

ORIGINAL ARTICLE

Comparative ecological transcriptomics and the contribution of gene expression to the evolutionary potential of a threatened fish

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Abstract

Understanding whether small populations with low genetic diversity can respond to rapid environmental change via phenotypic plasticity is an outstanding research question in biology. RNA sequencing (RNA-seq) has recently provided the opportunity to examine variation in gene expression, a surrogate for phenotypic variation, in nonmodel species. We used a comparative RNA-seq approach to assess expression variation within and among adaptively divergent populations of a threatened freshwater fish, *Nannoperca australis*, found across a steep hydroclimatic gradient in the Murray–Darling Basin, Australia. These populations evolved under contrasting selective environments (e.g., dry/hot lowland; wet/cold upland) and represent opposite ends of the species' spectrum of genetic diversity and population size. We tested the hypothesis that environmental variation among isolated populations has driven the evolution of divergent expression at ecologically important genes using differential expression (DE) analysis and an ANOVA-based comparative phylogenetic expression variance and evolution model framework based on 27,425 de novo assembled transcripts. Additionally, we tested whether gene expression variance within populations was correlated with levels of standing genetic diversity. We identified 290 DE candidate transcripts, 33 transcripts with evidence for high expression plasticity, and 50 candidates for divergent selection on gene expression after accounting for phylogenetic structure. Variance in gene expression appeared unrelated to levels of genetic diversity. Functional annotation of the candidate transcripts revealed that variation in water quality is an important factor influencing expression variation for *N. australis*. Our findings suggest that gene expression variation can contribute to the evolutionary potential of small populations.

KEYWORDS

Australia, climate change, comparative transcriptomics, conservation genomics, *Nannoperca australis*, RNA-seq

1 | INTRODUCTION

Understanding the mechanisms by which species may persist in variable, and often degraded habitats, is vital for identifying populations at risk of extinction and to mitigate the loss of biodiversity

(Hoffmann & Sgro, 2011). As more land is converted from a natural state for agricultural, industrial and urban uses, habitats have become fragmented, limiting species opportunity for dispersal and migration (Fischer & Lindenmayer, 2007). Adaptation from standing genetic variation is one way species can respond to environmental

change, and it has been increasingly suggested that even very small populations may retain variation at adaptive loci and respond to rapid change (Brauer, Hammer, & Beheregaray, 2016; Fraser, Debes, Bernatchez, & Hutchings, 2014; Koskinen, Haugen, & Primmer, 2002; Wood, Yates, & Fraser, 2016). Phenotypic plasticity—the ability for multiple phenotypes to arise from a single genotype—is another mechanism that may facilitate population persistence in altered environments and potentially lead to evolutionary adaptation (Chevin, Lande, & Mace, 2010; Dayan, Crawford, & Oleksiak, 2015; Ghalambor et al., 2015). These two mechanisms are not mutually exclusive and empirical examples featuring wild populations suggest that rapid phenotypic changes often involve a combination of genetic adaptation and phenotypic plasticity (Charmantier et al., 2008; Gienapp, Teplitsky, Alho, Mills, & Merila, 2008; van de Pol, Osmond, & Cockburn, 2012; Réale, McAdam, Boutin, & Berteaux, 2003). While the former has received substantial recent attention, relatively few studies have examined the role or extent of phenotypic plasticity in small populations in the wild (Wood & Fraser, 2015).

Our ability to predict species potential for phenotypic responses to environmental change depends on the identification of traits affecting fitness in the new environment. For cryptic or threatened species especially, information linking phenotypic variation and fitness is often scarce or nonexistent. In such cases, any consideration of environment–phenotype interactions will always represent a balance between exploring and explaining trait variation (Houle, Govindaraju, & Omholt, 2010). An alternative strategy for study systems for which the knowledge of important traits is lacking or the ability to measure them is limited is to consider gene expression measurements as phenotypic traits (Houle et al., 2010). The advent of high-throughput genomic methods such as microarrays and, more recently, RNA sequencing (RNA-seq) has seen an increase in gene expression studies for nonmodel species (Alvarez, Schrey, & Richards, 2015). RNA-seq measures global levels of mRNA transcription that are often used as a surrogate for gene expression (e.g., Leder et al., 2015). These data can provide insight into the most basic link between genotypes and complex phenotypic traits shaped by ecological and evolutionary processes (Whitehead, 2012). Additionally, the simultaneous measurement of vast numbers of traits facilitated by RNA-seq may reveal cryptic evolutionary patterns not discernible when fewer phenotypic traits are considered (Houle et al., 2010).

Comparative gene expression or transcriptomic analyses of wild populations can contribute to our understanding of the molecular basis (both plastic and evolved) of physiological responses to environmental stressors (Romero, Ruvinsky, & Gilad, 2012; Whitehead, Triant, Champlin, & Nacci, 2010). However, several challenges exist in analysing and interpreting comparative gene expression data, especially in the case of wild populations of nonmodel species. For instance, phylogenetic distance needs to be accounted for when comparing gene expression among groups (Dunn, Luo, & Wu, 2013); cryptic or transient environmental factors or developmental effects may bias results due to sampling just one time point (DeBiasse &

Kelly, 2016); and the biological interpretation of functional annotations derived from distantly related taxa may be misleading (Pavey, Bernatchez, Aubin-Horth, & Landry, 2012). Nevertheless, comparative studies of wild populations can provide information concerning the effects of multiple and dynamic environmental conditions on gene expression not otherwise obtainable in more controlled experimental conditions (Alvarez et al., 2015). However, when populations sampled over environmentally heterogeneous landscapes are also isolated by geographic and phylogenetic distance, it becomes difficult to determine whether differences in expression represent plastic or adaptive responses to variation in the environment, or are simply due to neutral drift (Khaitovich et al., 2004).

While significant research effort has been directed towards understanding the effects of population size and drift on evolutionary potential, comparatively few studies have considered the impact of genetic drift on species capacity for phenotypic plasticity (Chevin, Gallet, Gomulkiewicz, Holt, & Fellous, 2013; Wood & Fraser, 2015). On the one hand, small fragmented populations are likely to exhibit reduced genetic diversity. Given the mounting evidence for an underlying heritable component of variation in gene expression (Gibson & Weir, 2005; Leder et al., 2015; McCairns, Smith, Sasaki, Bernatchez, & Beheregaray, 2016), it might be reasonable to expect that where genetic diversity has been eroded by drift, plasticity in gene expression may also be impaired (Bijlsma & Loeschcke, 2012). If, on the other hand, fragmentation causes an overall decrease in habitat quality and a concurrent increase in environmental variation, natural selection may maintain phenotypic plasticity in small populations for traits important in responding to environmental stressors (Chevin & Lande, 2011; Paschke, Bernasconi, Schmid, & Williams, 2003).

Studies of differential expression among wild populations can potentially identify ecologically important genes involved in evolutionary or plastic responses to environmental variation. Leder et al. (2015) recently suggested that the effects of demography and natural selection may exert the greatest influence on expression divergence during, or immediately following, lineage divergence. Comparative gene expression studies among recently isolated but demographically independent populations undergoing rapid environmental, ecological and evolutionary change may therefore offer the best opportunity to examine key genetic and environmental elements of expression variation. The southern pygmy perch, *Nannoperca australis*, is an Australian freshwater fish that represents a good system for examining variation in gene expression in heterogeneous landscapes and in the context of conservation. Despite indications that populations across the Murray–Darling Basin (MDB) in south-eastern Australia were more connected in the past (Attard et al., 2016; Cole et al., 2016), the impact of drift due to recent human-driven demographic decline and isolation of populations has resulted in remarkably strong contemporary population divergence (Brauer et al., 2016; Cole et al., 2016). Additionally, genome-wide SNP (Brauer et al., 2016) and reproductive phenotypic (Morronegiello, Bond, Crook, & Wong, 2012) data provided evidence for adaptive divergence of *N. australis* populations occupying a range of naturally variable hydroclimatic environments also subjected to varying

degrees of human impacts. The long-term hydroclimatic variability and unpredictability that characterize the MDB also suggest that selection may not only lead to local adaptation in *N. australis*, but may also favour plasticity in traits related to maintaining fitness in a variable environment (Brauer et al., 2016). Determining the role of gene expression plasticity in facilitating population persistence and adaptive evolution in changing environments is a key research question in ecology and evolution (Alvarez et al., 2015; DeBiasse & Kelly, 2016). An initial step towards this goal is to understand how patterns of gene expression vary within and among populations, and to examine the relative contribution of plastic (environmental) and evolutionary (genetic) components in shaping these patterns in the wild. Here, we used an RNA-seq approach to construct and functionally annotate a de novo liver transcriptome for *N. australis*. This resource then provided the foundation to examine patterns of global (i.e., transcriptome-wide) expression variation among select wild ecotypes. The question of how population size affects the evolutionary potential of populations has received recent attention, with some evidence now suggesting that some adaptive potential can be maintained in even very small populations (Fraser et al., 2014; Wood, Tezel, Joyal, & Fraser, 2015). This subsequently raises the important question of whether plastic responses to environmental change are also affected by population size.

