Hepatic transcriptomic profiles from barramundi, Lates calcarifer, as a means of assessing organism health and identifying stressors in rivers in northern Queensland

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2   health and identifying stressors in rivers in northern Queensland

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19

20 **Abstract**

21 Resource managers need to differentiate between sites with and without contaminants and those
22 where contaminants cause impacts. Potentially, transcriptomes could be used to evaluate sites
23 where contaminant-induced effects may occur, to identify causative stressors of effects and
24 potential adverse outcomes. To test this hypothesis, the hepatic transcriptomes in Barramundi, a
25 perciforme teleost fish, (*Lates calcarifer*) from two reference sites, two agriculturally impacted sites
26 sampled during the dry season, and an impacted site sampled during the wet season were
27 compared. The hepatic transcriptome was profiled using RNA-Seq. Multivariate analysis showed
28 that transcriptomes were clustered based on site and by inference water quality, but not sampling
29 time. The largest differences in transcriptomic profile were between reference sites and a site
30 sampled during high run-off, showing that impacted sites can be identified via RNA-Seq. Transcripts
31 with altered abundance were linked to xenobiotic metabolism, peroxisome proliferation and stress
32 responses, indicating putative stressors with the potential for adverse outcomes in barramundi.

33 Graphical Abstract:

34
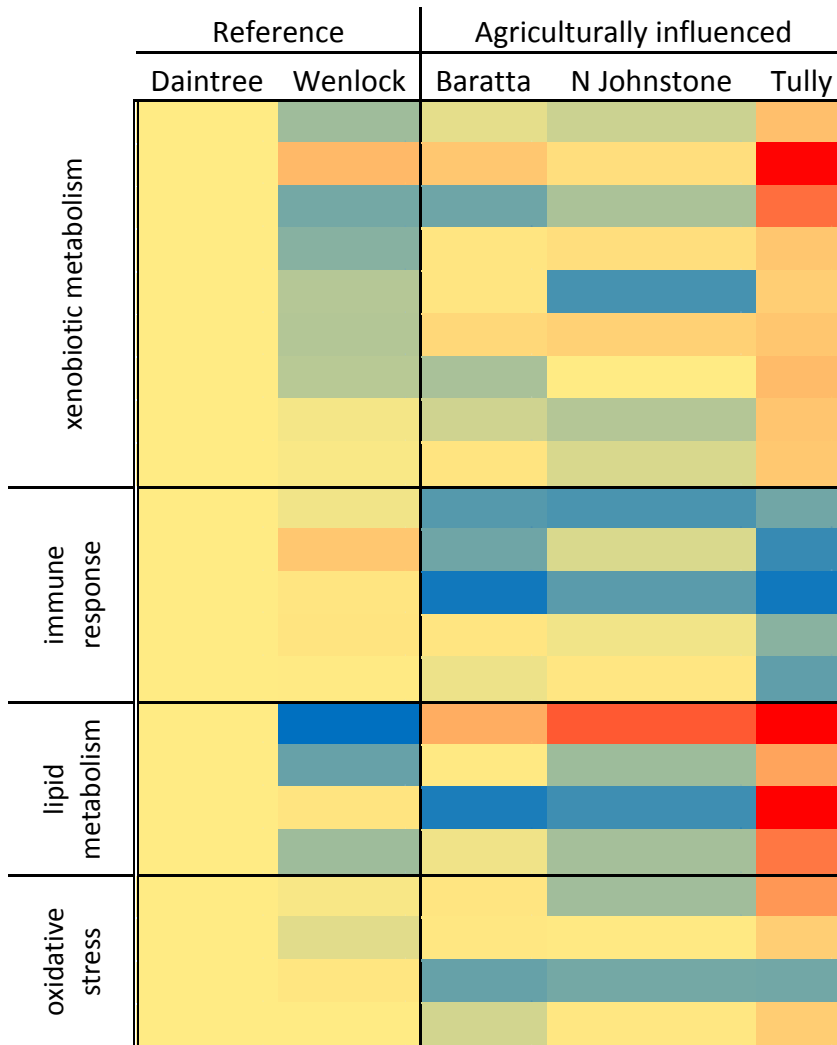
Highlights:

- Fish were collected from catchments with different agricultural land use patterns
- The hepatic transcriptome differed with different land use and water quality
- Transcripts for exposure biomarkers were used to tentatively identify stressors
- Functional pathways could contribute to predictions of adverse health outcomes

Keywords:

1. Introduction:

Few aquatic ecosystems are truly pristine. Many face increased pressure from modifications of waterways, changes in land-use patterns as a consequence of coastal development, industrial and agricultural discharges, introduced species and global climate change (Dafforn et al., 2012). Determining what impact, if any, these stressors are having on the health of key species or on ecosystem function as a whole can be challenging for resource managers, who often lack the logisitic support to characterise every aspect of a system. Since many of the stressors in the system may interact, their behaviour in the field may not be easily predicted from laboratory-based studies. For example, the impact of stressors such as endocrine disrupting chemicals (EDCs) may not be apparent from traditional toxicity tests (Hook et al., 2014a). Resource managers need metrics to use, both to classify systems as "unimpacted" or "impacted" so that they can be prioritised for protection or remediation, as well as to identify the causative agents of any declines in either organism or ecosystem health.

Several metrics for classifying the health of ecosystems already exist. One of these is the index of biotic integrity (IBI), which measures the capacity of an area to sustain populations of organisms with comparable composition, diversity, and function to natural areas within the same system. These comparisons are done by comparing the composition and biomass of selected taxa from impacted and unimpacted sites (Hughes et al., 1998; Bilkovic et al., 2006). This approach has been effectively used in a variety of systems (e.g. Hughes et al., 1998; Mebane et al., 2003; Bilkovic et al., 2006; An et al., 2002). However, this approach has disadvantages in that it cannot convey causal relationships between anthropogenic or natural stressors and changes in biotic integrity (Hughes et al., 1998). Also, this metric must be developed independently for each system in which it is intended to be used, and intensive field sampling, as well as in-depth prior knowledge of ecosystem composition and function, are required (Hughes et al., 1998).

Individual biomarker-based responses in fish have been used to differentiate between impacted and unimpacted sites (reviewed in Hook et al., 2014a), for example vitellogenin (Jobling and Tyler, 2003; Kroon et al., 2015a; Sumpter and Jobling, 1995) or the condition of gonads (Blazer et al., 2014), both of which were used to infer exposure to EDCs and the potential for impacts on reproduction. Vitellogenin, cytochrome p450 1A, biliary Fluorescent Aromatic Compounds, DNA damage, and histology have also been used to indicate exposure to sewage borne contaminants near waste water outfalls in Orange County, CA, USA (Roy et al., 2003). However, choosing the appropriate biomarker-based assay requires knowledge of the causative agent of toxicity and in some cases a change at the molecular level may be difficult to link to population level outcomes (reviewed in Hook et al., 2014a).

DNA metabarcoding has also been used recently to classify systems as being more or less degraded (e.g. Chariton et al., 2010; Baird and Hajibabaei, 2012; Dafforn et al., 2014; Gibson et al., 2015). While this approach has the advantage of being high throughput and taking more of a "whole ecosystem" approach (Dafforn et al., 2014; Gibson et al., 2015; Chariton et al., 2015), it only reports on the presence or absence of organisms, nothing about their condition, so these approaches cannot act as "early warning" of impending species loss. Further, while there are approaches to correlate changes in species abundance with environmental parameters (Chariton et al., 2015), it can be difficult to determine causality as many these parameters co-vary, such as increased nutrient levels,

85　decreased oxygen levels and increased micropollutants (as per Luo et al., 2014) that would all be
86　expected to be found near sewage treatment plants (Dafforn et al., 2014).

87　Here, we hypothesise that the transcriptome of a selected species could be used to not only classify
88　ecological sites as comparatively pristine or impacted, but also to identify potential causative agents
89　of changes in organism physiology. Since all transcripts are profiled, this approach does not require
90　a priori knowledge of the stressor or its modes of action. Since there is a vast amount of literature
91　on gene expression and ecotoxicology (Hook, 2010; Bahamonde et al., 2016;), transcripts that are
92　indicative of exposure to specific contaminants or classes of contaminants, such as those that
93　correspond to established exposure biomarkers (Hook et al., 2014a), could be identified. Also,
94　changes in the transcriptome could be used to identify potential population level impacts, via the
95　adverse outcome pathway concept (Ankley et al., 2010). A transcriptomic approach has been used
96　successfully in prior studies. For example, caged fathead minnow have been used to profile
97　differences in microarray based gene expression profiles due to agricultural land use patterns in
98　Nebraska, USA, watersheds (Jeffries et al., 2012). Multivariate models of microarray--based gene
99　expression fingerprints have also been used to predict the causative agent of toxicity in flounder
100　collected from contaminated sites in the United Kingdom (Falciani et al., 2008). In addition, qPCR
101　based transcriptional profiles have been used to predict the health outcomes of steelhead
102　Oncorhynchus mykiss from the Columbia River basin (Connon et al., 2012). However, both of these
103　approaches require prior knowledge of the genome sequence of the organism being studied, which
104　is frequently not available for non model organisms, especially in Australia. Instead, we used RNA
105　Seq to characterise the transcriptome (reviewed in Mehinto et al., 2012). RNA Seq, where
106　transcripts are reverse transcribed into cDNA libraries and quantified directly, has the advantage of
107　being able to be performed on organisms with an uncharacterised genome (Mehinto et al., 2012;
108　Hook et al., 2014b) and has a greater dynamic range than either qPCR or microarray hybridisation
109　(Tarazona et al., 2011).

110　To test the hypothesis that the transcriptome would indicate the presence of stressors in an
111　organism's habitat, the hepatic transcriptome of fish collected from different rivers in tropical North
112　Queensland was examined. Most of the study sites discharge into the Great Barrier Reef (GBR).
113　Some rivers in the system, including those included in this study, have poor water quality principally
114　due to increased loads of nutrients, sediment, and pesticides arising from agricultural land use
115　(reviewed in Brodie et al., 2012; Kroon et al., 2012; Smith et al., 2012). Rainfall in this area is
116　typically very seasonal, with frequent, heavy rain in November through March, and much lower
117　amounts the rest of the year. Concentrations of pesticides and other water quality related stressors,
118　such as sediments and high nutrient load, are typically highest during the rainy season (Smith et al.,
119　2012; Davis et al., 2012 Furthermore, aqueous concentrations of some stressors, such as diuron,
120　atrazine, terbuthiuron and metolachlor, are frequently measured above water quality guidelines
121　(Lewis et al., 2012; Smith et al., 2012). Concentrations of water soluble pesticides, such as atrazine
122　and imidacloprid, are typically below detection during the dry months. However, it is uncertain
123　whether elevated concentrations of stressors are sufficiently high to cause changes in the physiology
124　of fish species (Kroon et al., 2015a). In addition to changes in water quality, these watersheds have
125　been modified by changes in land use patterns, (e.g. deforestation, altered biogeochemical cycles),
126　which could have impacts on aquatic food webs, and as a consequence, fish health (Jeffrey et al.,
127　2015). We postulated that changes in the transcriptome that were a result of declining water quality
128　would be most apparent in fish collected from agriculturally impacted watersheds during the wet

season, whereas those that resulted from persistent contaminants or habitat changes would be apparent in all seasons.

In this paper, three hypotheses were tested: 1) that the hepatic transcriptome would differ between impacted and reference sites; 2) that the changes in the transcriptome would indicate seasonal stressor exposure; and 3) that changes in the transcriptome would contain transcripts for known contaminant exposure biomarkers (e.g. Hook et al., 2014a) that could be used to help identify putative causative stressors and other transcripts to could be related to physiological changes that might link to potential adverse outcomes. The study extended previous work examining changes in vitellogenin and aromatase levels in field-collected fish (Kroon et al., 2015a) and an initial survey of differences in global transcriptomic patterns at comparatively impacted (the Tully River) and a reference site (the Daintree River) (Hook et al., 2017). The previous study found large differences in the transcriptomic patterns, but we could not be certain that these were not just variability between different sites. We also could not be certain that the changes we measured in the previous study were related to water quality and not habitat modification. To address some of these uncertainties, this study examined an additional reference site but in this study two additional sites were examined during the dry season when the contaminant concentrations were expected to be low based on previous monitoring studies (Lewis et al., 2009) and the same two sites previously used by Hook et al. (2017). We used known exposure biomarkers in the transcriptomes and functional pathways with altered transcriptome abundance to identify sites and potential causative agents.

2.   Materials and methods:

2.1 Study organism

Barramundi *(Lates calcarifer)* (Bloch) (Family Latidae), a perciforme teleost fish, is of cultural importance to Australia's aboriginal peoples, and is fished extensively recreationally and commercially (Kailola et al., 1993). In northern Australia, barramundi lives in river and estuarine habitats. In northeast Queensland, they spawn near the mouths of estuaries prior to and during the start of the wet season, between November and January. Larval barramundi are found in coastal wetlands after December, with some migrating further upstream into freshwater habitats between March and June. The species is protandrous hermaphrotide, with juveniles maturing as males >250 mm total length (TL) in the first 2–4 years, and changing into females in the 680–900 mm TL size range.

2.2 Study site description

The five catchments from which barramundi were collected are located in North Queensland, Australia (Figure 1). These catchments differ in surface area, land uses, mean annual flow and river loads of sediment, nutrients and pesticides (Table 1). In the catchments of the two reference rivers agricultural land use is either completely absent (Wenlock) or covers a relatively small area (7%, Daintree). In contrast, agricultural land uses cover relatively larger areas in the Tully, North Johnstone and Barratta catchments, with horticulture crops (primarily bananas) and sugarcane being cultivated on the coastal floodplains. Conservation land use occupies large areas of the upper

169   catchments in the Daintree, North Johnstone and Tully Rivers. All rivers are perennial, with most of
170   the discharge occurring during the summer wet season (December – April). River pollutant loads of
171   sediment and nutrients to the GBR lagoon have increased substantially since European settlement in
172   the 1850s (Kroon et al., 2012; Waters et al., 2016), with increases particularly evident in the North
173   Johnstone, Tully and Barratta. In addition, over 50 pesticides have been detected in these three
174   rivers, with substantial loads of pesticides being transported to the GBR lagoon every year (Waters
175   et al., 2016).

