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Transcriptomic, lipid, and histological profiles suggest changes in health in fish from a pesticide hot spot

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ABSTRACT

Barramundi (*Lates calcarifer*) were collected at the beginning (1st sampling) and end (2nd sampling) of the wet season from Sandy Creek, an agriculturally impacted catchment in the Mackay Whitsundays region of the Great Barrier Reef catchment area, and from Repulse Creek, located approximately 100 km north in Conway National Park, to assess the impacts of pesticide exposure. Gill and liver histology, lipid class composition in muscle, and the hepatic transcriptome were examined. The first sample of Repulse Creek fish showed little tissue damage and low transcript levels of xenobiotic metabolism enzymes. Sandy Creek fish showed altered transcriptomic patterns, including those that regulate lipid metabolism, xenobiotic metabolism, and immune response; gross histological alterations including lipidosis; and differences in some lipid classes. The second sampling of Repulse Creek fish showed similar alterations in hepatic transcriptome and tissue structure as fish from Sandy Creek. These changes may indicate a decrease in health of pesticide exposed fish.

1. Introduction

Poor water quality from agricultural runoff is a concern for the Great Barrier Reef and the adjacent catchment area in northern and central Queensland, Australia (Brodie et al., 2012; Kroon et al., 2012; Smith et al., 2012). Elevated levels of sediment, nutrient, and pesticides are transported from agricultural land to freshwater and estuarine ecosystems, and subsequently discharged to the Great Barrier Reef (GBR) lagoon (Devlin and Schaffelke, 2009; Kroon et al., 2012; Smith et al., 2012). The presence of these agricultural contaminants in the GBR has been linked to loss of coral cover and species from this iconic ecosystem (e.g. Brodie and Pearson, 2016). Notably, catchments within the Mackay Whitsunday region have been recognised as ecosystems with a high risk from pesticides (Brodie et al., 2013a; b), with elevated levels of photosystem II inhibiting herbicides, such as atrazine, diuron and hexazinone, as well as the neonicotinoid imidacloprid (e.g., Garzon-Garcia et al., 2015; Wallace et al., 2016). In particular, Sandy Creek in the Plane basin, has recorded some of the highest pesticide

concentrations of any monitored catchment that discharge to the GBR (Smith et al., 2012, 2015; Brodie et al., 2013a; b; Garzon-Garcia et al., 2015; Wallace et al., 2016).

The economic and ecological importance of the GBR to Queensland and Australia, as well as the threats of climate change to reef ecosystems, has driven most research on the GBR to focus on the marine ecosystems of the GBR (Schaffelke et al., 2012; Brodie and Pearson, 2016). Far less emphasis has been directed towards evaluating the impacts from poor water quality on ecosystem health within catchments that discharge to the GBR (Kroon et al., 2015). Concerningly, pesticide concentrations, and therefore risk, are higher in freshwater and estuarine ecosystems as these ecosystems are closer to the pesticide source (Devlin et al., 2015; Waterhouse et al., 2017). Much of the contemporary research concerning pesticide risks in the region has focussed on impacts to photosynthetic species, such as algae and seagrass (e.g., Magnusson et al., 2010; Magnusson et al., 2012; Flores et al., 2013) due to the proliferation of photosystem II (PSII) inhibiting herbicides in aquatic ecosystems (Lewis et al., 2009). However, there are