Our aims in this study were to test the hypotheses that among-sample variance in global gene expression, in this case a surrogate for phenotypic plasticity, differs among isolated populations and that this variance is correlated with levels of genetic diversity. Specifically, we applied a comparative phylogenetic ANOVA-based expression variance and evolution model to five populations of *N. australis*. Our samples include ecotype populations that evolved under contrasting selective environments (e.g., dry/hot lowland vs. wet/cold upland) and that also represent opposite ends of the spectrum of adaptive genetic diversity and effective population size of the species in the MDB (Brauer et al., 2016). We identified candidate transcripts potentially under divergent selection for expression level, and others with a signal of high expression-level plasticity. Multivariate models of global and candidate gene expression profiles were then used to examine the relationship between expression variance and genetic diversity within and among populations.

2 | METHODS

2.1 | Sampling

Nannoperca australis were collected from 25 locations, encompassing 13 catchments across the entire current MDB distribution of the species (Figure 1; Table 1). From those, five ecotypes were selected for RNA sampling on the basis of capturing maximum hydroclimatic variation across the MDB (Figure 1c) and to include populations with relatively high (LMR, SEV), intermediate (LIM, KIN) and low (MER) levels of genetic diversity (Brauer et al., 2016) (Table 1). The lower Murray (LMR) is a semi-arid environment with warmer winter temperatures and far less rainfall than elsewhere in the MDB (Chiew et al., 2008).

The relatively well-connected lakes and wetlands of this region contrast with the small, isolated rivers and creeks typical of headwater habitats (Hammer et al., 2013). In these higher-elevation headwater sites (especially KIN), hydroclimatic conditions are much wetter, with generally cooler winter temperatures (Brauer et al., 2016).

RNA was sampled directly from the wild at four upper MDB sites during a single field expedition in 2013 at a similar time of day in each case. A combination of dip netting and electrofishing was used to collect approximately six adult males of similar size from each site (Table 1). Only males were used to account for putative differences in expression between males and females (Smith, Bernatchez, & Beheregaray, 2013). To reduce the potential for increased variance in gene expression associated with variation in ontogeny, sexual maturity of each individual was confirmed by visual inspection of the gonads following dissection. Wild-born but captive-held individuals were sampled from the lower Murray. Although the use of these fish may potentially influence some results, there was no other alternative as the population representing the lower Murray ecotype is critically endangered after being locally extirpated from the wild during a recent catastrophic drought. The fish sampled here were rescued prior to the complete loss of habitat in the lower Murray and were part of a founding captive breeding population (Attard et al., 2016; Hammer et al., 2013). It was, nonetheless, considered important to include this population, as these are the only representatives of this geographically isolated, environmentally divergent and ecologically important region (Figure 1). Fish were euthanized in an overdose of AQUI-S[®] solution (50% isoeugenol) and immediately dissected to extract the liver. Liver tissue was incubated at 4°C for 12 hr in RNeasy lysis buffer (Qiagen) following the manufacturer's protocol before freezing in liquid nitrogen for transport and subsequent laboratory storage at -80°C. Liver tissue was selected because gene expression in liver is known to respond to environmental stimuli in fish, such as variation in temperature (McCairns et al., 2016; Rabergh et al., 2000; Smith et al., 2013).

2.2 | RNA extraction and library preparation

Total RNA was extracted from approximately 5 mg of liver tissue for each sample using a MAGMAX 96 Total RNA Extraction Kit (Life Sciences) following the manufacturer's protocol. Integrity (minimum acceptable RIN of 7.0) and concentration were evaluated with an RNA Nano assay kit on an Agilent Bioanalyzer 2100 (Agilent Technologies) and purity assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific).

Samples were normalized to a starting quantity of 0.9 µg total RNA and individual libraries were prepared for 26 samples using a TruSeq RNA sample preparation kit (Illumina) following the low sample protocol. Briefly, poly-A-containing mRNA was first purified with magnetic beads before fragmenting the RNA by incubating at 94°C. SuperScript II reverse transcriptase was used to synthesize the first strand of cDNA after which the RNA template was removed and replaced with a second cDNA strand to produce double-strand cDNA. Illumina adapter indices 2, 4–7, 12–16, 18 and 19 were ligated to the cDNA with the 12 barcodes assigned to samples for

