish are able to provide multiple lines of evidence for anthropogenic impact studies, which is an important aspect when considering politically sensitive studies such as this one, due to the importance of the study area.

In the present study the ecological and economically important tigerfish (*Hydrocynus vittatus*) was chosen as the bio-indicator species. *Hydrocynus vittatus* are an important, dominant piscivorous predator in many African rivers (Smit et al., 2009) and by virtue of its position as the top predatory fish it is important to elucidate the health of these populations considering what has occurred in the Olifants River regarding *C. niloticus*. Tigerfish fulfil the requirements of a good aquatic bio-indicator organism (Bervoets et al., 2004; Burger and Gochfeld 2001; Zhou et al., 2008). Tigerfish are abundant and are widely distributed within the sampling area, are easy to sample and target, occupy an important position in the food chain (tigerfish are the top predators in these systems – Skelton, 2001; McHugh et al., 2011) and may provide a good indication of human linked activities on ecosystems, as cascading effects along the food web may manifest within *H. vittatus*. Tigerfish are relatively long lived (up to 20 years, Gerber et al., 2009; Soekoe et al., 2013) and as such should provide a time integrated view of the selected systems. Tigerfish have also been shown to accumulate pollutants; which include DDTs, various other OCPs, various metals and organohalogens (Bouwman et al., 1990; du Preez and Steyn, 1992; Mhlanga 2000; Wepener et al., 2012; Barnhoorn et al., 2015; Gerber et al., 2016a, 2016b). Although tigerfish are not sedentary and often migrate over vast areas (Thorstad et al., 2003), tigerfish in the sampled area are limited in their movements because of migration barriers such as dams and weirs, and therefore contaminant levels in these fish and the resultant fish health can be regarded as representative of the area.

The aim of this research was firstly to determine the health of the tigerfish from two lowland rivers in KNP and to compare the health of these fish between the two rivers that currently have different anthropogenic impacts on them, secondly to attempt to explain the histological changes observed, through the application of a suite of multivariate statistics to relate the changes to biotic levels of selected metals and OCPs and thirdly to determine the suitability of *H. vittatus* as a bio-indicator of riverine health.

2. Material and methods

2.1. Study area and sampling

Prior to data sampling, ethical clearance for fish collection and processing were obtained from the University of Johannesburg, Faculty Ethics Committee. Sampling of tigerfish by rod and line angling techniques took place during the period September 2009 to June 2011 at sites along the Olifants (S23° 59′ 25.2′′ E31° 49′ 33.3′′) and Luvuvhu (S22° 27′ 04.3′′ E31° 04′ 47.7′′) rivers within the KNP (Fig. 1). Tigerfish were sampled during three different surveys on the Olifants River (OLI), i.e. November 2009 (LF2009), May 2010 (HF2010) and June 2011 (HF2011); and sampling surveys on the Luvuvhu River (LUV) were conducted during September 2009 (LF2009), April 2010 (HF2010), and May 2011 (HF2011).

2.2. Sampling of fish tissue

All fish were transported to a nearby field laboratory for processing. The body mass (g) and the total length (mm) of each fish were recorded and sacrificed by severing the spinal cord anterior to the dorsal fin. A ventral incision was made to expose the visceral organs where after a standard necropsy was performed. Any macroscopic abnormalities were noted (Goede and Barton 1990; Adams et al., 1993). The liver, gonad and spleen masses were recorded to calculate the hepato-somatic index (HSI), the gonado-somatic index (GSI) and the spleno-somatic index (SSI) respectively for each fish using the formula: organ mass/body weight X 100 (Marchand et al., 2012). The body mass and length measurements were used to calculate a condition factor (CF) per fish (Carlander, 1969). Muscle tissue samples were removed for metal and organochlorine determination as described in Gerber et al. (2016b) and Gerber et al. (2016a), respectively.

2.3. Histology techniques and microscopy

Gill, liver, kidney and gonad samples were collected for histopathological analysis, fixed in 10% neutrally buffered formalin (gills, livers and kidneys) for 48 h and in Bouins solution (gonads) for 24 h. Following fixation, tissue samples were washed in tap water