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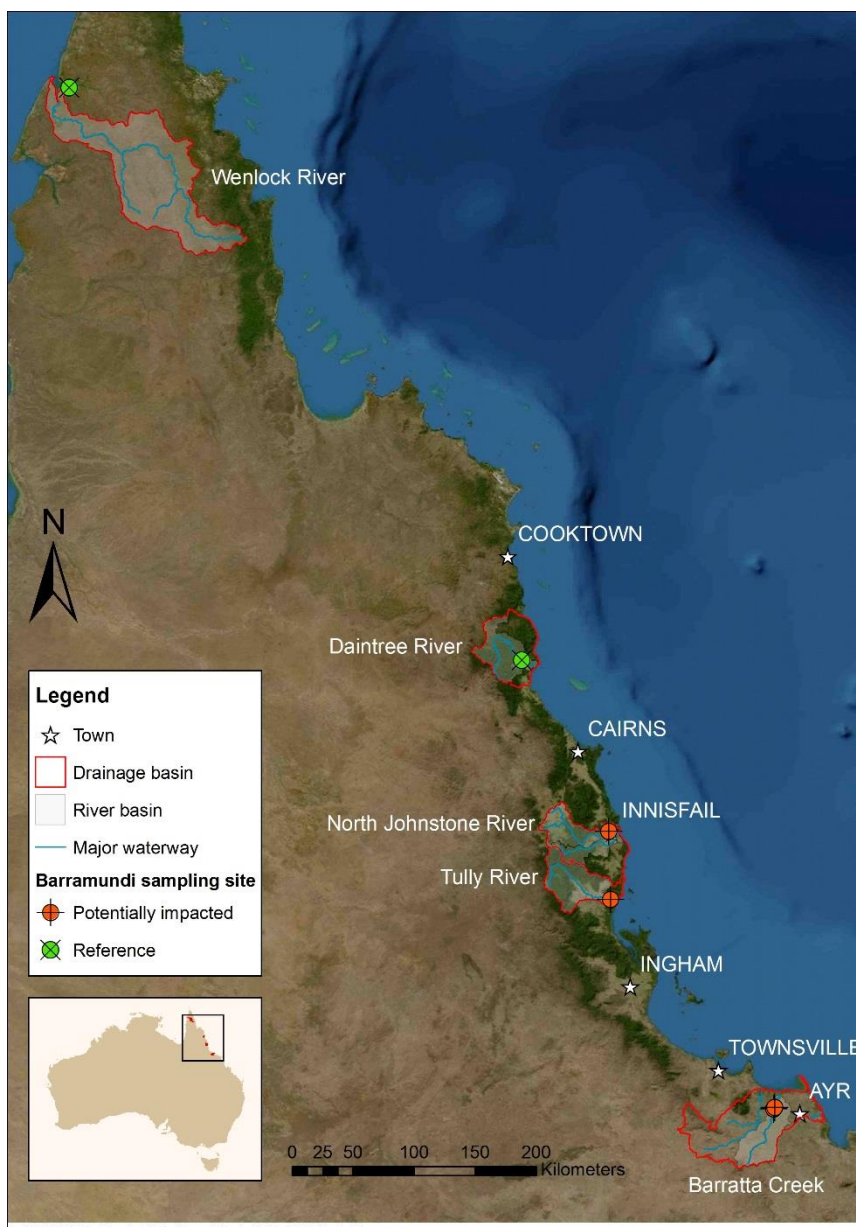
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179   Figure 1. Barramundi collection sites. Drainage basins are outlined in red.

180   Green spots indicate reference sites, whereas red spots indicate potentially impacted sites.

181   Table 1. Catchment information for collection sites of barramundi (*Lates calcarifer*). Catchment surface area, mean annual discharge, main land uses, and

182   river pollutant loads are given. TSS is total suspended sediment, TN is total nitrogen, TP is total phosphorus, and PSII herbicides is photosystem II herbicides.

183   PSII herbicide load is expressed as the annual toxicity-based herbicide loads (diuron equivalent loads, Escher et al., 2008). Land use data was taken from

184   Kroon et al., (2015); river pollutant loads from Waters et al. (2014).

River	Catchment surface area (km^2)	Main land uses (% of total catchment area)					Long-term mean annual discharge (GL)	River pollutant loads			
		Conser-vation	Forestry	Grazing	Horti-culture	Sugar-cane		TSS (t km^{-2})	TN (kg km^{-2})	TP (kg km^{-2})	PSII herbicides (kg y^{-2})
Wenlock~	7,525	64	<1	33	<1	<1	3,200[d]	Not measured	Not measured	Not measured	Not measured
Daintree	2,107	67	23	10	<1	2	925~	29	642	45	Not measured
North Johnstone	2,325	53	<1	40	<1	1	1,800	150	1,100	290	79
Tully	1,683	72	2	5	<1	13	3,100	67	1,100	100	270
Barratta (Haughton)	4,051	0	<1	73	<1	25	160	10	240	25	38

185   ~ based on long term monitoring from 108002A Daintree River at Bairds, note this will be an underestimate of total discharge from the basin

186

187   .

188 *2.3 Fish collections*

189 Barramundi were collected using monofilament gill nets (50 mm stretched mesh) in 2011 or 2012 via
190 purse seining, where fish were captured and immediately transferred to a water bath containing
191 anaesthetic (Kroon et al., 2015a) with the approval of the CSIRO Sustainable Ecosystems animal
192 ethics committee (permit #13-12) (Table 2). All fish collected were immature or mature males, and
193 smaller than the smallest length at which female gonadal features are first recognizable (680 mm TL;
194 Kailola et al., 1993), Fish were anaesthetised with clove oil (Kroon, 2015), then sacrificed by gill
195 slitting and cervical dislocation. Fish were immediately measured (mm, total length, TL), weighed
196 (g), then liver tissues were collected and small samples preserved in *RNA later*[©] (Ambion). Samples
197 were kept on ice until delivery to the laboratory then stored at -20°C until further processing.

198 Table 2. Collection information for barramundi (*Lates calcarifer*).

Collections		Classification				Fish	
Rivers	Dates	Status	Season	Number of fish	Size range (mm, TL)	K^1 (x 10^{-3})	Sample ID
Wenlock	23/04/2012	Reference	Changing towards dry	5	357–492	1.1–1.3	WC2–4; 55, 56
Daintree	22/03/2011	Reference	Wet	6	295–460	1.0–1.2	2–7
North Johnstone	27/06/2011	Impacted	Dry	5	192–350	1.0–1.2	402–406, 408
Tully	23/03/2012	Impacted	Wet	8	264–445	1.1–1.2	97–104
Barratta	25/05/2011	Impacted	Dry	6	240–421	1.0–1.2	353–355, 358–360

199 [1.] Fulton's condition index (K) was calculated as per Froese et al. (2006).

200 *2.4 RNA extraction*

201 The workflow used during the RNA-Seq experiment is described in Hook et al (2017) (Fig. S1). RNA
202 was extracted as described previously (Kroon et al., 2014; Kroon et al., 2015a;). Briefly,
203 approximately 20 mg of liver tissue was immersed in TRIzol[©] (Invitrogen) reagent, homogenized
204 using MP Biomedical[©] bead beater and lysing matrix E tubes, then extracted following the TRIzol[©]
205 protocol through the removal of the aqueous phase. The RNA was subsequently purified using the
206 Ambion Purelink kit After extraction, the TURBO DNA free[©] (Ambion) kit was used to eliminate
207 genomic DNA contamination. RNA purity was determined using a nanodrop spectrophotometer
208 (260/280 ratio greater than 2.0) and integrity determined using an Agilent bioanalyzer[©] (RIN greater
209 than 7.0).

210 *2.5 Sequencing*

211 Libraries for sequencing were created from hepatic RNA from each fish in Table 2 as an individual
212 (samples were not pooled). Sequencing for this project was performed at the Australian Genome
213 Research Facility. Briefly, cDNA synthesis and library preparation was carried out using a starting
214 template concentration of 1 µg RNA. Illumina's TruSeq stranded mRNA sample preparation was
215 used and the manufacturer's protocol was followed. The samples were run on an Illumina HiSeq
216 2000 with 100 base pair reads. The sample reads were deposited in the CSIRO data access portal and
217 at NCBI at PRJNA352735 (SRP093513). Data are also available via the CSIRO data access portal at
218 http://doi.org/10.4225/08/583766535a240.

219   *2.6 Quality Assessment and Read Mapping*

220 The Trimmomatic algorithm (Bolger et al., 2014) on the CSIRO instance of Galaxy (Goecks et al.,
221 2010) was used to filter reads for quality (Figure S1). Sequences were trimmed on a sliding window,
222 with a window size of 20 bases, a minimum quality score of 20 (Blankenberg et al., 2010) and the
223 number of bases to exclude set to 1. Read mapping against the whole barramundi transcriptome
224 derived in Hook et al. (submitted) (PRJNA290400) was performed using Bowtie (Langmead et al.,
225 2009) and quantified using the RSEM abundance estimation algorithm (Li and Dewey, 2011) with the
226 aid of scripts provided with Trinity (Grabherr et al., 2011; Haas et al., 2013); (r2014_04_13). The
227 numbers of total reads and mapped reads for each individual fish are in Table S1.

228   *2.7 Differential Expression Analysis*

229 Data (from both this and the previous study, Hook et al., 2017) were loaded into CLC Genomics
230 workbench version 8.0.3 for differential expression analysis. The previously published data were
231 included to expand the number of sites surveyed, and to ensure that more than one reference site
232 was examined. The previous data had a greater sequencing depth (22–31 M reads per sample) than
233 the current study did (12–14 M reads per sample). To account for differences in overall number of
234 reads per sample, FPKM (fragments per kilobase of transcript per million mapped reads) normalised
235 values were used in all subsequent analysis. Transcripts that did not have an average FPKM value of
236 one across all treatments were discarded to eliminate problems with false positives associated with
237 low read abundances (Tarazona et al., 2011). A total of 18,031 transcripts were included in the
238 differential abundance analysis. Approximately 95% of mapped reads (or 137.4 million reads
239 overall) mapped to these 18,031 contigs.   Differential expression was calculated using Gaussian
240 statistics, with an FDR rate of 0.05.

241   *2.8 Annotation*

242 In addition to annotating with BLAST2GO, as described previously (Hook et al., 2017), the
243 differentially abundant transcript lists were annotated by BLAST against the *Oreochromis niloticus,*
244 another perciforme fish, Ref Seq transcriptome, downloaded from NCBI on March 2, 2016 with an e
245 value cut off of $1e^{-5}$. Both annotations are included in lists of differentially expressed genes for
246 completeness. Differentially abundant contigs (putative transcripts assembled from sequencing
247 reads) were also BLASTx searched with an evalue cut off of $1e^{-5}$ against the UniProtKB/Swiss-prot
248 database using the CSIRO instance of Galaxy, their closest orthologue (or best match) identified, and
249 submitted to DAVID against a background of all contigs with the minimum read count subjected to
250 the same search criteria (Huang et al., 2009) for further functional annotation. Functional pathways
251 were also annotated by analysis against KEGG pathways in BLAST2GO (Conesa et al., 2005).

3. Results

3.1 Sequencing results

The Illumina 2500 HiSeq run generated 241 million reads. Once the reads were trimmed to remove the first base pairs that are non-random due to primer bias, virtually all reads were of sufficient quality to be used in mapping (Table S1). The number of reads per sample was lower in this study (12–14 million) than in our previous study (25–31 million) because the number of samples multiplexed per sequencing lane was higher in this study (16) than in the previous (14). The percentage that mapped in this study (53–64%), with the exception of one sample, was higher than the previous study (40–48%), despite the fact that the same Perl script and mapping algorithm were used. To account for these differences in read number and percent mapping, FPKM-corrected values were used in all subsequent analyses. Since large fold changes in low abundance transcripts are often artificial, only contigs with an average FPKM of one in at least one condition were considered. A total of 18,031 transcripts met this condition.

3.2 Transcriptomic profiling

The reads were mapped onto a previously developed transcriptome (Hook et al., submitted) which is available via the CSIRO DAP available via the CSIRO data access portal (http://doi.org/10.4225/08/583b5570b27e5). Briefly, that transcriptome contains 102,061 contigs. 27891 ORFs could be extracted from these contigs, representing coding sequences. Of these transcripts, 27,034 (96.9%) have an orthologue in the BLAST database. The read mapping revealed differences in the transcriptomic profiles between different fishes (Figure 2). There are large differences in the hepatic transcriptomes of fish collected from the Tully River relative to fish collected from other sites. These trends are also apparent if the data are visualised using hierarchical clustering (Figure 3) instead of PCA. If data are input based on the size of the fish collected or the date the fish was collected, no clear clusters are apparent (Figures S2 and S3).
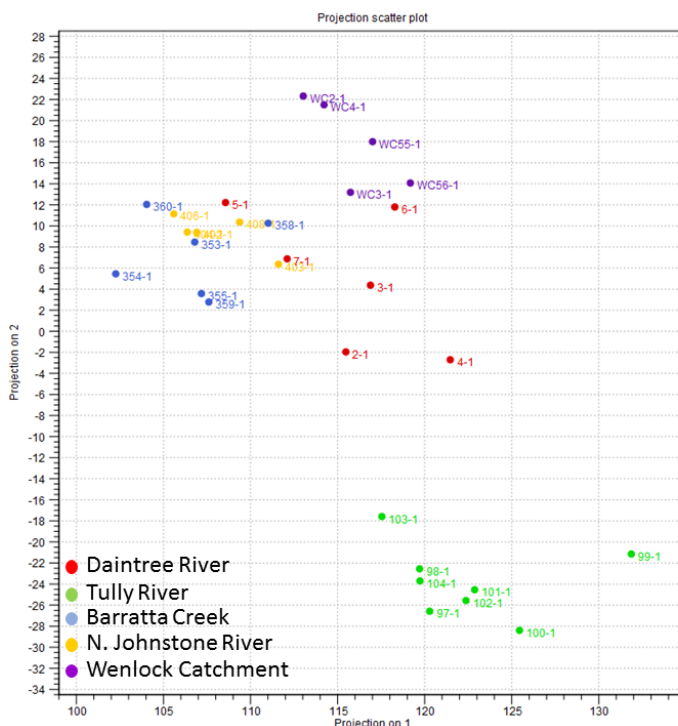
277   Figure 2. Principal components based visualisation of the plus one, log 10-transformed hepatic

278   transcriptome based on site where the fish was collected. Each dot represents an individual fish.
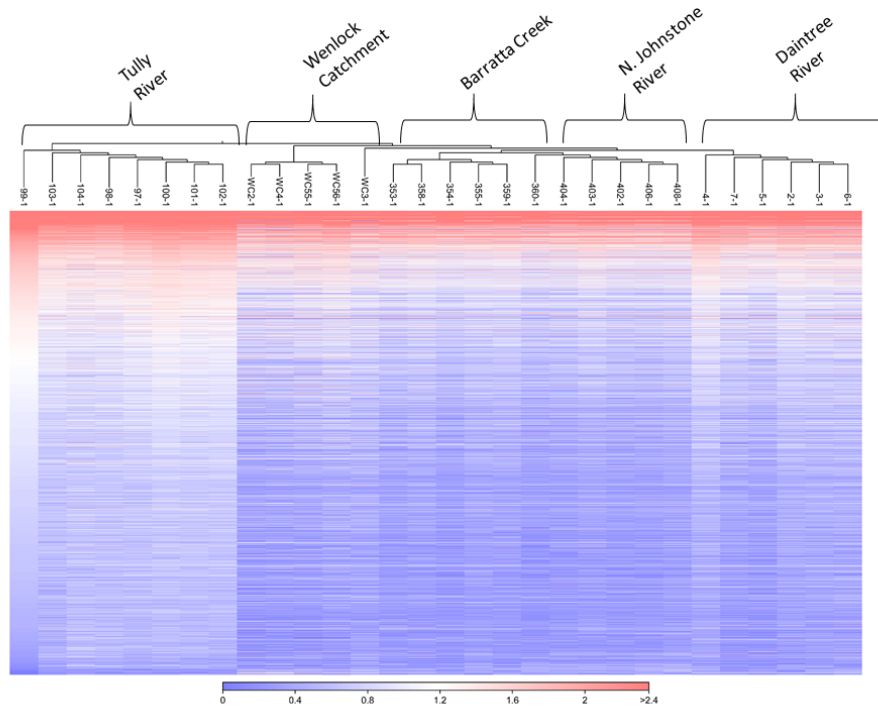
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280   Figure 3. Hierarchical clustering algorithm showing relationships between the hepatic transcriptome

281   of barramundi collected from different sites. Only transcripts with significantly different abundances

282   relative to both reference sites are plotted. The colour bar indicates +1 log transformed FPKM read

283   count.