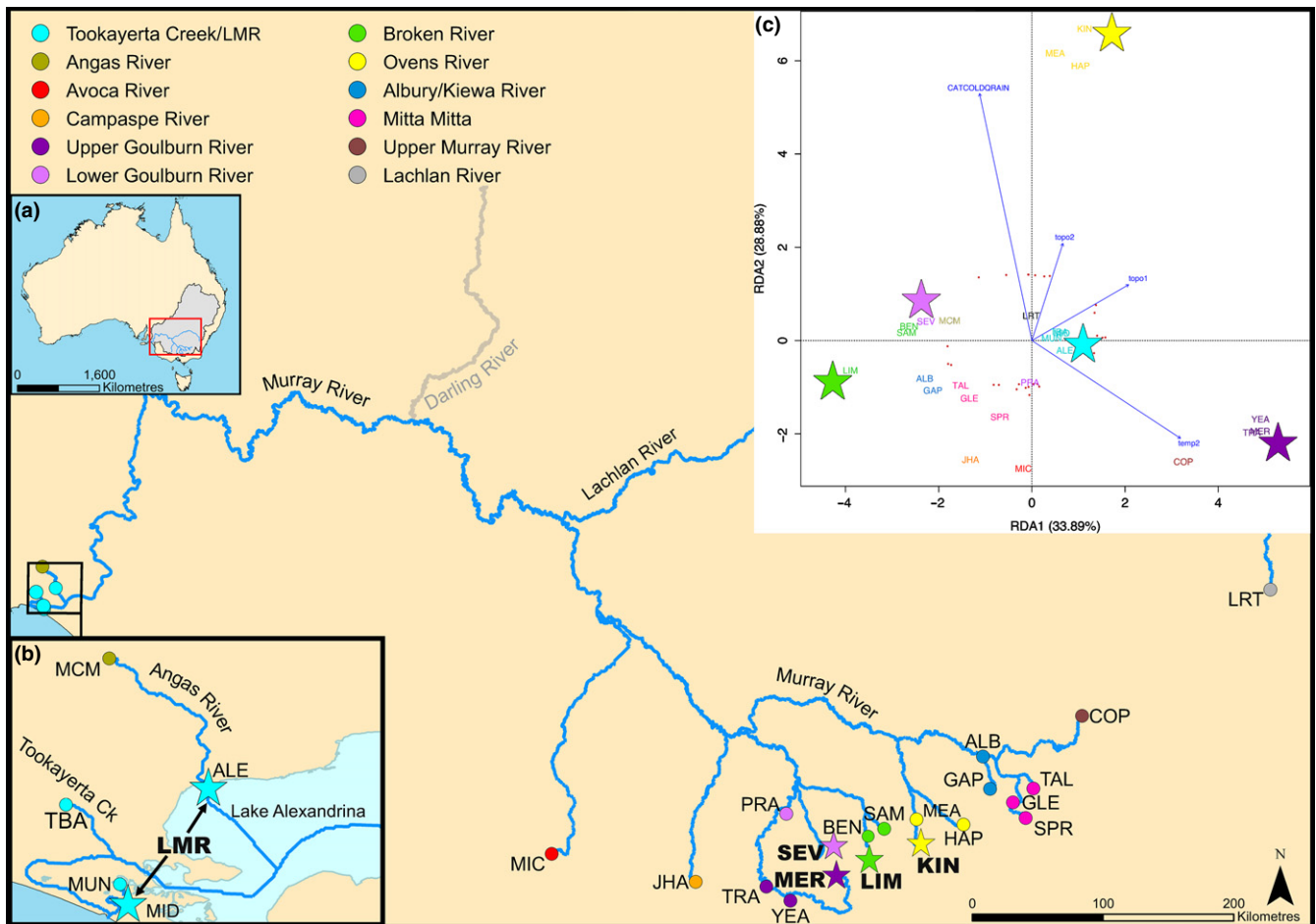


FIGURE 1 *Nannoperca australis* distribution in the Murray–Darling Basin (MDB) with sites colour-coded by catchment and locations where RNA was sampled for this study depicted with a star (★). Inset a shows the location of the MDB (shaded area), and inset b shows the location of sites in the lower Murray. Inset c highlights that the ecotypes sampled here span the full spectrum of adaptive genetic diversity associated with hydroclimatic variation depicted by a genotype-by-environment association redundancy analysis model summarizing candidate SNP loci for *N. australis* (reproduced from Brauer et al., 2016)

pooled sequencing across four Illumina lanes (along with 22 additional libraries not included in this study). Fragments with adapters at both ends were amplified using PCR and the resulting libraries validated using an Agilent Bioanalyzer 2100 before normalizing and pooling 12 individual libraries for each sequencing lane. Paired-end, 100-base pair sequencing was performed on an Illumina HiSeq2000.

2.3 | Read trimming, de novo transcriptome assembly and quality assessment

Raw sequence reads were demultiplexed according to individual indices at the sequencing facility. TRIMMOMATIC version 0.36 (Bolger, Lohse, & Usadel, 2014) was used to remove adapter sequences and trim low-quality bases using the TRINITY version 2.4.0 (Grabherr et al., 2011) default parameters. Reads from one individual per ecotype (five in total) were combined and assembled using TRINITY. Prior to assembly, in silico normalization was implemented to reduce redundancy in the data by limiting read coverage to a maximum of 50× in order to reduce memory requirements and improve computational time.

Statistics describing read representation, N50 values, and the number of BUSCO (Benchmarking Universal Single-Copy Orthologs) conserved orthologs (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) were generated to assess quality of the transcriptome assembly. Sequence reads retained after quality filtering were mapped back to the assembled transcripts using BOWTIE2 version 2.2.7 (Langmead, Trapnell, Pop, & Salzberg, 2009) to examine the overall number of reads mapping to the assembly and also the proportion of those mapped reads occurring as proper forward and reverse pairs. Finally, to quantify completeness of the assembly in terms of gene content, the transcriptome was assessed against the BUSCO vertebrata_odb9 database (<http://busco.ezlab.org/>). This database consists of 2586 evolutionarily conserved genes expected to be found as single-copy orthologs in >90% of vertebrate species (Simão et al., 2015).

2.4 | Functional annotation and gene ontology

Homology searches of several sequence and protein databases were performed using TRINOTATE version 3.0.2 to assign functional

TABLE 1 Information about sampling localities, number of RNA samples (N_{RNA}), number of DNA samples (N_{DNA}) and population mean individual heterozygosity (IH) for *Nannoperca australis* from the Murray–Darling Basin (MDB). Sites sampled for the present study are indicated in boldface, while additional sites sampled from across the species range in the MDB in Brauer et al. (2016) are included below for comparison

Site	Location	N_{RNA}	N_{DNA}	IH ($\pm$ SD)	Latitude	Longitude
LMR	Turvey's Drain, L. Alexandrina	4	10	0.16 (0.04)	−35.395	139.008
MER	Merton Ck	6	17	0.06 (0.01)	−36.981	145.727
SEV	Trib to Seven Creeks	6	11	0.17 (0.04)	−36.875	145.701
LIM	Unnamed Ck, Lima South	5	18	0.09 (0.03)	−36.826	146.008
KIN	King R., Cheshunt	5	16	0.08 (0.02)	−36.795	146.424
TBA	Tookayerta Ck, Black Swamp	—	7	0.15 (0.05)	−35.428	138.834
MCM	Middle Ck	—	9	0.09 (0.02)	−35.250	138.887
MIC	Trib to Middle Ck, Warrenmang	—	11	0.10 (0.03)	−37.028	143.338
JHA	Jews Harp Ck, Sidonia	—	12	0.07 (0.01)	−37.139	144.578
TRA	Trawool Ck	—	10	0.06 (0.01)	−37.135	145.193
YEA	Yea R., Yea	—	8	0.06 (0.02)	−37.213	145.414
PRA	Pranjip Ck	—	9	0.18 (0.03)	−36.623	145.309
BEN	Swanpool Ck, Swanpool	—	10	0.18 (0.06)	−36.723	146.022
SAM	Sam Ck	—	10	0.20 (0.04)	−36.661	146.152
HAP	Happy Valley Ck	—	9	0.09 (0.02)	−36.579	146.824
MEA	Meadow Ck, Moyhu	—	8	0.13 (0.04)	−36.573	146.423
GAP	Gap Ck, Kergunyah	—	12	0.14 (0.03)	−36.317	147.022
ALB	Murray R. lagoon, Albury	—	12	0.18 (0.03)	−36.098	146.928
SPR	Spring Ck	—	10	0.11 (0.07)	−36.499	147.349
GLE	Glencoe Ck	—	10	0.12 (0.03)	−36.393	147.221
TAL	Tallangatta Ck	—	7	0.14 (0.05)	−36.281	147.382
COP	Coppabella Ck	—	16	0.11 (0.03)	−35.746	147.729
LRT	Blakney Ck	—	8	0.04 (0.01)	−34.736	149.180

annotations to the transcriptome. TRANSDCODER version 4.0 was first used to extract open reading frames (ORFs) >100 amino acids in length from the TRINITY assembly and identify candidate protein-coding regions. BLASTX (Trinity transcripts) and BLASTP (Transdecoder predicted protein-coding regions) were used to search (default *e*-value threshold) the SWISSPROT sequence database (UniProt Consortium 2015) to provide gene annotation and assign functional gene ontology (GO) terms (Tao, 2014). BLASTP queries against Ensembl genomes for zebra fish (*Danio rerio*), three-spined stickleback (*Gasterosteus aculeatus*) and Japanese puffer (*Takifugu rubripes*) (Yates et al., 2016) were also performed (default *e*-value threshold) to provide additional support for annotations derived from more distantly related species. Finally, the predicted protein-coding regions were also searched for homologies with the Pfam protein family domain (Bateman et al., 2004), protein signal peptide (Petersen, Brunak, von Heijne, & Nielsen, 2011) and transmembrane protein domain (Krogh, Larsson, Von Heijne, & Sonnhammer, 2001) databases (*e*-value thresholds of 1×10^{-5}). The resulting BLAST homologies were loaded into a SQLite database along with the transcriptome to generate an annotation report and to provide GO information (Botstein et al., 2000) for downstream functional enrichment analyses.

2.5 | Transcript quantification and differential expression analysis

To quantify the level of transcription for individual samples, reads for each sample were first mapped back to the transcriptome using BOWTIE2 version 2.2.7 (Langmead et al., 2009), before gene-level abundance estimations were performed with RSEM version 1.2.19 (Li & Dewey, 2011). To enable comparison of expression level among samples, the resulting read count estimations were also cross-sample-normalized using the trimmed mean of *M*-values method (TMM).