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Abbreviations

BHMT	Betaine–homocysteine S-methyltransferase 1	endoU	Poly(U)-specific endoribonuclease
CYP	Cytochrome p450	C1 Inh	Plasma protease C1 inhibitor
Cep85	Centrosomal protein of 85 kDa	CEPS	Ubiquitin-60S ribosomal protein L40
UDPGT	Uridine diphosphate glucuronosyltransferase	SPT	Serine–pyruvate aminotransferase
GCS	Glutamate–cysteine ligase catalytic subunit	CRBP-II	Cellular retinol-binding protein II
PAPSS 2	Functional 3′-phosphoadenosine 5′-phosphosulfate synthase 2	DECRI	2,4-dienoyl-CoA reductase
LDM	Lanosterol 14-alpha demethylase	FABP	Fatty Acid Binding Protein
RARRE	Retinoic acid receptor responder protein	EL	Endothelial lipase
MAT 2	Methionine adenosyltransferase 2	PAF-AH	Platelet-activating factor acetylhydrolase
TAT	Tyrosine aminotransferase	GT	Gastrotropin
CAT	Catalase	LBP	Lipopolysaccharide-binding protein
GST	Glutathione S Transferase	AOX	Peroxisomal acyl-coenzyme A oxidase
m GST	microsomal Glutathione S Transferase	LPIN1	Lipin-1
zDJ-1	Protein deglycase DJ-1zDJ-1	ACSBG2	Long-chain-fatty-acid–CoA ligase
GPx-1	Glutathione peroxidase 1	FERMT2	Fermitin family homolog 2
PHGPx	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	PLC	Phospholipase C
C1-B17.2	NADH-ubiquinone oxidoreductase subunit B17.2	gdpd 2	Glycerophosphodiester phosphodiesterase 2
FGH	Formylglutathione hydrolase	LK4	Lipid kinase 4
FALDH	Glutathione-dependent formaldehyde dehydrogenase	StARD11	StAR-related lipid transfer protein 11
GCS	Glutamate–cysteine ligase catalytic subunit	INP54	Phosphatidylinositol 4,5-bisphosphate 5-phosphatase
DHCR24	Delta(24)-sterol reductase	VLACS	Very long-chain acyl-CoA synthetase
Glx II	Glyoxalase II	SCD	Acyl-CoA desaturase
ndufa6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 6	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GRB2	Growth factor receptor-bound protein 2	PP1	Protein phosphatase 1
park2	E3 ubiquitin-protein ligase	PGM 1	Phosphoglucomutase-1
tmem161a	Transmembrane protein 161A	PGK1	Phosphoglycerate kinase 1
ZnF	Zinc finger like protein	TIM-B	Triosephosphate isomerase B
FASN	Fatty acid synthase	PGAM1	Phosphoglycerate mutase 1
CF	Complement factor	ATF	cAMP-dependent transcription factor
MyD88	Myeloid differentiation primary response protein	G6PD	Glucose-6-phosphate 1-dehydrogenase
CCL	CC- chemokine	GYS	Glycogen synthase
NF-κ	Nuclear factor NF-kappa	MDH	Malate dehydrogenase
IgE Fc γ ₁	High affinity immunoglobulin epsilon receptor subunit gamma	EMAP-2	Endothelial monocyte-activating polypeptide 2
RP	RNA polymerase	MAX	Myc-associated factor X
pS100B	S100 calcium-binding protein B	EGR-1	Early growth response protein 1
RSAD	Radical S-adenosyl methionine domain-containing protein	PKM	Pyruvate kinase
p53Lyn	Tyrosine-protein kinase Lyn	AKR1A1	Aldo-keto reductase
SPRK1	Serine/arginine-rich protein-specific kinase 1	GTase	Glucanotransferase
THBS-1	Thrombospondin-1	HPX	Hemopexin
Apo	Apoptosis-inducing ligand	CaM	Calmodulin
GPBP	Goodpasture antigen-binding protein	PI3K	Phosphatidylinositol 3-kinase
CAR	Coxsackievirus and adenovirus receptor homolog	PKB	Protein kinase B beta
		p53Lyn	Tyrosine-protein kinase Lyn
		GSK	Glycogen synthase kinase
		PCB	Pyruvic carboxylase
		GNMT	Glycine N-methyltransferase

also concerns relating to the impact of poor water quality on the health of local fish populations (e.g. Kroon et al., 2015; Hook et al., 2017a; b). The herbicides measured at elevated concentrations in GBR catchments have been shown to cause changes in fish health in laboratory studies. For example, exposure to atrazine caused decreased fecundity in fish continuously exposed to environmentally realistic (0.5 µg/L) concentrations of atrazine in laboratory studies (Rohr and McCoy, 2010; Tillitt et al., 2010), although the mechanism by which this occurs has not been established. However, the sublethal impacts on fish from exposure to many of the compounds present in the GBR catchments have not been determined (Kroon et al., 2015). Moreover, impacts on fish health from exposures to complex mixtures of herbicides, their breakdown products, and the adjuvants present in commercial pesticide products, that exist in the GBR catchments, also have not been elucidated.

Global gene expression is increasingly analysed via RNA-Seq

(Mehinto et al., 2012). Analysing global gene expression has the benefit of being able to identify changes in transcript levels, which suggest changes at the physiological level, without an *a priori* hypothesis as to which pathways are altered by changes in water quality (e.g., Hook et al., 2017a; b). The ability to measure physiological changes without a pre-identified mode of toxic action is an advantage when working with pesticides, many of which have not been studied at the molecular level in fish, and in field studies, where contaminants exist in complex mixtures and may interact (e.g., Gustavsson et al., 2017; Scott et al., 2018). In theory, changes in transcript abundance can also be used as evidence of altered signalling, a cellular response in adverse outcome pathways (e.g. Ankley et al., 2010), and be used to predict potential higher organism responses (Villeneuve et al., 2014). However, to be ecologically relevant, changes in the transcriptome need to be linked to a “higher level” change, such as increased incidence of disease. Changes in transcript abundance do not necessarily reflect changes in gene

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