284

285   *3.3 Differentially Abundant Transcripts*

286   There were more differentially abundant transcripts in the transcriptome of barramundi collected

287   from the Tully River compared to either Barratta Creek or the North Johnstone River (Table 4).

288   These transcripts, their closest orthologue as determined via BLAST against the *Oreochromis*

289   *niloticus* Ref Seq transcriptome, their mean abundances (as FPKM) in fish from each river, are

290   presented in Table S2. The Venn diagram shows little overlap amongst the transcripts differentially

291   abundant in each river (Figure S4).

292

293 Table 4. Matrixes showing the number of transcripts with significantly different abundances for each
294 comparison, relative to a total number of 18,031 transcripts.

295 By site:

Reference sites	Impacted sites			Reference Sites	
	Tully River	North Johnstone River	Barratta Creek	Daintree River	Wenlock River
Daintree River	4974	1846	817	-	84
Wenlock River	6964	4037	3591	88	-
Both	2610	303	861	-	-

296

297

298 Differences in the transcriptome in fish of different size were difficult to discern, as the main
299 differences were between fish captured at different sites (Figure S2). When the transcriptome was
300 compared by date collected, the patterns were compounded by site (Figure S3).

301 *3.4 Functional Annotation of Differentially Abundant Transcripts*

302 Transcripts for the pathway of excretion of xenobiotic compounds, which are established biomarkers
303 for exposure to organic contaminants, were elevated in fish from the agriculturally impacted North
304 Johnstone, Tully, and Barratta relative to the Daintree and Wenlock rivers (Figures 4A –C).
305 Transcripts encoding pesticide and nicotine-metabolising enzymes (cytochrome p450 2J,(CYP2J) a
306 Phase I oxidoreductase, and multiple isoforms of glutathione S transferase (GST) and UDPGT (uridine
307 diphosphate glucuronosyltransferase), conjugating enzymes involved in phase II metabolism), are all
308 significantly more abundant in fish from the Tully River relative to all other rivers (Figures 4 A and B
309 respectively). Transcripts encoding ATP binding cassettes (ABC transporters), which excrete
310 xenobiotic compounds from cells, were also more abundant in the hepatic transcriptomes of fish
311 from the Tully River (Figure 4C).

312 As reported previously (Hook et al., 2017), barramundi transcripts encoding gene products with an
313 immune related function were less abundant in fish from the agriculturally intensive rivers, the
314 North Johnstone, Tully and Barratta relative to the Daintree and Wenlock rivers (Figure 4D).
315 Chemokines, chemokine receptors, granzyme and t cell receptors encoding transcripts are all less
316 abundant in fish collected from the North Johnstone, Tully and Barratta compared to the Daintree
317 and Wenlock (Figure 4D and Table S2). A large number of transcripts encoding enzymes involved in
318 the metabolism of energy stores (lipid, protein or carbohydrate) were elevated in the livers of fish
319 collected from the Tully River, as reported previously (Hook et al., 2017), but not from the North
320 Johnstone and Barratta (Figure 4E and Table S2). These transcripts encoded peroxisome proliferator

321   activated receptor (PPAR) alpha, tyrosine aminotransferase, long-chain fatty acid ligase (lpl), and

322   apolipoproteins (apo - lipid binding proteins). Growth function genes, such as insulin-like growth

323   factor binding factor protein (igfbp), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), had

324   altered abundance in the Tully River when compared to the other rivers (Figure 4F). Transcripts

325   encoding leptin, the saiety hormone, was less abundant in the Tully River compared to other sites

326   (Table S2). Oxidative stress related transcripts, catalase (CAT), heme oxygenase (HO) and superoxide

327   dismutase (SOD), were also measured in increased abundances in the transcriptomes of fish

328   collected from the Tully River only (Figure 4G).

329   Transcripts encoding estrogen responsive gene products such as *vtg* and the zona pellucida (zp)

330   proteins were elevated in all rivers relative to the Daintree River (Figure 4H) , although not to the

331   Wenlock Catchment – the other reference site. Transcripts involved oogenesis including vitellogenin

332   were elevated in the North Johnstone, Tully and Barratta, while transcripts encoding the egg

333   membrane proteins were elevated in the Tully and Barratta. Cytochrome p450 3A (CYP3A)

334   transcripts were less abundant in fish from the Tully and Barratta compared to the Daintree and

335   Wenlock.

336   The majority of transcripts with differential abundance between the two reference sites could not be

337   annotated, making their functional significance difficult to discern. However, several ATPases were

338   approximately 5 fold more abundant in the transcriptomes of barramundi collected from the

339   Wenlock catchment relative to the Daintree River. Not enough of the transcripts with differential

340   abundance between reference sites could be sufficiently annotated to allow for mapping to

341   functional pathways.

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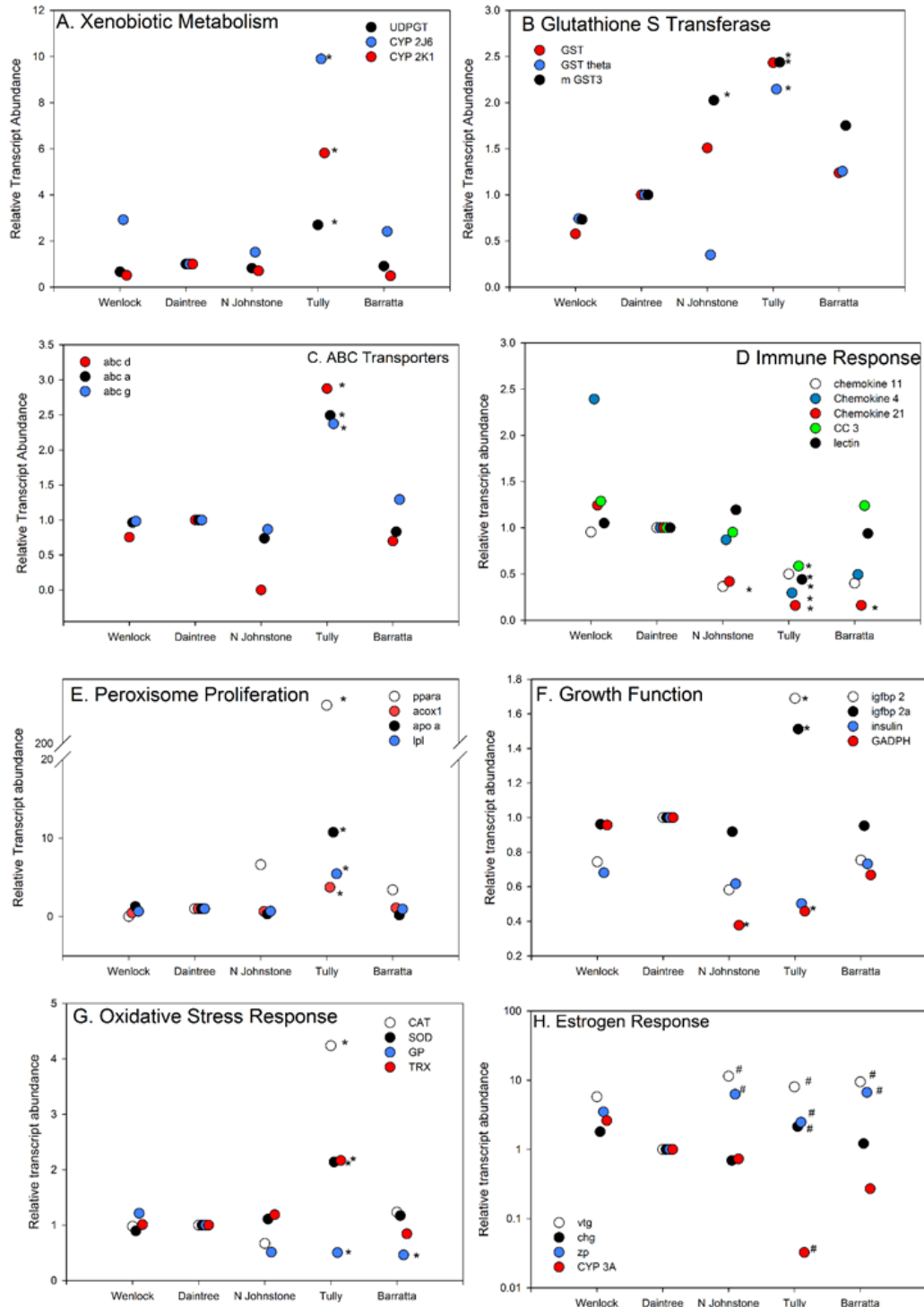
Figure 4. Mean transcript abundance for fish collected from each river, relative to the mean
abundance in the hepatic transcriptome of fish from the Daintree River (chosen because of the
larger samples size) of selected transcripts in different functional categories. Panel A: xenobiotic
metabolising enzymes; B: s glutathione S transferases C: ABC transporters; D: immune function
transcripts; E the peroxisome proliferator activated receptor alpha pathway; F growth function
transcripts; G: transcripts for oxidative stress response genes and panel H shows estrogen
responsive transcripts* indicate statistical significance FDR corrected p<0.05 from both the Daintree

350 and Wenlock values, # indicates statistical significance from the Daintree fish only. Standard
351 deviations are not shown for clarity. Data are slightly offset so that all symbols are visible.

352

353   *3.5 Alignment of Differentially Expressed Transcripts to Functional Pathways*

354 Because comparatively few contigs had an orthologue in the UniProt_Swiss Prot database, as
355 identified via a BLASTx search, the DAVID functional annotation platform (Huang et al., 2009)
356 identified very few functional categories that were significantly enriched amongst the differentially
357 abundant transcripts (Table 5, Table S3). Transcripts involved in protein tagging (phosphoproteins)
358 and alternative splicing, as well as hydrolases, were most significantly enriched (lowest p value ,
359 Benjamani Hochberg multiple test correction) in fish from the Tully River (Table S3). Transcripts
360 involved in disease, lipid metabolism and oxido-reductase activity were also significantly different (p
361 value <0.05 , Benjamani Hochberg multiple test correction) (Table 5). Immune-related transcripts,
362 including chemokines and cytokines, had decreased abundance in the transcriptomes of fish
363 collected from the Tully River (Table 5 and S3). No functional groups were significantly enriched
364 amongst those transcripts with increased abundance in the North Johnstone River or Barratta Creek,
365 and only cell adhesion was significantly enriched among the transcripts that were less abundant in
366 fish from the North Johnstone River (Table 5 and S3). In Barratta Creek, Pleckstrin homology (cell
367 signalling) and GTPase activity were the only functional categories significantly less abundant in the
368 transcriptome at that site (Table 5 and S3).

369 Table 5. A summary of the results of DAVID and KEGG pathways functional mapping. Full results
370 (including counts and p values) are given in Tables S3 and S4.

River	Relative abundance compared to the reference sites	Pathway	Evidence from
Tully	More abundant	Hydrolase, transferase, oxidoreductase, lipid metabolism	DAVID
		Lipid metabolism, glycolysis, glutathione metabolism, amino acid metabolism, Metabolism of xenobiotics by cytochrome P450, Steroid hormone biosynthesis	KEGG

	Less abundant	Chemokines (immune related transcripts)	DAVID
		glycolysis, amino acid metabolism	KEGG
N Johnstone	More abundant	N.A.	
	Less abundant	Cell adhesion	DAVID
Barratta Creek	More abundant	N.A.	
	Less abundant	GTPases, actin binding	DAVID
		Glycolysis / gluconeogenesis, cell signalling pathways	KEGG

371 N.A., not applicable, no significantly enriched functional categories were identified.

372

373 As with the DAVID analysis, comparatively few of the differentially abundant contigs could be
374 assigned an enzyme code, which is required for mapping to KEGG pathways. Nevertheless, a few
375 trends were apparent from this analysis, as summarised in Table 5. More transcripts that were
376 differentially abundant in the hepatic transcriptomes of Tully River fish could be mapped than the
377 other impacted or reference collection sites. As discussed previously (Hook et al., 2017), transcripts
378 that were more abundant in the hepatic transcriptomes of fish collected from the Tully River could
379 be mapped to a variety of pathways, including fatty acid degradation, glycolysis, metabolism of
380 xenobiotics via cytochrome p450, and steroid hormone biosynthesis (Table 5 and S4). Some of the
381 transcripts that were less abundant in the hepatic transcriptome of fish from the Tully could be
382 mapped to oxidative phosphorylation, the metabolism of different amino acids, and glycolysis (Table
383 5 and S4). By comparison, far fewer KEGG pathways were identified in the transcripts from the
384 other impacted rivers. Few transcripts with increased abundance that could be mapped to KEGG
385 pathways were observed in the hepatic transcriptomes from fish from North Johnstone River and
386 Barratta Creek. KEGG pathways for the metabolism of simple sugars such as galactose, starch and
387 pentose were less abundant in fish from the North Johnstone, whereas fish from Barratta Creek had
388 fewer transcripts involved in oxidative phosphorylation, T cell receptor signalling and the oxidation
389 of simple sugars (Table 5 and S4).