Pairwise comparisons of differential expression (DE) among populations were estimated using the Transdecoder predicted protein-coding regions in both EDGER (Robinson, McCarthy, & Smyth, 2010) and DESEQ2 version 1.10.1 (Love, Huber, & Anders, 2014). Transcripts with a minimum $\log_2$ fold change of two between any two populations were considered differentially expressed at a false discovery threshold of 0.05. Heatmaps describing the correlation among samples, and gene expression per sample, were generated using the TRINITY analyze_diff_expr.pl utility to allow visual analysis of patterns of expression.

Functional GO enrichment analysis for DE genes was performed using the Bioconductor R package GOSEQ version 1.22.0 (Young,

Wakefield, Smyth, & Oshlack, 2010). GSESEQ can account for the bias in DE detection for long and highly expressed transcripts common to RNA-seq data (Young et al., 2010). Gene ontology terms for the DE transcripts were retrieved from the earlier BLAST annotation results and tested for enrichment compared to all GO term assignments for the transcriptome assembly.

2.6 | Gene expression plasticity and divergent selection

The advent of RNA-seq provides a powerful platform for examining the mechanisms behind nonmodel species' capacity to persist in variable environments (Harrison, Pavlova, Telonis-Scott, & Sunnucks, 2014). Analyses based on tests of ANOVA have often been used in comparative transcriptomics studies that contrast variation in gene expression within species with variation among species. In many cases, these analyses have incorporated a correction for phylogenetic effects (Dayan et al., 2015; Oleksiak, Churchill, & Crawford, 2002; Oleksiak, Roach, & Crawford, 2005; Uebbing et al., 2016) and a recent extension of such ANOVA-based methods is the Expression Variance and Evolution Model (EVE) (Rohlf & Nielsen, 2015). EVE models gene expression as a quantitative trait across a phylogeny; considering the ratio (β) of among-lineage expression divergence to within-lineage expression diversity in a similar manner to the Hudson–Kreitman–Aguadé (HKA) test used to detect molecular evolution in DNA sequences (Hudson, Kreitman, & Aguadé, 1987). The expectation is that β should be consistent among the majority of genes that have undergone similar evolutionary and demographic processes, but higher for genes with more variance within than among lineages, and lower for genes with more variance among compared to within lineages. Here, the EVE model was used to parameterize the ratio β across the five populations of *N. australis*, to identify transcripts potentially under divergent selection for expression level (low β) or transcripts with high expression plasticity (high β). The model utilizes gene expression data and a phylogeny as input. A TMM normalized expression matrix based on the 27,425 TRANSDCODER predicted protein-coding regions was used for the EVE analysis. To construct a phylogenetic tree, PYRAD version 3.0.6 (Eaton, 2014) was first used to align ddRAD sequences from the same 26 individuals and an additional five Yarra pygmy perch (*N. obscura*), included as an outgroup (Unmack, Hammer, Adams, & Dowling, 2011) (pyRAD parameters are specified in Appendix S1, Supporting information). Maximum-likelihood phylogenetic analyses were then run with RAXML version 8.0.26 (Stamatakis, 2014), specifying a GTRGAMMA model and 1000 bootstrap replicates. The majority-rule consensus tree was used as the input phylogeny for EVE.

For each transcript (i), maximum-likelihood values were calculated and a likelihood ratio test (LRT) was performed to assess the null hypothesis that β_i is equal to β for all transcripts. Under the null model, the LRT statistic follows a χ^2_1 distribution (Rohlf & Nielsen, 2015) and a custom R script (Appendix S2, Supporting information) was used to identify candidate transcripts where the LRT statistic deviated from this distribution at a FDR of 10%.

2.7 | Is variance in gene expression constrained by drift?

A multivariate analogue of Levene's test for homogeneity of variances was used to test for differences in intrapopulation expression variance among populations. Bray–Curtis dissimilarity matrices were constructed for all samples based on (i) the expression matrix for all predicted protein-coding regions and (ii) the two candidate gene sets identified using the EVE model (divergent selection on expression and high expression plasticity). Using the BETADISPER function in the VEGAN R package (Oksanen, Blanchet, Kindt, Oksanen, & Suggests, 2015), the matrices were reduced to principal coordinates and the distance of each individual to the population centroid (average population multivariate expression profile) was calculated and subjected to ANOVA. A total of 9,999 permutations were used to test for significant departure from the null hypothesis of no difference in variation among populations. Tukey's test for significant differences between groups was also applied using the TUKEYHSD R function (also in VEGAN) to test for pairwise population differences in within-population mean expression variance.

To test the hypothesis that gene expression variance, here a surrogate for phenotypic plasticity (Bijlsma & Loeschcke, 2012), is constrained by genetic diversity, individual heterozygosity was regressed against gene expression, again based on all predicted protein-coding regions, and the two candidate gene sets. The calculations were made using the ADONIS function in VEGAN. This function performs an analysis of variance using distance matrices and allows linear models to be fitted to multiple matrices. Individual heterozygosity was calculated as the proportion of heterozygous loci per individual at 3443 neutral and at 216 genotype–environment association (GEA) candidate SNP loci previously identified in a riverscape genomics study of the species across the MDB (Brauer et al., 2016). The test was performed separately for each data set to assess the possibility that expression variance may respond differently to putatively neutral and to candidate adaptive loci. One individual from site MER was dropped from these analyses, as reliable estimates of genetic diversity were unable to be obtained due to a high proportion of missing data in this sample's SNP data set. A stratified permutation test with pseudo-F ratios was performed to test significance of the portion of gene expression attributed to variation in genetic diversity using 9,999 permutations within each population.

3 | RESULTS

3.1 | Sequencing and assembly

Illumina sequencing of the 26 individual libraries produced over 443 million paired-end reads (2×100 bp) and, after trimming and quality filtering, 425 million read pairs (95.9%) were retained (Table 2). This resulted in an average of 16,354,889 read pairs per individual (min = 5,722,753, max = 35,333,360) for downstream analyses (Table S1, Supporting information).

TABLE 2 Sequencing, de novo assembly and annotation statistics for the *Nannoperca australis* liver transcriptome

Sequencing	Total read pairs (2 × 100 bp)	443,386,380
	Retained trimmed read pairs	425,227,106
Assembly	Total aligned reads	1,191,758,692
	Trinity transcripts	201,037
	Trinity genes	96,717
	Percentage GC	45.24
	Complete BUSCO conserved orthologs	1598 (62%)
	N50 (bp)	2,021
	Median contig length (bp)	601
	Mean contig length (bp)	1107
	Total assembled bases	222,596,762
Annotation	Trinity genes with open reading frames (ORFs)	27,425
	ORFs assigned functional gene ontology terms	26,638

Five samples with a total of 272,199,744 reads were used to assemble the transcriptome de novo (Table S1, Supporting information). Over 247 million of these reads (90.8%) mapped to the assembly with 80.6% aligning as proper pairs (Table S2, Supporting information). The final assembly consisted of 201,037 unique transcripts that clustered into 96,717 Trinity genes (Table 2). Based on all transcript contigs, a N50 of 2021 bases (mean = 1107, total assembled bases = 222,596,762) was achieved (Table 2; Table S3, Supporting information). A search of the vertebrate BUSCO database revealed at

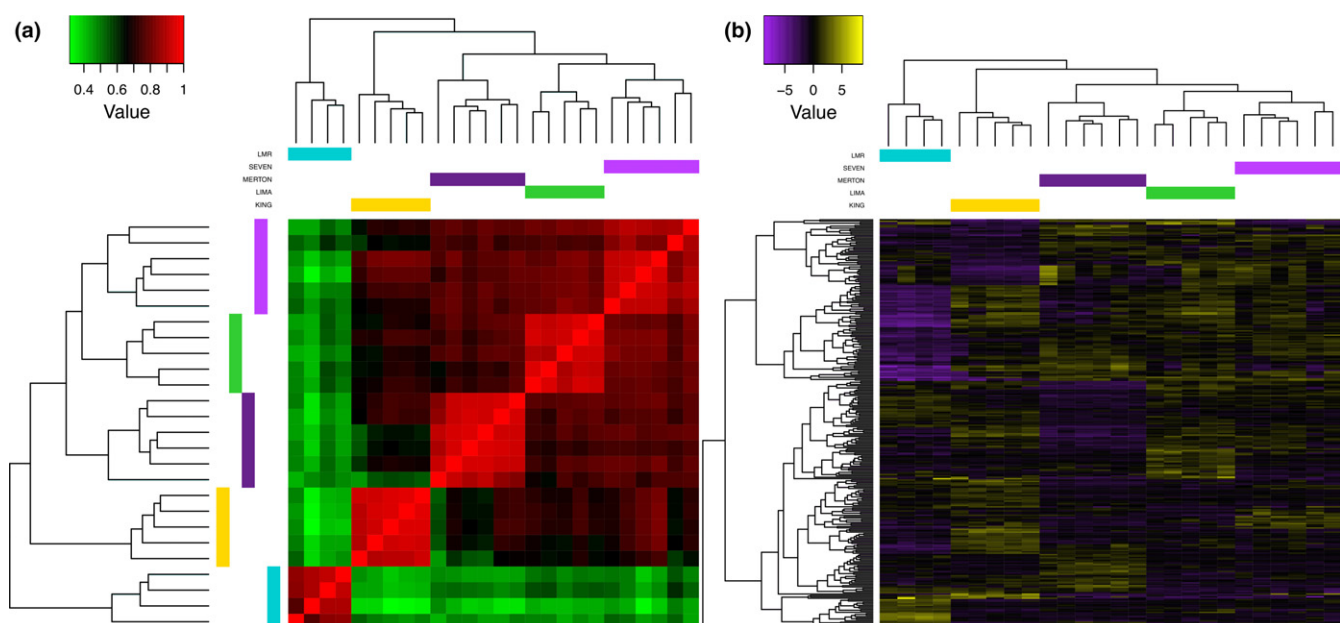
least partial hits for 2236 (87%) orthologs, including 1598 (62%) complete orthologs (Table 2; Table S4, Supporting information).

3.2 | Functional annotation, gene ontology and differential expression analysis

The BLAST search to the SwissProt sequence database resulted in annotations of 168,360 unique transcripts, while 20,771 Trinity genes annotated to the zebra fish genome, 20,409 to the stickleback genome and 20,091 to the Japanese puffer genome. TRANSDCODER predicted protein-coding regions of at least 100 amino acids that aligned to 27,425 Trinity genes (these genes were used for all downstream analyses) (Table 2). Of these genes, 26,638 could be assigned functional GO terms (Table 2). A full annotation report can be accessed on Dryad: <https://doi.org/10.5061/dryad.6gh7b>.

DESEQ and EDGER results were remarkably similar, with DESEQ2 identifying 290 transcripts differentially expressed in at least one pairwise population comparison (FDR 5%) compared to 299 for EDGER, with 256 common to both methods (Figure S1; Tables S5–6, Supporting information). The slightly more conservative DESEQ2 results were retained for downstream analyses. Within populations, expression profiles of DE transcripts among samples were similar with all individuals clustering within their population of origin, and clear distinctions among populations (Figure 2a).

Expression levels for the top 50 DE transcripts are contrasted in Figure 2b where clear patterns emerge among populations for several clusters of genes. Plots depicting the log₂ fold change in expression vs. the log₂ mean expression counts for each pairwise comparison are shown in Figure S2, Supporting information.

**FIGURE 2** Heatmaps summarizing (a) correlation among samples in log₂ gene expression profiles based on the top 50 differentially expressed transcripts and (b) log₂ gene expression levels for samples (columns) based on the top 50 differentially expressed transcripts (rows) identified with DESEQ2. Coloured bars under the sample dendrograms represent the five *Nannoperca australis* populations and are based on colours used in Figure 1

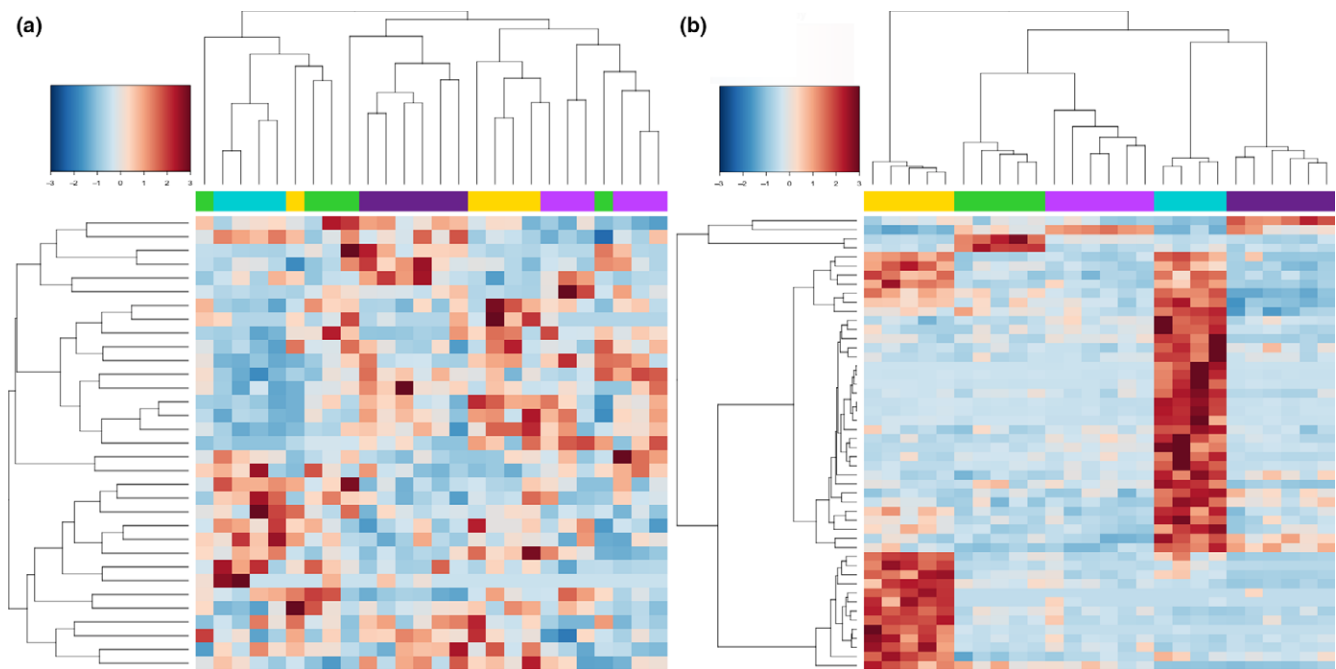


FIGURE 3 Hierarchical clusters of (a) 33 transcripts identified as candidates (FDR 10%) demonstrating high intrapopulation expression-level plasticity and (b) 50 transcripts identified as candidates (FDR 10%) for divergent selection for expression level with the EVE model. Individual samples with similar patterns of expression among transcripts cluster together (columns), and transcripts with similar expression profiles among individuals are also clustered (rows). Coloured bars under the sample dendrograms represent the five *Nannoperca australis* populations and are based on colours used in Figure 1

Functional annotation enrichment analysis of GO terms for the DE transcripts identified 643 significantly enriched terms ($p < .05$) with 54 remaining significant at a FDR of 10% (Table S7, Supporting information).

3.3 | Gene expression plasticity and divergent selection

PYRAD processing of the ddRAD sequences (Brauer et al., 2016) to generate the phylogeny for EVE resulted in 30,870 distinct alignments with a total of 384,998 sites, of which 14,997 were variable and 12,244 were parsimony informative. Phylogenetic analysis supported reciprocal monophyly for all populations (Figure S3, Supporting information). The RAXML majority-rule consensus tree was used as the input phylogeny for the EVE analysis (Figure S4, Supporting information).