390 4. **Discussion**

391 In this study, we used the hepatic transcriptomic profiles of barramundi from rivers with different
392 land use patterns collected during the dry (low run off) and wet (high run off) seasons both to (i)
393 differentiate between impacted and reference sites; (ii) determine the extent to which the
394 transcriptome reflected seasonal changes in water quality; and (iii) identify potential stressors in
395 these systems using transcripts encoding known exposure biomarkers. Overall, the largest

differences in the global hepatic transcriptome were in barramundi from the Tully River collected during the wet season, demonstrating that the transcriptome can be used to identify impacted sites, supporting the first hypothesis. Compared to the Daintree catchment (a reference site), where barramundi were also collected during the wet season, the Tully (an impacted site) contains more sugarcane and horticulture upstream of the collection site than the Daintree collection site (Waters et al., 2016). Barramundi collected from the Tully River during the wet season had more differences in their transcriptomes relative to the two reference catchments and to those collected from the two other agriculturally impacted catchments (i.e., North Johnstone and Barratta) during periods of low run off, when pollutant levels are lower, supporting the second hypothesis. Few differences in the transcriptome could be attributed to wet versus dry season overall (e.g. pooling both reference and impacted areas) or attributed to different sequencing runs.

4.1 Transcripts for xenobiotic metabolising enzymes

Xenobiotic metabolising enzymes are commonly used, well established exposure biomarkers, and increases in their transcription levels can be used to surmise exposure to pharmacologically relevant doses of their substrate (Schlenk et al., 2008, Hook et al., 2014). The presence of these exposure biomarkers in the transcriptome can assist with putative stressor identification and thus supports the third hypothesis. Xenobiotics are often metabolised in a three phase pathway (Livingstone, 1991; Schlenk et al., 2008). The first phase occurs via oxidation, typically performed by a member of the cytochrome p450 family (Schlenk et al., 2008). Transcripts encoding CYP 2j and k transcripts of enzymes that metabolise pesticides in fish (Haasch et al., 1998; Lemaire et al., 2004) and nicotine in mammals (Hukkanen et al., 2005) were elevated in the hepatic transcriptomes of fish from all the agriculturally impacted sites compared to the reference sites – but only significantly ($p \leq 0.05$) in the fish from the Tully River. The second phase of metabolism is glucorondiation, which is often performed by a member of the GST family (Schlenk et al., 2008). Different transcript isoforms of GST were significantly ($p \leq 0.05$) more abundant in fish collected from the Tully River than the reference sites, although some contigs were elevated in fish from all agriculturally impacted rivers, which suggests exposure to lower levels of pesticides year round, consistent with the known patterns of contamination (e.g. Lewis et al., 2009) or another oxidative stressor. Exposure to atrazine is known to induce GST in fish (Egaas et al., 1993; Wiegand et al., 2000). Transcripts of UDPGT are also more abundant in fish from the Tully River, and like CYP2 proteins they are also involved in the metabolism of nicotine (Hukkanen et al., 2005) and potentially neonictinoid insecticides such as imidacloprid. The third phase of metabolism is excretion from the cell, which is typically carried out by an ABC transmembrane protein (Schlenk et al., 2008). Several different members of the ABC transporters, which excrete organic contaminants from cells (Leslie et al., 2005) had increased abundance in fish from the Tully River (Figure 4C). Collectively, transcripts of fish from the impacted sites indicate exposure to organic contaminants, most likely to commercial pesticide formulations that include both active ingredients and additives such as solvents, surfactants, or preservatives, as there are no other known sources of organic contaminants in the area (Cox and Surgan, 2006; Kroon et al., 2015a, b). Other xenobiotic metabolising enzymes were also induced in fish from the impacted sites. The elevations in these transcripts in the Tully River barramundi were discussed in our previous study (Hook et al., 2017).

4.2 Estrogen-responsive transcripts

439 Estrogen-responsive transcripts, such as zona pellucida and vitellogenin, were amongst the most
440 differentially abundant transcripts (Table S2). Normally, in female fish approaching spawning,
441 circulating levels of estrogen increase, setting off a cascade of events, including stimulating the liver
442 to start synthesising proteins required for oogenesis (reviewed in Swanson et al., 2003; Villeneuve et
443 al., 2007). These transcripts can also become more abundant following exposure to xenoestrogens,
444 including in juveniles and males (Jobling and Tyler, 2003). Transcripts encoding vitellogenin and the
445 egg shell proteins were significantly elevated in the male fish from the agricultural catchments
446 relative to male fish from the Daintree (Figure 4H). A common commercial herbicide formulation
447 used in the area contains surfactants such as alkylphenol ethoxylates (Kroon et al., 2015b).
448 Akylphenol ethoxylates are weakly estrogenic, and have been shown to cause elevations in the
449 abundances of transcripts encoding *vtg* and egg yolk proteins in both lab and field studies (Meucci
450 and Arukwe, 2005; Li et al., 2012; Schlenk et al., 2012). This includes controlled laboratory studies
451 on juvenile barramundi, during which similar, modest changes in transcript abundance were
452 observed following exposure to both the "surfactant booster", and the combination of herbicides
453 and the "surfactant booster" (Kroon et al., 2015b). In addition to an aqueous pathway, alkylphenol
454 ethoxylates have been shown to be persistent in sediments (Ferguson et al., 2003; Ferguson and
455 Brownawell, 2003; Ferguson et al., 2001) so fish could be exposed during foraging or feeding during
456 the dry season. The increased abundance of these transcripts in livers of male fish suggests
457 exposure to a xenoestrogen such as alkylphenol ethoxylates and thus supports the third hypothesis.

458 *4.3 Immune responsive transcripts*
459
460 Decreased abundance of some immune related transcripts were measured in all of the impacted
461 rivers. Decreased abundances of immune related transcripts may indicate toxicant induced
462 immunomodulation (reviewed in Segner et al., 2012). Previous studies have also noted changes in
463 the abundance of immune related transcripts following contaminant exposure. For instance,
464 following exposure to crude oil, decreased abundances of immune related transcripts was noted in
465 Japanese flounder (Nakayama et al., 2008), and similar changes in the transcriptome were also
466 measured in the head kidney of olive flounder exposed to benzo[α]pyrene (Hur et al., 2008) and in
467 rainbow trout exposed to xenoestrogens (Hook et al., 2008). Decreased levels of immune related
468 transcripts may also be a generalised stress response (Aluru and Vijayan, 2009). Without histological
469 examination, it can not be determined whether these changes indicate increased incidence of
470 disease.

471 *4.4 Transcripts with a metabolic function*

472 Some of the other changes in the fish transcriptome at agriculturally impacted sites suggested the
473 potential for adverse outcomes on both individuals and populations, also supporting the third
474 hypothesis. Changes in metabolism can be indicative of increased energy expenditure via stress.
475 Stressed animals would be expected to have increased utilisation of energy stores (lipid and protein),
476 as well as increased levels of gluconeogenesis (Philip et al., 2012). Increases in transcript abundance
477 of genes involved in lipid and amino acid metabolism, which is an expected stress response in fish
478 (Aluru and Vijayan, 2009), were measured only in the Tully River (Table 5, tables S3 and S4). A
479 previous study has noted an increase in protein metabolism in fish exposed to atrazine (McCarthy
480 and Fuiman, 2008), which is frequently detected in the Tully River (Smith et al., 2012). Other studies

481 have noted changes in osmoregulatory capacity and as a consequence decreased feeding in fish
482 exposed to atrazine (Nieves-Puigdoller et al., 2007). Whether the metabolic changes noted are
483 directly attributable to exposure to stressors or a consequence of changed feeding behaviour is not
484 known.

485 Changes in lipid metabolism and energy utilisation can also be indicative of a metabolic disorder.
486 PPARs regulate lipid and carbohydrate metabolism (Urbatzka et al., 2015), so changes in PPARs are
487 considered a form of metabolic endocrine disruption (Casals-Casas et al., 2008). Activation of PPAR
488 alpha has been shown to alter insulin resistance and alter patterns of lipid storage (Pavlikova et al.,
489 2010). Notably, these included an increase in the abundance of PPAR and acyl-coenzyme oxidase
490 (Pavlikova et al., 2010). Changes in PPARs have been noted following exposure to a variety of
491 anthropogenic contaminants, including organotins (Pavlikova et al., 2010), oil (Bilbao et al., 2010),
492 and nonylphenol (Cocci et al., 2013). A large increase (greater than 200 fold, table S2, figure 4E) in
493 the abundance of the transcript for PPARα was measured in fish from the Tully River, as well as an
494 increased abundance of transcripts for acyl-coenzyme a oxidase, fatty acid synthase, and for several
495 lipases. All of these transcripts have been induced in a previous study following exposure to
496 environmental contaminants and implicated in metabolic disorders such as steatosis disease
497 (Maradonna et al., 2015). Changes in transcript abundance for insulin-like growth factor-binding
498 protein and GADPH were also measured in fish from the Tully River. Previous studies have
499 hypothesised these proteins have a negative effect on gametogenesis, heart rate, and energy supply
500 for swimming (Urbatzka et al., 2015).

501 Both generalised stress and induction of the peroxisome proliferator pathway are thought to have
502 deleterious consequences for the immune system (reviewed in Aluru and Vijayan, 2009, DeWitt et
503 al., 2009). For example, exposure to Perfluorooctanoic acid and Perfluorooctane sulfonate, two
504 well-known peroxisome proliferating compounds, altered transcription of immune related genes in
505 cultured hepatocytes of the minnow (*Gobiocypris rarus*) (Wei et al., 2009). Cortisol was shown to
506 modulate the expression of cytokines and alter the inflammatory response in trout hepatocytes
507 (Philip et al., 2012). Previous studies have measured a decreased abundance of immune function
508 related transcripts such as various complement components and macroglobulin in rainbow trout
509 exposed to ethinyl estradiol (Hook et al., 2007), in flounder exposed to fuel oil (Nakayama et al.,
510 2008), in juvenile rainbow trout exposed to both atrazine and nonylphenol (Shelley et al., 2012) and
511 in delta smelt exposed to permethrin, a pyrethroid pesticide (Jeffries et al., 2015). We recorded a
512 decrease in the abundance of immune related transcripts in fish from the three agriculturally
513 impacted rivers relative to the fish from the reference rivers, with these decreases typically greatest
514 in fish collected from the Tully River, as was noted in our previous study (Hook et al., 2017).

515 Elevations in the levels of oxidative stress related transcripts (Table 5) could be a direct contaminant
516 response, or they could be indicative of higher levels of stress experienced in fish from the
517 agricultural catchments. For instance, in fish from the Tully River, an increased abundance of
518 transcripts of SOD and CAT were measured. These enzymes act in concert to detoxify superoxides
519 with superoxide dismutase reducing radical oxygen species to hydrogen peroxide, then catalase
520 reducing hydrogen peroxide to water and oxygen (Dalton et al., 2002). Oxidative stress responses
521 are a well known indicator of exposure to and effects of environmental contaminants (e.g. Maria et
522 al., 2009; Marigomez et al., 2013; Regoli et al., 2002). Previous studies have found increased
523 abundance of these transcripts in the hepatic transcriptome of brown trout exposed to the herbicide

glyphosate (Uren Webster and Santos, 2015). These transcripts were only measured in fish from the Tully River that were collected during the wet season. Changes in oxidative stress related transcripts may also be indicative of stress. For instance, increased abundance of transcripts for catalase were measured in migrating salmon in the Fraser River in British Columbia (Veldhoen et al., 2010), though the authors were not certain whether these were associated with contaminant body burdens or the metabolic costs of migration. Typically, in North Queensland, pesticide, nutrient, and sediment loads in the catchments are highest during the wet season (Turner et al., 2012, 2013; Wallace et al., 2014, 2015, 2016 and Garzon-Garcia et al., 2015). Hence, we suggest that Tully fish were exposed to the highest levels of stressors in the study.

Changes in transcript abundance could be a direct response of exposure to commercial pesticide formulations used in North Queensland (Kroon et al., 2015b), as previous studies have measured these changes in fish exposed to active ingredients such as atrazine and additives such as nonylphenol (Cocci et al., 2013). Alternatively, changes in transcript abundance could be a response to turbidity or other stressors in the environment known to correlate with elevated pesticide levels (Garzon-Garcia et al., 2015). Elevations in stress-related transcripts (though not the ones measured in this study) have been measured in delta smelt larvae exposed to high levels of turbidity (Hasenbien, 2016). Other changes that may result in stress, such as changes in habitat or loss of a prey species could also underpin the changes observed (Aluru and Vijayan, 2009). Nevertheless, changes in the abundance of stress related transcripts were only apparent in fish collected in the Tully during the wet season, and not in the two other agricultural catchments during the dry season. This suggests that any potential effect of habitat or prey alterations is overridden by effects of water quality. Regardless of the cause, prolonged exposure to stress can lead to the allocation of energy away from growth, reproduction and development, and could have negative consequences for the population (Hasenbien et al., 2016).

4.5 *Using transcriptomic signatures in environmental management*

At the onset of this research, it was not certain if there would be any differences between sites or if the site related differences would be masked by inter-individual variability. The results of the previous efforts (Hook et al., 2017) and this research clearly show that despite individual differences and presumably, different population structures (e.g. Keenan, 1994), the transcriptomic profiles had greater differences between sites than between individuals. The number of individual replicates (5-8) used in this study likely contributed to being able to differentiate between sites, individual variability would have had more influence if fewer individuals were being compared.