Of the 27,425 ORFs assessed with the EVE phylogenetic ANOVA, 83 showed a significant departure from the null hypothesis of a constant expression divergence-to-diversity ratio. Of these, 33 were identified as candidates for high expression-level plasticity, demonstrating significantly (FDR 10%) greater expression variance within than among lineages. The hierarchical sample dendrogram that clusters individuals based on these genes was inconsistent with spatial phylogenetic patterns (Figure 3a). This suggests that the expression of these genes is highly plastic at the species level and can vary in response to local variations in environmental conditions.

Functional annotation enrichment analysis of GO terms for these transcripts identified 170 significantly enriched terms ($p < .05$);

however, none remained significant at a FDR of 10%. Functional categories consisted mainly of terms related to general metabolic activities and cell cycle regulation, but several terms involving responses to oxidative stress and immune responses stand out as key biological processes associated with these genes (Table S8, Supporting information).

The remaining 50 candidates identified with EVE showed significantly (FDR 10%) greater expression variance among than within lineages as indicated by the highly consistent spatial phylogenetic patterns (Figure 3b). This is suggestive of adaptive evolution of expression level of these genes in response to environmental differences among catchments. Enrichment analysis of GO terms assigned to this group of transcripts recovered 137 significantly enriched terms ($p < .05$), with 10 remaining significant at a FDR of 10% (Table S9, Supporting information).

3.4 | Gene expression variance and genetic diversity

The multivariate homogeneity of variances tests identified no significant differences in gene expression variance among populations based on all 27,425 transcripts ($p = .778$), the 50 EVE candidate divergent transcripts ($p = .233$) or the 33 high expression plasticity transcripts ($p = .150$) (Table 3). For each pairwise Tukey's test, the 95% confidence intervals included zero, supporting the null hypothesis of no difference in expression variance among any populations (Figure S5, Supporting information).

TABLE 3 Multivariate analysis of homogeneity of variance of gene expression based on 27,425 ORFs, 50 divergent and 33 plastic candidate transcripts for populations of *Nannoperca australis*

Transcripts		df	SumsOfSqs	MeanSqs	F value	p
27,425	Groups	4	0.00303	0.00076	0.441	.778
	Residuals	20	0.03433	0.00172		
50	Groups	4	0.01027	0.00257	1.523	.233
	Residuals	20	0.03370	0.00168		
33	Groups	4	0.04704	0.01176	1.896	.150
	Residuals	20	0.12403	0.00620		

The analysis of variance using distance matrices permutation test found no significant relationship between genetic diversity and gene expression. Variation in individual heterozygosity (i.e., the proportion

of heterozygous loci per individual) based on 3443 neutral and 216 candidate SNPs is summarized in Figure 4a. Variance in population multivariate expression profiles is summarized in Figure 4b–d. Individual heterozygosity at both neutral and candidate SNP loci was a poor predictor of expression variance for the 27,425 transcripts, 50 EVE candidate divergent transcripts, and the 33 high expression plasticity transcripts (Table 4).

4 | DISCUSSION

The long-term persistence of populations trapped by habitat fragmentation and threatened by the combination of rapid climate change and habitat degradation likely depends on their ability to

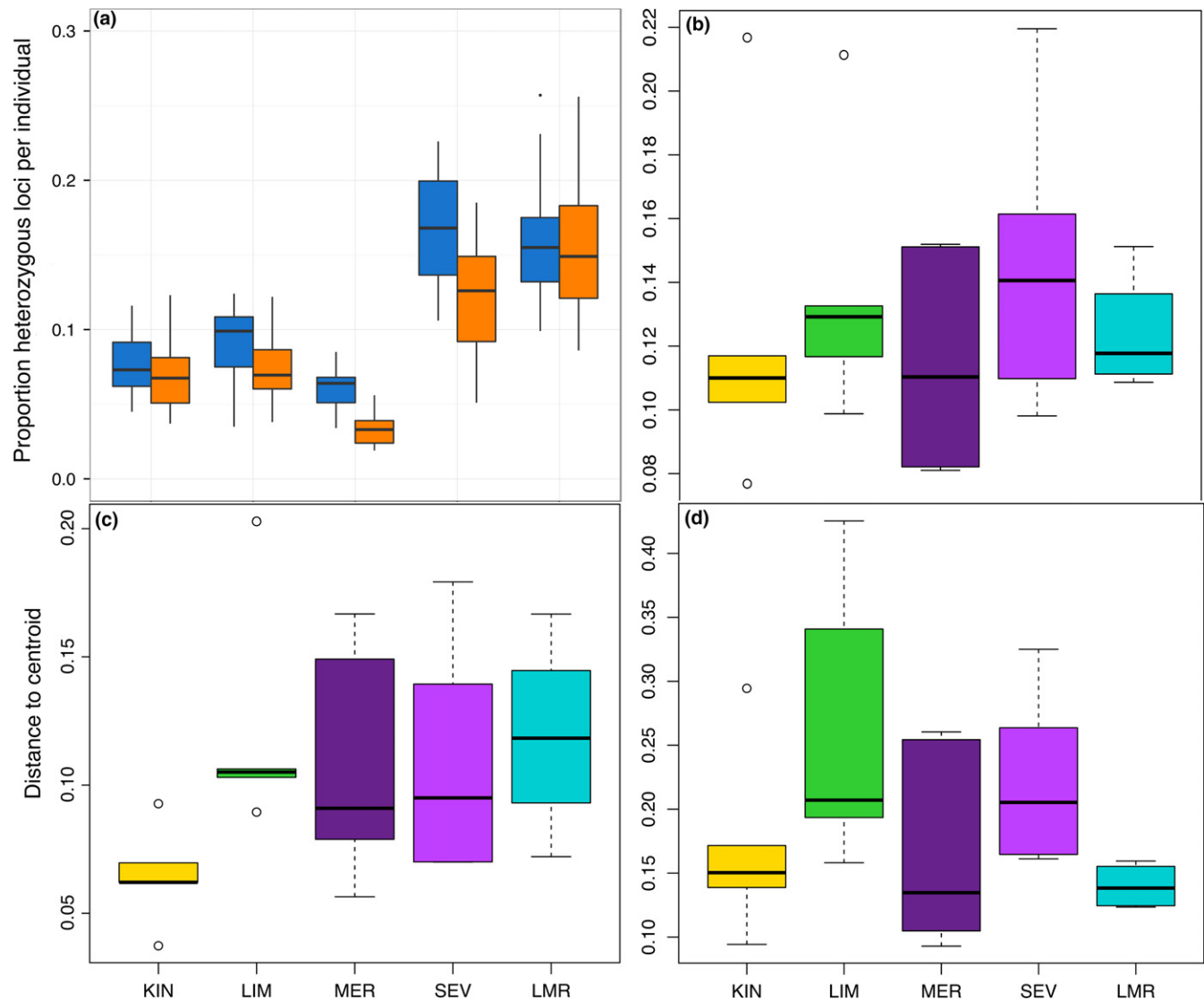


FIGURE 4 Boxplots summarizing (a) population variance in individual heterozygosity at 3443 neutral (blue) and 216 genotype–environment association candidate (orange) SNP loci (Brauer et al., 2016), (b) population variance in gene expression based on the first two principal coordinate axes summarizing 27,425 ORFs, (c) population variance in expression based on the first two principal coordinate axes summarizing 50 transcripts identified as candidates for divergent selection for expression level and (d) population variance in expression based on the first two principal coordinate axes summarizing 33 transcripts identified as candidates demonstrating high intrapopulation expression-level plasticity. Colours in (b), (c) and (d) are based on those used in Figure 1

TABLE 4 Multivariate analysis of variance test for association between gene expression variance based on 27,425 ORFs, 50 divergent and 33 plastic candidate transcripts and genetic diversity (proportion of heterozygous loci at 3443 putatively neutral and 216 GEA candidate SNPs) for *Nannoperca australis*

Transcripts	SNPs		df	SumsOfSqs	MeanSqs	F.Model	R ²	p
27,425	3443	H _E	1	0.050110	0.050110	1.551	0.063	.503
		Residuals	23	0.743173	0.032312		0.937	
		Total	24	0.793283			1	
	216	H _E	1	0.084370	0.084370	2.737	0.106	.492
		Residuals	23	0.708912	0.030822		0.894	
		Total	24	0.793283			1	
50	3443	H _E	1	0.193556	0.193556	2.201	0.087	.463
		Residuals	23	2.022549	0.087937		0.913	
		Total	24	2.216105			1	
	216	H _E	1	0.442324	0.442324	5.735	0.200	.125
		Residuals	23	1.773782	0.077121		0.800	
		Total	24	2.216105			1	
33	3443	H _E	1	0.143784	0.143784	2.253	0.089	.761
		Residuals	23	1.467629	0.063810		0.911	
		Total	24	1.611413			1	
	216	H _E	1	0.210906	0.210906	3.464	0.131	.723
		Residuals	23	1.400507	0.060892		0.869	
		Total	24	1.611413			1	

mount both adaptive genetic and phenotypic responses (Chevin et al., 2010). The extent to which phenotypic plasticity contributes to evolutionary potential of wild populations, and the relationship between plastic and evolved responses to environmental variation, however, remains unresolved and is a key research priority (Alvarez et al., 2015; Merilä & Hendry, 2014). Comparative transcriptomics provides a powerful platform with which to address these issues, as gene expression measurements can be considered as phenotypic traits resulting from a combination of genotype, environment and genotype–environment interactions (DeBiasse & Kelly, 2016). Here, we first present a de novo liver transcriptome for *Nannoperca australis*, a member of Percichthyidae, one of the dominant freshwater fish families in Australia. We then examined baseline patterns of transcript expression variation within and among populations sampled across a marked gradient of hydroclimatic variability in the highly impacted Murray–Darling Basin, Australia. A combination of DE and ANOVA-based EVE analyses identified 373 candidate transcripts with 83 of these demonstrating expression profiles consistent with either high plasticity or divergent selection on expression among ecotypes. Functional GO analyses revealed that many of these candidates may be involved in responses to environmental challenges including oxidative stress and metabolism of a range of natural organic and xenobiotic compounds (Tables S7–9, Supporting information). Finally, we found no significant relationship between global gene expression variance and genetic diversity for *N. australis*, suggesting that despite reduced genetic diversity, small and isolated populations retain similar capacity for gene expression plasticity as larger populations.

4.1 | Variance in gene expression does not appear constrained by genetic diversity

A growing body of evidence indicates that gene expression has a large heritable component (Gibson & Weir, 2005; Leder et al., 2015; McCairns et al., 2016), suggesting that if genetic diversity is lost due to drift, plasticity in gene expression may also be reduced (Bijlsma & Loeschcke, 2012). Very few studies have addressed this issue using wild populations, and the relationship between genetic diversity and phenotypic plasticity remains unclear (Chevin et al., 2013). Wood and Fraser (2015) recently examined the relationship between population size and plasticity in several life history traits using a common garden experiment with populations of brook trout (*Salvelinus fontinalis*). They found little evidence to suggest that phenotypic plasticity was constrained by population size and proposed that increased habitat variability in smaller habitat fragments likely favours higher plasticity.

Small populations with reduced genetic diversity are expected to exhibit lower fitness and less capacity for adaptive evolutionary responses than large populations (Hoffmann, Sgrò, & Kristensen, 2017). Many populations do persist with low genetic diversity, however (e.g., Robinson et al., 2016). While these populations are vulnerable to stochastic demographic declines due to extreme weather events, disease and pollution, there must be evolutionary processes and mechanisms that allow small populations to survive. Phenotypic plasticity, for instance, including variation in gene expression, can facilitate population persistence in rapidly changing and poor-quality environments (Whitehead et al., 2010). Theoretical predictions also

suggest that higher levels of plasticity should evolve in marginal and highly variable environments, despite reduced population sizes (Chevin & Lande, 2011). These predictions are supported by empirical studies of range-margin populations where genetic diversity is often reduced, and low habitat quality in combination with high habitat variability are common (Lázaro-Nogal et al., 2015; Nilsson-Örtman, Stoks, De Block, & Johansson, 2012; Valladares et al., 2014). The populations examined in our study span the range of diversity found for the species in the MDB (Table 1), including some of the lowest levels of population genetic diversity reported for a freshwater fish (Cole et al., 2016). They also include sites at the extreme ends of the hydroclimatic gradient that characterizes the basin (Figure 1). When considered in the context of the naturally highly variable environment that *N. australis* have evolved in, along with more recent impacts of fragmentation and population size reductions (Attard et al., 2016; Brauer et al., 2016; Cole et al., 2016), our finding that gene expression variance is not constrained by genetic diversity suggests that *N. australis* may use this mechanism to respond to environmental challenges, despite reduced levels of genetic variation.

4.2 | Comparative transcriptomics in the wild

Disentangling genetic and environmental components of transcriptional variation in the wild remains an important question in evolutionary and conservation biology. While it is increasingly recognized that effective conservation strategies need to incorporate information concerning adaptive and functional genetic variation (Harrisson et al., 2014; Sgrò, Lowe, & Hoffmann, 2011), extending this concept to also include gene expression variation has the potential to further improve conservation efforts. Hoffmann et al. (2017) recently outlined the difficulties faced in tracking adaptive genomic variation in small populations, and studies of gene expression in the wild present even greater challenges, particularly for small and threatened populations. Despite these challenges, Hoffmann et al. (2017) conclude by highlighting the importance of continued efforts to measure and map genetic diversity across the landscape to increase understanding of demographic and adaptive processes contributing to evolutionary potential. Similarly, we argue here it is equally important to begin to build our understanding of broad patterns of gene expression variation in the wild. Comparative transcriptomics is one approach that can provide insight (Harrisson et al., 2014), and results in the present study raise the possibility that gene expression variation contributes to population persistence and the evolutionary potential of *N. australis*.

Transcriptomic responses to environmental stressors are well documented in fishes (Baillon et al., 2015; Bozinovic & Oleksiak, 2011; Leder et al., 2015; Oleksiak, 2008; Pujolar et al., 2012; Smith et al., 2013; Whitehead et al., 2010). For species evolving in variable and naturally harsh environments, the ability to respond rapidly to often-abrupt changes in water quality should provide a distinct evolutionary advantage. Accordingly, several studies have provided evidence that natural selection can influence patterns of gene expression variation. For example, killifish (*Fundulus heteroclitus*)

inhabit highly variable tidal marshes and are well known for their ability to tolerate extreme conditions and rapid changes in water quality such as variation in pH, temperature, salinity and dissolved oxygen (Burnett et al., 2007). Experimental work revealed that complex patterns of gene expression and genetic variation in killifish are underpinned by locally adapted transcriptomic responses to osmotic shock (Whitehead et al., 2010). Similarly, Leder et al. (2015) found substantial genetic variance in gene expression among populations of three-spined stickleback (*Gasterosteus aculeatus*) for genes associated with temperature stress. Heritable patterns of gene expression have also been documented for an Australian rainbowfish (*Melano- taenia duboulayi*) at candidate genes for thermal adaptation (McCairns et al., 2016). In that study, additive genetic variance and transcriptional plasticity explained variation in gene expression associated with long-term exposure to a predicted future climate, providing pedigree-based support that transcriptional variation has an underlying heritable basis. In our study, one of the differentially expressed candidate genes (TBX2) appears homologous with a previously identified GEA candidate locus thought to be under selection in *N. australis* due to hydroclimatic variation (Brauer et al., 2016). The TBX2 gene is known to influence fin development in zebra fish (Ruvinsky, Oates, Silver, & Ho, 2000). This is suggestive of heritable genotype–environment interactions and provides a strong candidate for adaptive plasticity in gene expression. Our findings are consistent with those previous studies supporting the hypotheses that gene expression can evolve in response to natural selection and that both genomic and transcriptomic variations contribute to species' evolutionary potential.