The results of this study also demonstrate the potential utility in using transcriptomic profiles in site management. As we stated in the introduction, at the onset of this study water quality in the GBR catchment area was known to be degraded (reviewed in Brodie et al., 2012; Kroon et al., 2012; Smith et al., 2012), but it was not clear whether these changes were having any impact on the fish in the catchment. Because of natural variability in fish populations, declines in abundance and their causative agents can be difficult to identify (Hamilton et al., 2016). Measuring changes in fish physiology has advantages over traditional endpoints such as length at age or abundance because these endpoints are slow to respond to stressors and do not provide any information about causality (Jeffreys et al., 2015).   However, since most of the pesticides in the catchment have not been well studied in fish, such that mode of action has been identified (Kroon et al., 2015; Pathiratne and

566   Kroon, 2016) there were a large number of physiological endpoints that could be monitored. The
567   RNA Seq study helped to resolve the uncertainty by showing that fish were exposed to a
568   pharmacologically relevant dose of pesticides (e.g. transcripts for xenobiotic metabolising enzymes
569   were more abundant). However, demonstrating exposure is not sufficient to prove chronic impact, a
570   link need to be made to another ecologically relevant endpoint, such as altered reproduction,
571   disease incidence or growth (Hamilton et al., 2016). The transcriptomic data suggested physiological
572   pathways that may be altered, including lipid, amino acid and primary metabolism, and these may
573   indicate a reduced energy budget (Groh et al., 2015a). This study also helped to direct future
574   monitoring efforts by identifying molecular endpoints (changes in lipid content and inflammation)
575   that will be surveyed in future studies. Also, since our previous work (Hook et al., 2017) was a single
576   comparison, this study provides further evidence that the changes observed can be attributed to
577   differences in water quality, as opposed to site specific changes.

578   This study also demonstrates how RNA Seq data can be integrated into adverse outcome pathway
579   (AOP) development to better understand the implications of contaminant exposure on fish
580   populations. As stated previously, a goal in ecotoxiciology is to translate from exposure (a molecular
581   initiating event in AOP terminology) to population level impacts (or adverse outcomes) (reviewed in
582   Garcia Reyero, 2014). The elevated transcripts for xenobiotic enzymes suggest exposure to
583   pesticides. This exposure may serve as a molecular initiating event in the adverse outcome pathway
584   (e.g. Villeneuve et al., 2014). The oxidative stress, altered metabolic pathways, and altered
585   abundance of immune transcripts are all suggestive of "key events" (e.g. physiological changes that
586   could alter organism health) (Villeneuve et al., 2014). If these effects are measurable at the whole
587   organism level and sufficient in duration and severity, may indicate a reduced energy budget in
588   these fish and reduced ecological resilience (Groh et al., 2015 et al., 2015 a,b, Hamilton et al., 2016).
589   In future work, we will integrate other physiological indices along our RNA Seq studies to assess the
590   potential for adverse outcomes.

591   Changes in water quality are likely only one of the factors driving differences in transcriptomic
592   profile. Other factors include natural variability as well as differences in hydrology, diet and other
593   natural variability (Hamilton et al., 2016). These factors may have contributed to the differences in
594   transcriptomic profile between the two reference sites, though the significance of these changes are
595   difficult to predict as many transcripts could not be identified.

596   *4.6 Conclusions*

597   The hepatic transcriptome profiles were able to indicate: 1) in which sites there is the greatest
598   potential for changes to fish health as indicated by alterations in transcript abundance (i.e. the Tully
599   River) supporting the first hypothesis; 2) the transcriptomes were most different in fish collected
600   from agriculturally impacted sites during the wet season, supporting the second hypothesis; 3) which
601   stressors are influencing the transcriptome (i.e., exposure biomarkers such as the xenobiotic
602   metabolising enzymes indicate exposure to pesticides, the xenoestrogens suggest exposure to
603   alkylphenol ethoxylates) supporting the third hypothesis; and 4) what are the potential adverse
604   outcomes associated with these stressors (i.e., decreased energetic reserves and immune capacity) if
605   substantiated in future studies using additional endpoints, also supporting the third hypothesis.
606   Collectively, these findings suggest that transcriptomic patterns may be a useful tool for resource
607   managers. The transcripts with altered abundance in the different rivers correspond to known

608 stressors in the catchments (e.g. pesticides and their surfactant co-factors), and confirms the
609 findings of our previous study (Hook et al., 2017). Furthermore, the transcripts suggest that
610 although some exposure to these compounds is occurring year round, barramundi may be
611 experiencing the most deleterious consequences during the wet season, as suggested by changes in
612 lipid metabolism and immune response. We hypothesise that since the altered transcript abundance
613 were most prevalent in fish collected from the Tully River during the wet season, pesticide runoff
614 from agriculture, as opposed to alterations in barramundi habitat, is the primary stressor. Our study
615 demonstrated that the hepatic transcriptome, as determined via RNA-Seq, could be an effective
616 means of classifying the relative degree of degradation of a site, and should be considered as a
617 management tool. Overall, this study not only provides information on the health of barramundi in
618 selected Great Barrier Reef catchments, it also demonstrates the utility of using the transcriptome to
619 monitor organism health and act as an early warning of potential deleterious biological impacts.

References:

631 Aluru, N., Vijayan, M.M., 2009. Stress transcriptomics in fish: A role for genomic cortisol signaling.
632 General and Comparative Endocrinology 164, 142-150.

634 An, K.G., Park, S.S., Shin, J.Y., 2002. An evaluation of a river health using the index of biological
635 integrity along with relations to chemical and habitat conditions. Environment International 28, 411-
636 420.

638 Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R.,
639 Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010.
640 Adverse Outcome Pathways: A Conceptual Framework to Support Ecotoxicology Research and Risk
641 Assessment. Environmental Toxicology and Chemistry 29, 730-741.

643 Bahamonde, P.A., Feswick, A., Isaacs, M.A., Munkittrick, K.R., Martyniuk, C.J., 2016. Defining the
644 Role of Omics in Assessing Ecosystem Health: Perspectives from the Canadian Environmental
645 Monitoring Program: Environmental Toxicology and Chemistry 35, 20-35.

647 Baird, D.J., Hajibabaei, M., 2012. Biomonitoring 2.0: a new paradigm in ecosystem assessment made
648 possible by next-generation DNA sequencing. Molecular Ecology 21, 2039-2044.

650 Bilbao, E., Raingeard, D., de Cerio, O.D., Ortiz-Zarragoitia, M., Ruiz, P., Izagirre, U., Orbea, A.,
651 Marigomez, I., Cajaraville, M.P., Cancio, I., 2010. Effects of exposure to Prestige-like heavy fuel oil

652 and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the
653 thicklip grey mullet Chelon labrosus. Aquatic Toxicology 98, 282-296.
654
655 Bilkovic, D.M., Roggero, M., Hershner, C.H., Havens, K.H., 2006. Influence of land use on
656 macrobenthic communities in nearshore estuarine habitats. Estuaries and Coasts 29, 1185-1195.
657 Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., Nekrutenko, A., Galaxy, T., 2010.
658 Manipulation of FASTQ data with Galaxy. Bioinformatics 26, 1783-1785.
659
660 Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., Nekrutenko, A., Galaxy, T., 2010.
661 Manipulation of FASTQ data with Galaxy. Bioinformatics 26, 1783-1785.
662
663 Blazer, V.S., Iwanowicz, D.D., Walsh, H.L., Sperry, A.J., Iwanowicz, L.R., Alvarez, D.A., Brightbill, R.A.,
664 Smith, G., Foreman, W.T., Manning, R., 2014. Reproductive health indicators of fishes from
665 Pennsylvania watersheds: association with chemicals of emerging concern. Environmental
666 Monitoring and Assessment 186, 6471-6491.
667
668 Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence
669 data. Bioinformatics 30, 2114-2120.
670
671 Brodie, J.E., Kroon, F.J., Schaffelke, B., Wolanski, E.C., Lewis, S.E., Devlin, M.J., Bohnet, I.C.,
672 Bainbridge, Z.T., Waterhouse, J., Davis, A.M., 2012. Terrestrial pollutant runoff to the Great Barrier
673 Reef: An update of issues, priorities and management responses. Marine Pollution Bulletin 65, 81-
674 100.
675
676 Casals-Casas, C., Feige, J.N., Desvergne, B., 2008. Interference of pollutants with PPARs: endocrine
677 disruption meets metabolism. International Journal of Obesity 32, S53-S61.
678
679 Chariton, A.A., Court, L.N., Hartley, D.M., Colloff, M.J., Hardy, C.M., 2010. Ecological assessment of
680 estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Frontiers in Ecology and the
681 Environment 8, 233-238.
682
683 Chariton, A.A., Stephenson, S., Morgan, M.J., Steven, A.D.L., Colloff, M.J., Court, L.N., Hardy, C.M.,
684 2015. Metabarcoding of benthic eukaryote communities predicts the ecological condition of
685 estuaries. Environmental Pollution 203, 165-174.
686
687 Cocci, P., Mosconi, G., Palermo, F.A., 2013. Effects of 4-nonylphenol on hepatic gene expression of
688 peroxisome proliferator-activated receptors and cytochrome P450 isoforms (CYP1A1 and CYP3A4) in
689 juvenile sole (Solea solea). Chemosphere 93, 1176-1181.
690
691 Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M., Robles, M., 2005. Blast2GO: a universal
692 tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21,
693 3674-3676.
694
695 Connon, R.E., D'Abronzo, L.S., Hostetter, N.J., Javidmehr, A., Roby, D.D., Evans, A.F., Loge, F.J.,
696 Werner, I., 2012. Transcription Profiling in Environmental Diagnostics: Health Assessments in
697 Columbia River Basin Steelhead (Oncorhynchus mykiss). Environmental Science & Technology 46,
698 6081-6087.
699
700 Cox, C. Surgan M. 2006. Unidentified inert ingredients in pesticides: Implications for human and
701 environmental health. Environmental Health Perspectives 12: 1803-1806.
702

703 Dafforn, K.A., Baird, D.J., Chariton, A.A., Sun, M.Y., Brown, M.V., Simpson, S.L., Kelaher, B.P.,
704 Johnston, E.L., 2014. Faster, Higher and Stronger? The Pros and Cons of Molecular Faunal Data for
705 Assessing Ecosystem Condition, in: Woodward, G., Dumbrell, A.J., Baird, D.J., Hajibabaei, M. (Eds.),
706 Advances in Ecological Research, Vol 51: Big Data in Ecology, pp. 1-40.

708 Dafforn, K.A., Simpson, S.L., Kelaher, B.P., Clark, G.F., Komyakova, V., Wong, C.K.C., Johnston, E.L.,
709 2012. The challenge of choosing environmental indicators of anthropogenic impacts in estuaries.
710 Environmental Pollution 163, 207-217.

712 Dalton, T.P., Puga, A., Shertzer, H.G., 2002. Induction of cellular oxidative stress by aryl hydrocarbon
713 receptor activation. Chemico-Biological Interactions 141, 77-95.

715 Davis, A.M., Lewis, S.E., Bainbridge, Z.T., Glendenning, L., Turner, R.D.R., Brodie, J.E., 2012. Dynamics
716 of herbicide transport and partitioning under event flow conditions in the lower Burdekin region,
717 Australia. Marine Pollution Bulletin 65, 182-193.

719 DeWitt, J.C., Shnyra, A, Badr, M, Loveless, SE, Hoban, D, Frame, S, Cunard, R, Anderson S, Meade, BJ,
720 et al. 2009. Immunotoxicity of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and the Role
721 of Peroxisome Proliferator-Activated Receptor Alpha. Critical reviews in Toxicology 39:76-94.

723 Egaas, E., Skaare, J.U., Svendsen, N.O., Sandvik, M., Falls, J.G., Dauterman, W.C., Collier, T.K.,
724 Netland, J., 1993.A compartive study of effects of atrazine on xenobiotic metabolisng enzymes in fish
725 and insect, and of the in vitro phase II atrazine metabolism in some fish, insects mammalsand one
726 plant species Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology
727 106, 141-149.

729 Falciani, F., Diab, A.M., Sabine, V., Williams, T.D., Ortega, F., George, S.G., Chipman, J.K., 2008.
730 Hepatic transcriptomic profiles of European flounder (Platichthys flesus) from field sites and
731 computational approaches to predict site from stress gene responses following exposure to model
732 toxicants. Aquatic Toxicology 90, 92-101.

734 Ferguson, P.L., Bopp, R.F., Chillrud, S.N., Aller, R.C., Brownawell, B.J., 2003. Biogeochemistry of
735 nonylphenol ethoxylates in urban estuarine sediments. Environmental Science & Technology 37,
736 3499-3506.

738 Ferguson, P.L., Brownawell, B.J., 2003. Degradation of nonylphenol ethoxylates in estuarine
739 sediment under aerobic and anaerobic conditions. Environmental Toxicology and Chemistry 22,
740 1189-1199.
741 Ferguson, P.L., Iden, C.R., Brownawell, B.J., 2001. Distribution and fate of neutral alkylphenol
742 ethoxylate metabolites in a sewage-impacted urban estuary. Environmental Science & Technology
743 35, 2428-2435.

745 Froese R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis
746 and recommendations. Journal of Applied Ichthyology. 2006;22:241–253. doi: 10.1111/j.1439-
747 0426.2006.00805.x

749 Garcia-Reyero, N. 2015. Are adverse outcome pathways here to stay? Environmental Science and
750 Technology 49: 3-9.

752 Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R,
753 Thomson B and Warne MStJ**. 2015. Total suspended solids, nutrients and pesticide loads (2013–

754 2014) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads
755 Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and
756 the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-
757 success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf .

759 Gibson, J.F., Shokralla, S., Curry, C., Baird, D.J., Monk, W.A., King, I., Hajibabaei, M., 2015. Large-Scale
760 Biomonitoring of Remote and Threatened Ecosystems via High-Throughput Sequencing. PloS one 10.

762 Goecks, J., Nekrutenko, A., Taylor, J., Galaxy, T., 2010. Galaxy: a comprehensive approach for
763 supporting accessible, reproducible, and transparent computational research in the life sciences.
764 Genome Biology 11.

766 Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L.,
767 Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F.,
768 Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome
769 assembly from RNA-Seq data without a reference genome. Nature Biotechnology 29, 644-U130.

771 Groh, K.J., Carvalho, R.N., Chipman, J.K., Denslow, N.D., Halder, M., Murphy, C.A., Roelofs, D., Rolaki,
772 A., Schirmer, K., Watanabe, K.H., 2015a. Development and application of the adverse outcome
773 pathway framework for understanding and predicting chronic toxicity: I. Challenges and research
774 needs in ecotoxicology. Chemosphere 120, 764-777.

776 Groh, K.J., Carvalho, R.N., Chipman, J.K., Denslow, N.D., Halder, M., Murphy, C.A., Roelofs, D., Rolaki,
777 A., Schirmer, K., Watanabe, K.H., 2015b. Development and application of the adverse outcome
778 pathway framework for understanding and predicting chronic toxicity: II. A focus on growth
779 impairment in fish. Chemosphere 120, 778-792.

781 Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles,
782 D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N.,
783 Westerman, R., William, T., Dewey, C.N., Henschel, R., Leduc, R.D., Friedman, N., Regev, A., 2013. De
784 novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference
785 generation and analysis. Nature Protocols 8, 1494-1512.

787 Haasch, M., Henderson, M., Buhler, D., 1998. Induction of CYP2M1 and CYP2K1 lauric acid
788 hydroxylase activities by peroxisome proliferating agents in certain fish species: Possible
789 implications. Marine Environmental Research 46, 37-40.

791 Hamilton, P.B., Cowx, I.G., Oleksiak, M.F., Griffiths, A.M., Grahn, M., Stevens, J.R., Carvahlo, G.R.,
792 Nicol, E., Tyler, C.R. 2016. Population-level consequences for a wild fish exposed to sublethal
793 concentrations of chemicals – a critical review. Fish and Fisheries 17: 545-566.

795 Hasenbien S, Lawler SP, Geist J, Connon RE 2016 A long-term assessment of pesticide mixture effects
796 on aquatic invertebrate communities. Environmental Toxicology and Chemistry 35: 218-232.

798 Hateley LR, Ellis R, Shaw M, Waters D, Carroll C. 2014. Modelling reductions of pollutant loads due to
799 improved management practices in the Great Barrier Reef catchments – Wet Tropics NRM region,
800 Cairns, Queensland.

802 Hook, S.E., Skillman, A.D., Small, J.A., Schultz, I.R., 2007. Temporal changes in gene expression in
803 rainbow trout exposed to ethynyl estradiol. Comparative Biochemistry and Physiology C-Toxicology
804 & Pharmacology 145, 73-85.

805

806   Hook, S.E., 2010. Promise and progress in environmental genomics: a status report on the
807   applications of gene expression-based microarray studies in ecologically relevant fish species.
808   Journal of Fish Biology 77, 1999-2022.

809

810   Hook, S.E., Gallagher, E.P., Batley, G.E., 2014. The role of biomarkers in the assessment of aquatic
811   ecosystem health. Integrated Environmental Assessment Management 10, 327-341.

812

813   Hook, S.E., Nagler, J.J., Cavileer, T., Verducci, J., Liu, Y., Hayton, W., Schultz, I.R., 2011. Relationships
814   between the transcriptome and physiological indicators of reproduction in female rainbow trout
815   over an annual cycle. Environmental Toxicology and Chemistry 30, 309-318.

816

817   Hook, S.E., Skillman, A.D., Small, J.A., Schultz, I.R., 2007. Temporal changes in gene expression in
818   rainbow trout exposed to ethynyl estradiol. Comparative Biochemistry and Physiology C-Toxicology
819   & Pharmacology 145, 73-85.

820

821   Hook, S.E., Kroon, F.J., Metcalfe, S., Moncuquet, P., McGrath, A., Greenfield, P., Smith, R., Warne, M.
822   St. J. Turner, R.D., McKeown, A., Westcott, D.A. in press. Global transcriptomic profiling in
823   barramundi *Lates calcarifer* from rivers impacted by differing agricultural land-uses*. Environmental
824   Toxicology and Chemistry 10.1002/etc.3505

825

826   Hook, S.E., Twine, N.A., Simpson, S.L., Spadaro, D.A., Moncuquet, P., Wilkins, M.R., 2014b. 454
827   pyrosequencing-based analysis of gene expression profiles in the amphipod Melita plumulosa:
828   Transcriptome assembly and toxicant induced changes. Aquatic toxicology 153, 73-88.

829

830   Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene
831   lists using DAVID bioinformatics resources. Nature Protocols 4, 44-57.

832

833   Hughes, R.M., Kaufmann, P.R., Herlihy, A.T., Kincaid, T.M., Reynolds, L., Larsen, D.P., 1998. A process
834   for developing and evaluating indices of fish assemblage integrity. Canadian Journal of Fisheries and
835   Aquatic Sciences 55, 1618-1631.

836

837   Hukkanen, J., Jacob, P., Benowitz, N.L., 2005. Metabolism and disposition kinetics of nicotine.
838   Pharmacological Reviews 57, 79-115.

839

840   Hur, D., Jeon, J-K., Hong, S. 2013. Analysis of immune gene expression modulated by
841   benzo[a]pyrene in head kidney of olive flounder (*Paralichthys olivaceus*). Comparative Biochemistry
842   and Physiology B 165: 49-57.

843

844   Jeffrey J.D., Hasler C.D., Chapman J.M., Cooke S.J., Suski C.D. 2015. Linking Landscape-Scale
845   Disturbances to Stress and Condition of Fish: Implications for Restoration and Conservation. Integr
846   Comp Biol. 55: 618-630.

847

848   Jeffries, K.M., Komoroske, L.M., Truong, J., Werner, I., Hasenbein, M., Hasenbein, S., Fangue, N.A.,
849   Connon, R.E., 2015. The transcriptome-wide effects of exposure to a pyrethroid pesticide on the
850   Critically Endangered delta smelt Hypomesus transpacificus. Endangered Species Research 28, 43-60.

851

852   Jeffries, M.K.S., Mehinto, A.C., Carter, B.J., Denslow, N.D., Kolok, A.S., 2012. Taking Microarrays to
853   the Field: Differential Hepatic Gene Expression of Caged Fathead Minnows from Nebraska
854   Watersheds. Environmental Science & Technology 46, 1877-1885.

855

856 Jobling, S., Tyler, C.R., 2003. Endocrine disruption in wild freshwater fish. Pure and Applied
857 Chemistry 75, 2219-2234.
858
859 Kailola, P.J., Williams, M.J., Stewart, P.C., Reichelt, R.E., McNee, A., and Grieve, C. (1993) 'Fisheries
860 Resources.' (Bureau of Resource Sciences, Department of Primary Industries and Energy, and the
861 Fisheries Research and Development Corporation: Canberra) 422 pp
862 Keenan, C.P. 1994. Recent evolution of population structure in Australian barramundi, *Lates*
863 *calcarifer* (Bloch): an example of isolation by distance in one dimension. Australian Journal of
864 Marine and Freshwater Research. 45:1123-1148.
865
866 Kroon, F.J., Hook, S.E., Jones, D., Metcalfe, S., Osborn, H.L., 2014. Effects of Atrazine on
867 Endocrinology and Physiology in Juvenile Barramundi Lates calcarifer (Bloch). Environmental
868 Toxicology and Chemistry 33, 1607-1614.
869
870 Kroon, F.J., Hook, S.E., Jones, D., Metcalfe, S., Henderson, B., Smith, R., Warne, M.S., Turner, R.D.,
871 McKeown, A., Westcott, D.A., 2015a. Altered transcription levels of endocrine associated genes in
872 two fisheries species collected from the Great Barrier Reef catchment and lagoon. Marine
873 Environmental Research 104, 51-61.
874
875 Kroon, F.J., Hook, S.E., Metcalfe, S., Jones, D., 2015b. Altered levels of endocrine biomarkers in
876 juvenile barramundi (Lates calcarifer; Bloch) following exposure to commercial herbicide and
877 surfactant formulations. Environmental Toxicology and Chemistry 34:1881-1890.
878
879 Kroon, FJ.. 2015. The efficacy of clove oil for anaesthesia of eight species of Australian tropical
880 freshwater teleosts. Limnology & Oceanography: Methods 13, 463-475. DOI: 10.1002/lom3.10040
881 Kroon, F.J., Kuhnert , P.M., Henderson, B.L., Wilkinson, S.N., Kinsey-Henderson, A., Abbott, B.,
882 Brodie, J.E., Turner, R.D.R., 2012. River loads of suspended solids, nitrogen, phosphoturs and
883 herbicides delivered to the Great Barrier Reef lagoon. Marine Pollution Bulletin 65, 167-181.
884
885 Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of
886 short DNA sequences to the human genome. Genome Biology 10.
887
888 Lemaire, G., de Sousa, G., Rahmani, R. 2004. A PXR reporter gene assay in a stable cell culture
889 system: CYP3A4 and CYP2B6 induction by pesticides. Biochemical Pharmacology 68, 2347-2358.
890 Leslie, E.M., Deeley, R.G., Cole, S.P.C., 2005. Multidrug resistance proteins: role of P-glycoprotein,
891 MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicology and Applied Pharmacology 204, 216-
892 237.
893
894 Lewis, S.E., Brodie, J.E., Bainbridge, Z.T., Rohde, K.W., Davis, A.M., Masters, B.L., Maughan, M.,
895 Devlin, M.J., Mueller, J.F., Schaffelke, B., 2009. Herbicides: A new threat to the Great Barrier Reef.
896 Environmental Pollution 157, 2470-2484.
897
898 Lewis, S.E., Schaffelke, B., Shaw, M., Bainbridge, Z.T., Rohde, K.W., Kennedy, K., Davis, A.M., Masters,
899 B.L., Devlin, M.J., Mueller, J.F., Brodie, J.E., 2012. Assessing the additive risks of PSII herbicide
900 exposure to the Great Barrier Reef. Marine Pollution Bulletin 65, 280-291.
901
902 Li, B., Dewey, C.N., 2011. RSEM: accurate transcript quantification from RNA-Seq data with or
903 without a reference genome. BMC Bioinformatics 12, 16.
904

905 Li, Z., Zhang, H., Gibson, M., Liu, P., 2012. An evaluation of the combined effects of phenolic
906 endocrine disruptors on vitellogenin induction in goldfish Carassius auratus. Ecotoxicology 21, 1919-
907 1927.
908
909 Livingstone, D.R., 1991. Organic xenobiotic metabolism in marine invertebrates. Advances in
910 Comparative and Environmental Physiology 7, 45-185.
911
912 Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C. 2014. A review on
913 the occurrence of micropollutants in the aquatic environment and their fate and removal during
914 wastewater treatment. Science of the Total Environment.
915
916 Maradonna, F., Nozzi, V., Santangeli, S., Traversi, I., Gallo, P., Fattore, E., Mita, D.G., Mandich, A.,
917 Carnevali, O., 2015. Xenobiotic-contaminated diets affect hepatic lipid metabolism: Implications for
918 liver steatosis in Sparus aurata juveniles. Aquatic Toxicology 167, 257-264.
919
920 Maria, V.L., Santos, M.A., Bebianno, M.J., 2009. Biomarkers of damage and protection in Mytilus
921 galloprovincialis cross transplanted in Ria Formosa Lagoon (Portugal). Ecotoxicology 18, 1018-1028.
922
923 Marigomez, I., Zorita, I., Izagirre, U., Ortiz-Zarragoitia, M., Navarro, P., Etxebarria, N., Orbea, A., Soto,
924 M., Cajaraville, M.P., 2013. Combined use of native and caged mussels to assess biological effects of
925 pollution through the integrative biomarker approach. Aquatic Toxicology 136, 32-48.
926
927 McCarthy, I.D., Fuiman, L.A., 2008. Growth and protein metabolism in red drum (Sciaenops
928 ocellatus) larvae exposed to environmental levels of atrazine and malathion. Aquatic Toxicology 88,
929 220-229.
930
931 Mebane, C.A., Maret, T.R., Hughes, R.M., 2003. An index of biological integrity (IBI) for Pacific
932 Northwest rivers. Transactions of the American Fisheries Society 132, 239-261.
933
934 Mehinto, A.C., Martyniuk, C.J., Spade, D.J., Denslow, N.D., 2012. Applications of next-generation
935 sequencing in fish ecotoxicogenomics. Frontiers in Genetics 3, 62-Article No.: 62.
936
937 Meucci, V., Arukwe, A., 2005. Detection of vitellogenin and zona radiata protein expressions in
938 surface mucus of immature juvenile Atlantic salmon (Salmo salar) exposed to waterborne
939 nonylphenol. Aquatic Toxicology 73, 1-10.
940
941 Nakayama, K., Kitamura, S.-I., Murakami, Y., Song, J.-Y., Jung, S.-J., Oh, M.-J., Iwata, H., Tanabe, S.,
942 2008. Toxicogenomic analysis of immune system-related genes in Japanese flounder (Paralichthys
943 olivaceus) exposed to heavy oil. Marine Pollution Bulletin 57, 445-452.
944
945 Nieves-Puigdoller, K., Bjornsson, B.T., McCormick, S.D., 2007. Effects of hexazinone and atrazine on
946 the physiology and endocrinology of smolt development in Atlantic salmon. Aquatic Toxicology 84,
947 27-37.
948
949 Pathiratne, A., Kroon, F.J., 2016. Using species sensitivity distribution approach to assess the risks of
950 commonly detected agricultural pesticides to Australia's tropical freshwater ecosystems.
951 Environmental Toxicology and Chemistry 35, 419-428.
952
953 Pavlikova, N., Kortner, T.M., Arukwe, A., 2010. Peroxisome proliferator-activated receptors,
954 estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and
955 second messenger activator. Aquatic Toxicology 99, 176-185.

956

957 Philip, A.M., Kim, S.D., Vijayan, M.M., 2012. Cortisol modulates the expression of cytokines and
958 suppressors of cytokine signaling (SOCS) in rainbow trout hepatocytes. Developmental and
959 Comparative Immunology 38, 360-367.

960

961 Regoli, F., Gorbi, S., Frenzilli, G., Nigro, M., Corsi, I., Focardi, S., Winston, G.W., 2002. Oxidative stress
962 in ecotoxicology: from the analysis of individual antioxidants to a more integrated approach. Marine
963 Environmental Research 54, 419-423.

964

965 Roy, L.A., Armstrong, J.L., Sakamoto, K., Steinert, S., Perkins, E., Lomax, D.P., Johnson, L.L., Schlenk,
966 D., 2003. The relationships of biochemical endpoints to histopathology and population metrics in
967 feral flatfish species collected near the municipal wastewater outfall of Orange County, California,
968 USA. Environmental Toxicology and Chemistry 22, 1309-1317.

969

970 Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., van den Hurk, P.,
971 Willett, K., 2008. Biotransformation in fishes. In: The Toxicology of Fishes (R. Di
972 Giulio and D. Hinton editors). Taylor and Francis Publishers, Washington DC pp 153-
973 234.

974

975 Schlenk, D., Lavado, R., Loyo-Rosales, J.E., Jones, W., Maryoung, L., Riar, N., Werner, I., Sedlak, D.,
976 2012. Reconstitution Studies of Pesticides and Surfactants Exploring the Cause of Estrogenic Activity
977 Observed in Surface Waters of the San Francisco Bay Delta. Environmental Science & Technology 46,
978 9106-9111.

979

980 Segner, H., Wenger, M., Moeller, A.M., Koellner, B., Casanova-Nakayama, A., 2012. Immunotoxic
981 effects of environmental toxicants in fish - how to assess them? Environmental Science and Pollution
982 Research 19, 2465-2476.

983

984 Shelley, L.K., Ross, P.S., Miller, K.M., Kaukinen, K.H., Kennedy, C.J., 2012. Toxicity of atrazine and
985 nonylphenol in juvenile rainbow trout (Oncorhynchus mykiss): Effects on general health, disease
986 susceptibility and gene expression. Aquatic Toxicology 124, 217-226.

987

988 Smith, R., Middlebrook, R., Turner, R., Huggins, R., Vardy, S., Warne, M.St.J., 2012. Large-scale
989 pesticide monitoring across Great Barrier Reef catchments - Paddock to Reef Integrated Monitoring,
990 Modelling and Reporting Program. Marine Pollution Bulletin 65, 117-127.

991

992 Sumpter, J.P., Jobling, S., 1995. Vitellogenesis as a biomarker for estrogenic contamination of the
993 aquatic environment. Environmental Health Perspectives 103, 173-178.

994

995 Swanson, P., Dickey, J.T., Campbell, B., 2003. Biochemistry and physiology of fish gonadotropins. Fish
996 Physiology and Biochemistry 28, 53-59.

997

998 Tarazona, S., Garcia-Alcalde, F., Dopazo, J., Ferrer, A., Conesa, A., 2011. Differential expression in
999 RNA-seq: A matter of depth. Genome Research 21, 2213-2223.

1000

1001 Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and **Warne MStJ**. 2013. Total suspended solids,
1002 nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef
1003 Loads Monitoring 2010–2011. Water Sciences Technical Report, Volume 2013, Number 1.
1004 Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland,
1005 Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from:

1006 http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-
1007 catchment-loads-report.pdf
1008
1009 Turner R, Huggins R, Wallace R, Smith R, Vardy S and **Warne MStJ**. 2012. Sediment, nutrient and
1010 pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010. Water Sciences Technical Report,
1011 Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts,
1012 Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from:
1013 http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-
1014 catchment-loads-report.pdf
1015
1016 Urbatzka, R., Galante-Oliveira, S., Rocha, E., Lobo-da-Cunha, A., Castro, L.F.C., Cunha, I., 2015. Effects
1017 of the PPAR alpha agonist WY-14,643 on plasma lipids, enzymatic activities and mRNA expression of
1018 lipid metabolism genes in a marine flatfish, Scophthalmus maximus. Aquatic Toxicology 164, 155-
1019 162.
1020
1021 Uren Webster TM, Santos, EM 2015 Global transcriptomic profiling demonstrates induction of
1022 oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate
1023 and Roundup. BMC Genomics 16: 32.
1024
1025 Veldhoen, N., Ikonomou, M.G., Dubetz, C., MacPherson, N., Sampson, T., Kelly, B.C., Helbing, C.C.,
1026 2010. Gene expression profiling and environmental contaminant assessment of migrating Pacific
1027 salmon in the Fraser River watershed of British Columbia. Aquatic Toxicology 97, 212-225.
1028
1029 Villeneuve, D.L., Larkin, P., Knoebl, I., Miracle, A.L., Kahl, M.D., Jensen, K.M., Makynen, E.A., Durhan,
1030 E.J., Carter, B.J., Denslow, N.D., Ankley, G.T., 2007. A graphical systems model to facilitate
1031 hypothesis-driven ecotoxicogenomics research on the teleost brain-pituitary-gonadal axis.
1032 Environmental Science & Technology 41, 321-330.
1033
1034 Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A.,
1035 Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., Ottinger, M.A., Vergauwen, L., Whelan, M.,
1036 2014. Adverse Outcome Pathway (AOP) Development I: Strategies and Principles. Toxicological
1037 Sciences 142, 312-320.
1038
1039 Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Severino Z,
1040 Smith RA, Warne MStJ, Turner RDR, and Mann RM. 2016. Total suspended solids, nutrients and
1041 pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef
1042 Catchment Loads Monitoring Program 2014–2015. Department of Science, Information Technology,
1043 Innovation and the Arts, Brisbane, Queensland, 111pp. DOI: 10.131140/RG.2.2.17851.62240.
1044 Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-
1045 2015-gbr-catchment-loads-summary.pdf
1046 Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and **Warne MStJ.** 2015. Total suspended
1047 solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef –
1048 Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science,
1049 Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from:
1050 http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-
1051 catchment-loads-report.pdf

1052   Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and **Warne MStJ**. 2014. Total suspended solids,
1053   nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef – Great
1054   Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information
1055   Technology, Innovation and the Arts. Brisbane, Queensland. 94p. Available from:
1056   http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-
1057   catchment-loads-report.pdf

1058   Waters, D.K., Carroll, C., Ellis, R., Hateley, L., McCloskey, G.L., Packett, R., Dougall, C., Fentie, B.,
1059   2014. Modelling reductions of pollutant loads due to improved management practices in the Great
1060   Barrier Reef catchments – Whole of GBR, Technical Report, Volume 1, Queensland Department of
1061   Natural Resources and Mines, Toowoomba, Queensland (ISBN: 978-1- 7423-0999). Available from:
1062   http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/great-barrier-reef-
1063   catchment-modelling-report.pdf .

1064   Webster, T.M.U., Bury, N., van Aerle, R., Santos, E.M., 2013. Global Transcriptome Profiling Reveals
1065   Molecular Mechanisms of Metal Tolerance in a Chronically Exposed Wild Population of Brown Trout.
1066   Environmental Science & Technology 47, 8869-8877.
1067
1068   Wei, YH, Shi, XJ, Zhang, HX, Wang, JS, Zhou, BS, Dai JY., 2009. Combined effects of polyfluorinated
1069   and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris*
1070   *rarus*) using toxicogenomic analysis. Aquatic Toxicology 95,27-36.

1071
1072   Wiegand, C., Pflugmacher, S., Giese, M., Frank, H., Steinberg, C., 2000. Uptake, toxicity, and effects
1073   on detoxication enzymes of atrazine and trifluoroacetate in embryos of zebrafish. Ecotoxicology and
1074   Environmental Safety 45, 122-131.

1075
1076
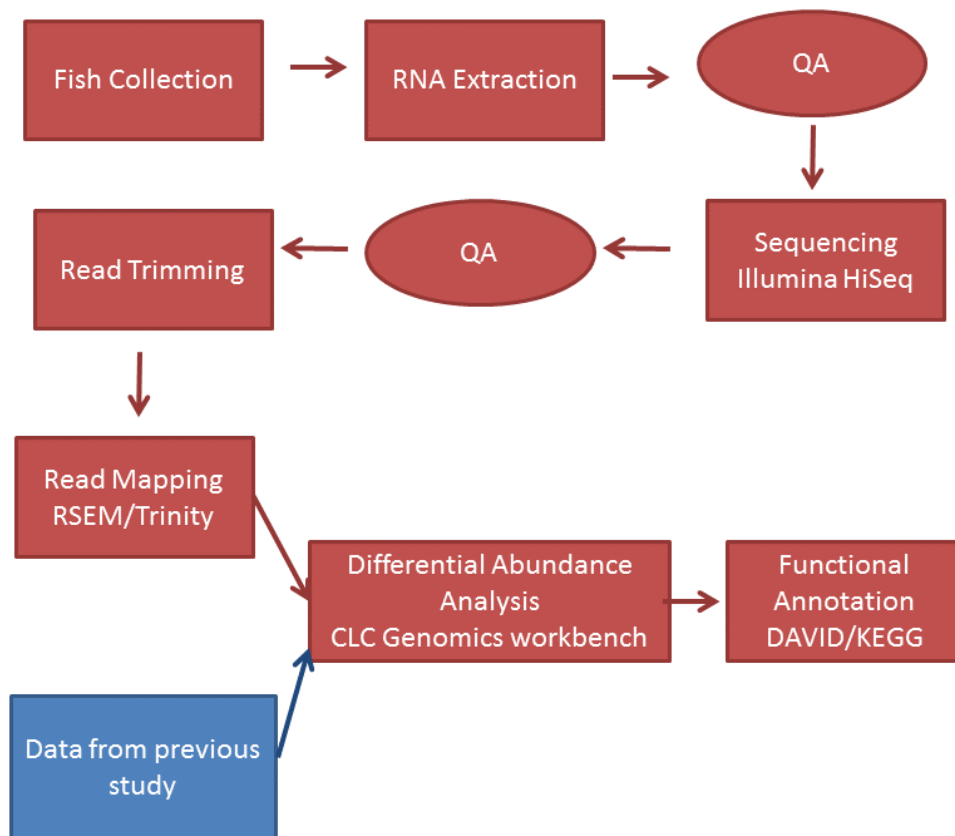
Figure S1. Workflow for this study, showing how data from the previous study were incorporated. These data were previously generated using the same analysis pipeline. Adapted from Hook et al., 2017.
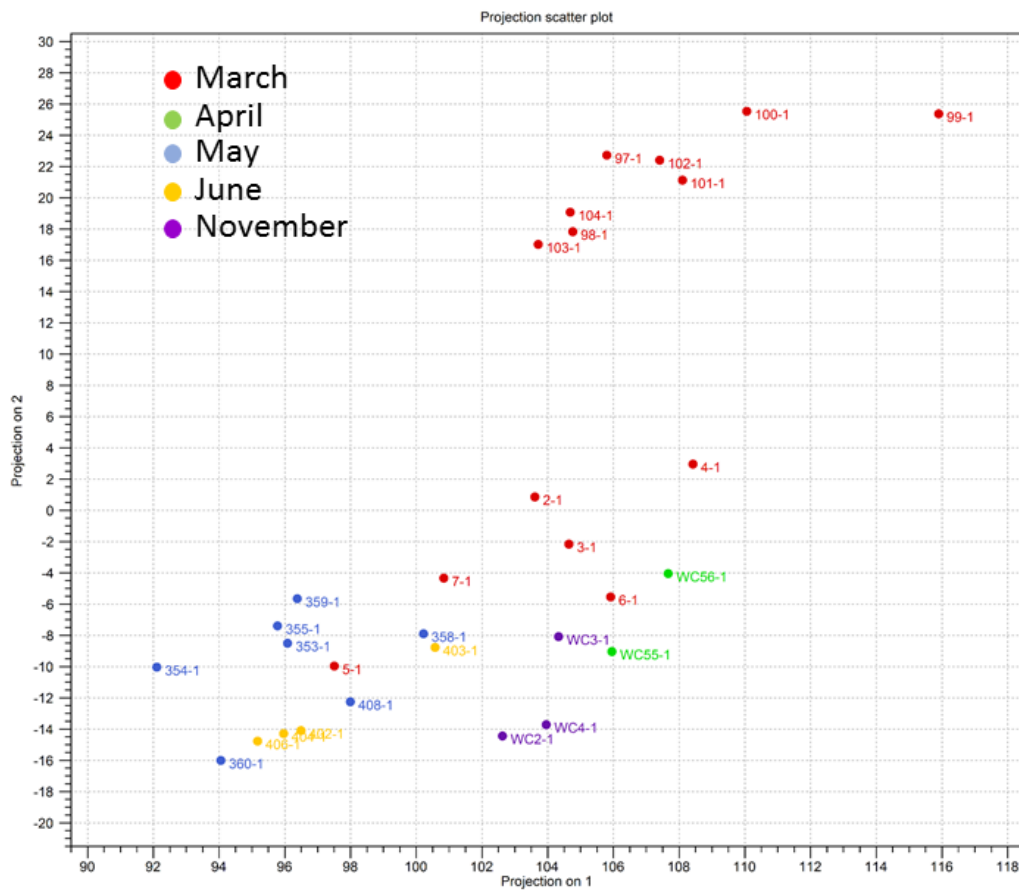
Figure S2. Principle components based visualisation of the plus one, log transformed hepatic transcriptome based on the time of year when the fish was collected. Each dot represents an individual fish.
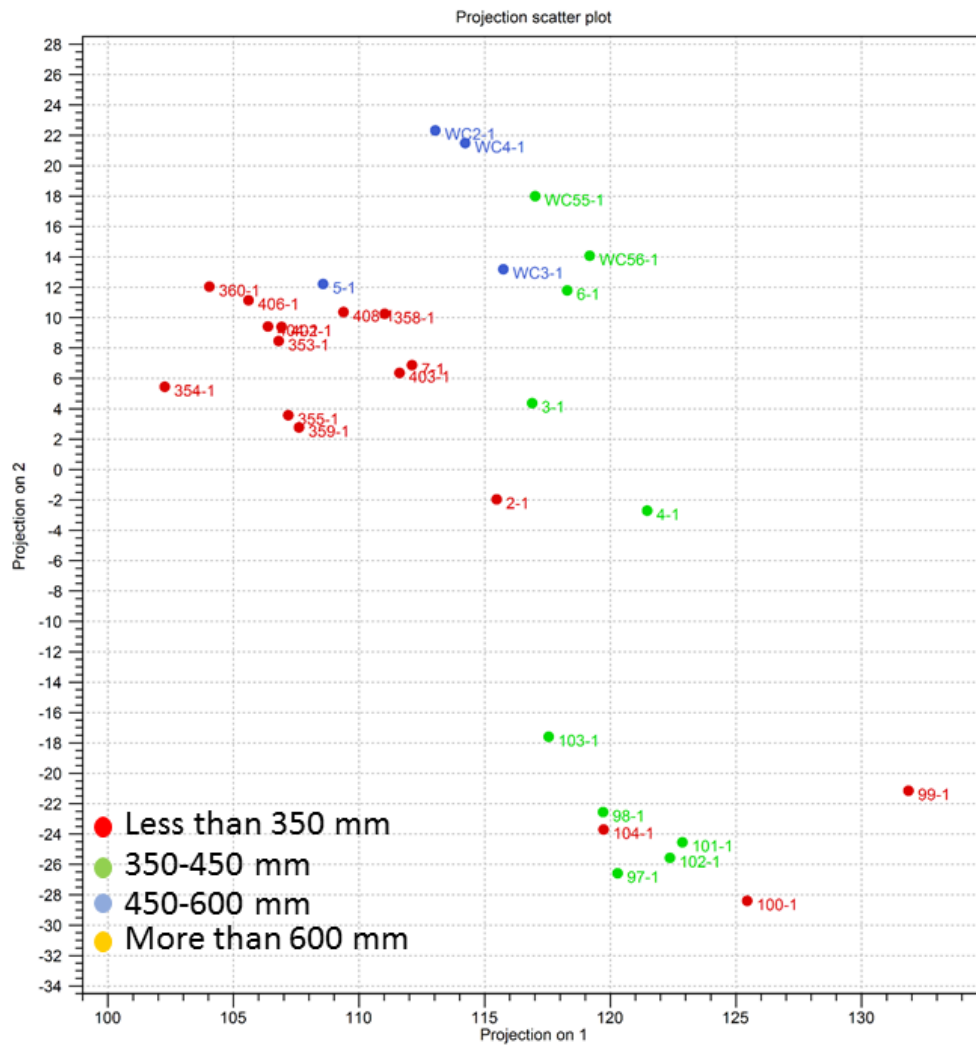
Figure S3. Principle components based visualisation of the plus one, log transformed hepatic transcriptome based on the size (in standard length) of the fish collected. Each dot represents an individual fish.
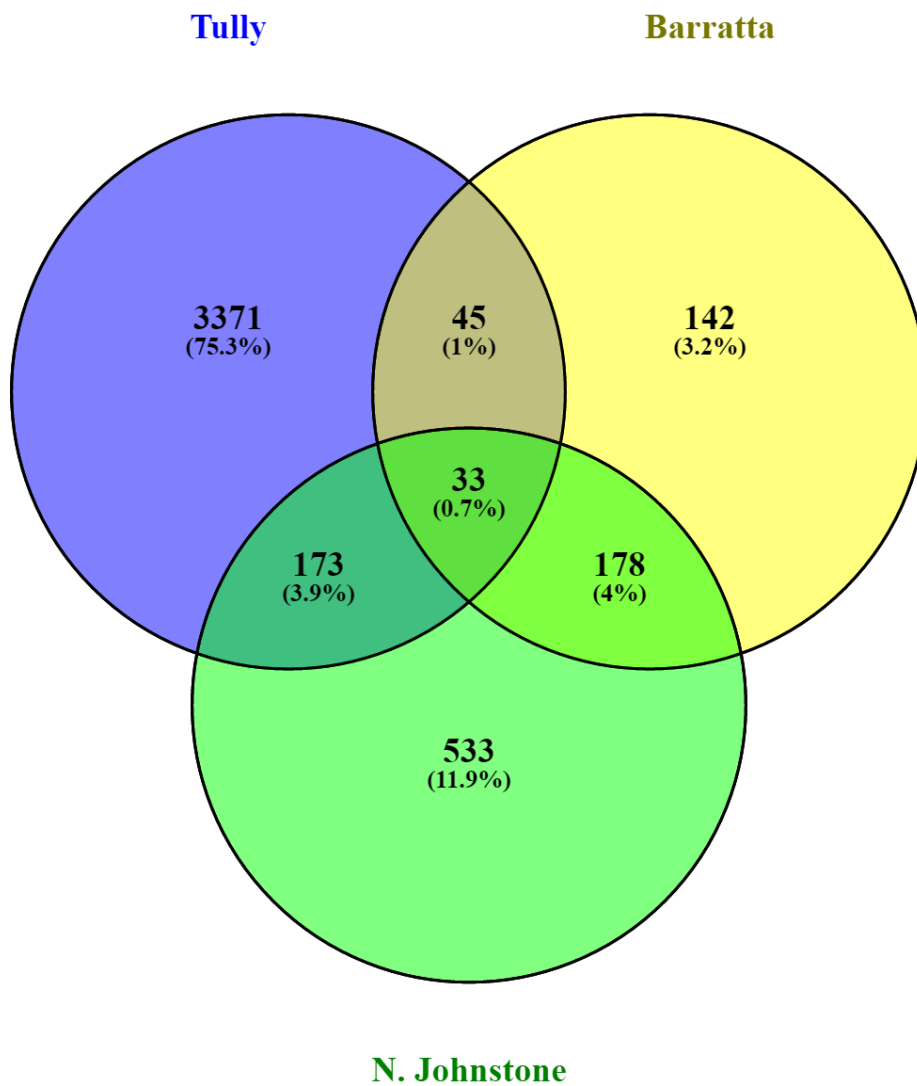
Figure S4. Similarities in the profiles of differentially abundant transcripts in livers for fish from each impacted river relative to both reference sites. Figure was created using Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Table S1. The number of reads for each sample and the percentage that aligned

M is million abbreviated. Fish numbers 2-7 were collected from the Daintree River, fish numbers 97-104 were collected from the Tully River.

Previous study (Hook et al., 2017)

Sample	2	3	4	5	6	7	97	98	99	100	101	102	103	104
Number of reads	28M	27M	26M	25M	25M	25M	28M	28M	31M	26M	28M	25M	26M	26M
% aligned	41.73%	47.50%	46.35%	47.28%	45.35%	42.35%	47.22%	47.81%	46.99%	46.17%	47.23%	47.92%	39.95%	47.88%

Current study

Sample	353	354	355	358	359	360	402	403	404	406	408	WC1	WC2	WC3	WC4	WC5	WC51	WC55	WC56
Number of reads	14M	14M	13M	13M	13M	13M	12M	13M	12M	12M	13M	12M	12M	12M	12M	12M	13M	13M	13M
% aligned	58.55%	60.22%	64.34%	59.7%	60.71%	57.89%	57.40%	56.01%	61.30%	62.12%	62.12%	62.97%	57.30%	58.17%	48.71%	58.59%	57.01%	53.14%	58.08%

Supplementary table S2. Gene lists go here.

Table S3. Functional pathways to which differentially abundant transcripts in the list of differentially abundant transcripts relative to the transcriptome as a whole could be significantly mapped

More abundant in the Tully River

Term	Number of transcripts	% of transcripts	Fold Enrichment	Benjamini-Hochberg corrected p value
phosphoprotein	362	19.30	1.60	1.45E-27
acetylation	151	8.05	1.84	7.16E-12
protein transport	40	2.13	2.65	9.64E-06
cytoplasm	154	8.21	1.48	1.75E-05
lysosome	19	1.01	4.09	8.06E-05
atp-binding	74	3.94	1.79	9.42E-05
nucleotide-binding	88	4.69	1.68	1.05E-04
alternative splicing	289	15.41	1.24	1.10E-04
hydrolase	82	4.37	1.69	1.40E-04
transferase	71	3.78	1.64	2.06E-03
compositionally biased region:Pro-rich	59	3.14	1.96	2.46E-03
splice variant	289	15.41	1.24	2.48E-03
actin-binding	21	1.12	2.73	3.90E-03
disease mutation	77	4.10	1.55	4.04E-03
endoplasmic reticulum	42	2.24	1.89	4.10E-03
oxidoreductase	35	1.87	2.00	5.62E-03
ligase	23	1.23	2.42	6.98E-03
lipid metabolism	15	0.80	3.19	7.39E-03
sequence variant	424	22.60	1.13	0.01
compositionally biased region:Glu-rich	24	1.28	2.71	0.02
kinase	38	2.03	1.77	0.02
GO:0005773~vacuole	22	1.17	2.57	0.03
GO:0005829~cytosol	72	3.84	1.59	0.03
nadp	14	0.75	2.88	0.03
GO:0000267~cell fraction	59	3.14	1.60	0.03
mutagenesis site	95	5.06	1.48	0.03
GO:0000166~nucleotide binding	113	6.02	1.43	0.03
polymorphism	395	21.06	1.10	0.04
GO:0001882~nucleoside binding	84	4.48	1.48	0.04
GO:0001883~purine nucleoside binding	83	4.42	1.47	0.04
GO:0005764~lysosome	19	1.01	2.65	0.04
GO:0000323~lytic vacuole	19	1.01	2.65	0.04
GO:0017076~purine nucleotide binding	97	5.17	1.43	0.04

Less abundant in the Tully

Term	Number of transcripts	% of transcripts	Fold Enrichment	Benjamini-Hochberg corrected p value
109.Chemokine_families	4	1.35	11.46	3.47E-03
PIRSF001950:small inducible chemokine, C/CC types	4	1.35	32.16	0.01
chemotaxis	5	1.69	19.08	0.02
IPR000827:Small chemokine, C-C group, conserved site	4	1.35	39.78	0.02
SM00199:SCY	4	1.35	22.71	0.03
cytokine	6	2.03	9.11	0.04
IPR001811:Small chemokine, interleukin-8-like	4	1.35	24.26	0.05

Less abundant in Barratta Creek

Term	Number of transcripts	% of transcripts	Fold Enrichment	Benjamini-Hochberg corrected p value
protein transport	36	2.18	2.78	1.37E-05
atp-binding	61	3.69	1.72	3.82E-03
IPR011993:Pleckstrin homology-type	25	1.51	2.92	4.59E-03
GO:0030695~GTPase regulator activity	28	1.69	2.39	0.01
GO:0060589~nucleoside-triphosphatase regulator activity	29	1.75	2.42	0.02
actin-binding	18	1.09	2.73	0.02
GO:0017048~Rho GTPase binding	8	0.48	7.25	0.02
SH2 domain	11	0.67	3.72	0.03
GO:0051020~GTPase binding	12	0.73	3.79	0.03
GO:0005096~GTPase activator activity	18	1.09	2.82	0.03
GO:0017016~Ras GTPase binding	11	0.67	4.16	0.04
IPR000198:RhoGAP	10	0.60	5.28	0.04
GO:0003779~actin binding	22	1.33	2.32	0.05
GO:0031267~small GTPase binding	11	0.67	3.75	0.05

Less abundant in N Johnstone

Term	Number of transcripts	% of transcripts	Fold Enrichment	Benjamini-Hochberg corrected p value
cell adhesion	12	3.07	3.99	0.02

Table S4. KEGG functional categories to which the enriched transcripts in fish from each transcriptome can be mapped.

Pathway	Number of sequences in gene list	Number of unique enzymes in gene list	Number of sequences in transcriptome	Expected number of sequences
More abundant in fish from the Tully River				
Purine metabolism	30	19	237	24
Glycerophospholipid metabolism	24	15	88	9
Glycerolipid metabolism	19	9	94	10
Glycolysis / gluconeogenesis	18	12	76	8
Fatty acid degradation	18	9	41	6
Lysine degradation	16	10	50	7
Glycine, serine and threonine metabolism	16	11	45	6
Pyruvate metabolism	16	11	47	5
Sphingolipid metabolism	15	6	59	8
Tryptophan metabolism	15	7	45	6
Pentose phosphate pathway	14	10	47	5
Citrate cycle (TCA cycle)	14	11	48	7
Glutathione metabolism	14	7	43	6
Arginine and proline metabolism	13	11	46	6
Ether lipid metabolism	13	6	27	4
Valine, leucine and isoleucine degradation	13	11	27	4
Phosphatidylinositol signalling system	13	8	109	11
Oxidative phosphorylation	13	8	63	9

Aminoacyl-tRNA biosynthesis	12	13	45	6
Inositol phosphate metabolism	12	7	94	10
Propanoate metabolism	12	9	36	5
Glyoxylate and dicarboxylate metabolism	12	9	26	4
Arachidonic acid metabolism	12	6	37	5
mTOR signalling	12	2	31	4
beta-Alanine metabolism	6	6	25	4
Amino sugar and nucleotide sugar metabolism	11	11	53	7
Tyrosine metabolism	11	12	26	4
Alanine, aspartate and glutamate metabolism	10	13	40	6
Biosynthesis of unsaturated fatty acids	10	4	25	4
Drug metabolism - cytochrome P450	10	5	32	5
Metabolism of xenobiotics by cytochrome P450	10	5	36	5
Aminobenzoate degradation	10	5	28	4
Starch and sucrose metabolism	10	11	37	5
alpha-linolenic acid metabolism	9	4	20	3
Chloroalkane and chloroalkene degradation	9	3	11	1
Pentose and glucuronate interconversions	6	3	24	3
Steroid hormone biosynthesis	8	5	30	4

Butanoate metabolism	8	10	20	3
Drug metabolism - other enzymes	8	8	53	5
Galactose metabolism	7	6	62	9
Retinol metabolism	7	4	32	4
Porphryrin metabolism	7	7	24	3
Steroid biosynthesis	7	5	10	1
Selenocompound metabolism	6	5	14	2
Nicotinate and nicotinamide metabolism	6	3	31	3
N-glycan biosynthesis	6	4	25	3
Other glycan degradation	6	5	34	5
Histidine metabolism	6	4	12	1
Ascorbate and aldarate metabolism	5	2	12	1
Folate biosynthesis	5	5	10	1
Cysteine and methionine metabolism	5	7	40	4
Phenylaline metabolism	5	7	32	4
Linoleic Acid	5	2	14	2
Various types of N-glycan biosynthesis	5	3	21	3
Limonene and pinene degradation	5	2	6	1
Arginine biosynthesis	5	5	46	6
Cyanoamino acid metabolism			20	3
Less abundant in fish from the Tully River				
Glycolysis / gluconeogenesis	18	12	76	8

Cysteine and methionine metabolism	5	4	40	4
Less abundant in fish from the N Johnstone River				
Glycolysis / gluconeogenesis	6	6	76	8
Less abundant in fish from Barratta Creek				
Purine metabolism	35	22	237	24
Glycolysis / gluconeogenesis	19	12	76	8
Phosphatidylinositol signalling system	16	7	109	11
Fructose and mannose metabolism	14	8	55	8
Inositol phosphate metabolism	13	6	94	13
Oxidative phosphorylation	13	4	63	9
Galactose metabolism	12	6	62	9
T cell receptor signalling pathway	12	2	82	8
Amino sugar and nucleotide sugar metabolism	9	12	53	7
Pyrimidine metabolism	11	8	105	10
Starch and sucrose metabolism	11	8	37	5
Pentose phosphate pathway	10	5	47	5
Pyruvate metabolism	7	6	47	5
Glycine, serine and threonine metabolism	8	8	45	5
Pentose phosphate pathway	8	3	47	5
Drug metabolism - other enzymes	7	5	53	5

Arginine and proline metabolism	6	4	46	6
Pentose and glucuronate interconversions	6	4	24	3
Ester lipid metabolism	6	5	27	4
Lysine degradation	6	4	50	7
Aminoacyl-tRNA biosynthesis	5	5	45	6
Nicotinate and nicotinamide metabolism	5	4	31	3
Glutathione metabolism	5	4	43	6
Trytophan metabolism	5	3	45	6

Only those pathways with five or more sequences that are relevant to eukaryotes are presented. The number of sequences expected to be included in the gene list due to chance alone is presented for comparison.