4.3 | Functional analysis and environmental stressors

Functional annotations based on distantly related species should be interpreted with caution as the extent to which gene functions are conserved among divergent taxa remains largely unknown (Primmer, Papakostas, Leder, Davis, & Ragan, 2013). A general assessment of putative functional categories characterizing candidate genes in an ecological context can, nonetheless, provide information and generate hypotheses regarding important environmental or ecological factors influencing patterns of gene expression (Pavey et al., 2012). Several candidate transcripts with enriched GO terms belong to a group of aspartic-type endopeptidase and peptidase enzymes involved in protein digestion (Table S7; Table S9). This class of enzyme is known to be important in other fishes for muscle proteolysis associated with physiological challenges such as starvation, migration or reproductive activity (Mommensen, 2004; Wang, Stenvik, Larsen, Mæhre, & Olsen, 2007), and is likely to play an important role in survival in variable environments.

Oestrogens and other endocrine-disrupting chemicals are recognized as a global issue for freshwater fishes. For instance, low concentrations of these substances have been implicated in the feminization of males in a population of fathead minnows (*Pimephales promelas*) in Canada, leading to the eventual collapse of the

population (Kidd et al., 2007). These chemicals are already known to adversely affect native fish reproduction in the MDB (Vajda et al., 2015), and several enriched GO terms (e.g., steroid biosynthetic process, steroid metabolic process, regulation of hormone levels, estrogen receptor activity) associated with candidate transcripts raise the possibility that environmental oestrogens are impacting reproductive health of *N. australis* and probably other MDB fishes.

Other enriched terms associated with candidate transcripts are involved in metabolism of organic and synthetic compounds and with response to stress (e.g., sterol biosynthetic process, response to organophosphorus, response to oxidative stress). Challenging environmental conditions such as thermal stress or exposure to pollution can induce oxidative stress (Hermes-Lima & Zenteno-Savin, 2002), and heritable variation in expression of genes associated with oxidative stress was identified in Australian *M. duboulayi* (McCairns et al., 2016). These responses can also be induced by industrial chemicals such as pesticides. Organochlorine pesticides were used heavily throughout the MDB during the mid-to-late 1900s, and residues remaining in sediments today are known to increase concentrations in waterways after heavy rainfall events (McKenzie-Smith, Tiller, & Allen, 1994). These chemicals have been linked to invertebrate larval deformities across the MDB (Pettigrove, 1989) and are known to cause oxidative stress in fish (Slaninova, Smutna, Modra, & Svobodova, 2009). Naphthalene is a water-soluble by-product of oil and gas production and is also a constituent of some pesticides (Gavin, Brooke, & Howe, 1996). This compound is toxic to fish and is known to induce developmental abnormalities and affect reproduction in another MDB fish, *Melanotaenia fluviatilis* (Pollino, Georgiades, & Holdway, 2009; Pollino & Holdway, 2002). Naturally occurring toxic compounds including endogenous cellular products and xenobiotics such as plant-based flavonoids and tannins can also influence gene expression (Buckley & Klaassen, 2007). Tannins and polyphenols leaching from *Eucalyptus* leaves are naturally present in waters inhabited by *N. australis* and are known to impact reproductive success, affect juvenile growth and survival and drive variation in male nuptial coloration in this species (Morrongiello, Bond, Crook, & Wong, 2010, 2011, 2013). The magnitude of response to *Eucalyptus* leachate exposure also varies for *N. australis* populations across a natural gradient of water quality (Morrongiello et al., 2013). The latter suggests that populations are adapted to local variations in water quality, which is consistent with our findings that transcripts with GO terms potentially associated with metabolizing flavonoids and tannins (e.g., terpenoid metabolic process, phenol-containing compound metabolic process) are divergently expressed among populations. Other organic compounds such as sulphides can originate in freshwaters from natural decomposition of organic matter, and also industrial and urban pollution such as sewage wastewater. Sulphides are toxic to fish and can reduce juvenile survival (Smith & Oseid, 1972). Smith and Oseid (1972) also found that the effects of sulphides were exacerbated by increased temperatures and reduced dissolved oxygen levels, suggesting that these toxins may become more virulent under a changed climate regime. This also points to the broader implications of widespread habitat degradation

concomitant with rapid climatic changes, and supports recent evidence that the compounding effects of climate change and pollution pose additional extinction risks for many threatened species (Brown et al., 2015).

While the divergent expression profiles of the candidate transcripts we identified are consistent with adaptive responses to environmental variation, there are several caveats that must be considered when inferring evolutionary and environmental responses from populations in the wild. Despite efforts to minimize variation in sampling, some uncontrolled environmental variation is unavoidable in studies of this nature. Accordingly, an alternative interpretation of our results is that some of the candidates may be simply responding plastically to local and transient environmental variation present at the time of sampling. This is plausible considering a number of these transcripts are putatively involved in responses to xenobiotic organic compounds and, more broadly, oxidative stress, both of which may be induced as a response to variations in water quality over relatively short timescales (Whitehead, Galvez, Zhang, Williams, & Oleksiak, 2011).

Another possibility is that genetic drift may be, at least partially, responsible for the patterns of expression divergence. Although the EVE model used here does account for phylogenetic divergence, it assumes one consistent phylogeny for all genes (Rohlf & Nielsen, 2015). The phylogeny for *N. australis* based on ddRAD sequences is well resolved, however (Figure S3), and we consider it highly unlikely to have biased the results. Additionally, EVE does not control for expression covariance among genes. This assumption of independence among transcripts is almost certainly violated in any global gene expression study, and developing new methods to account for the complex correlation structures present in RNA-seq data would undoubtedly prove beneficial. Notwithstanding these limitations, our findings support the hypothesis that divergent natural selection is driving patterns of expression for *N. australis* at a number of ecologically important genes. This provides a basis for further work, and examining the candidate transcripts within a common garden experimental framework (e.g., McCairns et al., 2016; Smith et al., 2013; Whitehead et al., 2011) represents a next step in assessing the potential role of natural selection in shaping gene expression in this system.

5 | CONCLUSIONS

Plastic and evolutionary components of gene expression can now be explored for nonmodel species using RNA-seq, and as new analytical approaches evolve, we stand to gain insight into how gene expression variation contributes to evolutionary potential. The comparative transcriptomic analysis here identified 373 candidate transcripts putatively contributing to the evolutionary potential of *N. australis* populations across a hydroclimatic environmental gradient. These included 290 DE transcripts, 50 candidates for divergent selection on expression level and 33 candidates for high expression plasticity after controlling for phylogenetic effects. Functional GO analyses of

the candidate transcripts suggest that variation in water quality is driving patterns of expression for genes potentially related to metabolic and reproductive traits for populations of *N. australis*. Our results also support the hypothesis that variation in gene expression may be one mechanism allowing small populations with depleted genetic diversity to persist in variable environments.

Proactive conservation management strategies for small and threatened populations need to incorporate assessments of evolutionary potential. How managers should best exploit this information to maintain or build evolutionarily resilient populations remains challenging (Webster et al., 2017). It is, nonetheless, important to work towards understanding the evolutionary and environmental determinants of gene expression variation among wild populations. Although the work presented here represents just an initial step towards that understanding, it is particularly important given that it pioneers this type of research in poorly studied but highly diverse Southern Hemisphere fishes.

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DATA ACCESSIBILITY

Final transcriptome assembly, ORF raw counts data and *Trinotate* annotation report can be accessed on Dryad: <https://doi.org/10.5061/dryad.6gh7b>.

AUTHOR CONTRIBUTIONS

C.J.B. and L.B.B. designed the study. C.J.B. generated and analysed the data with assistance from L.B.B. and P.J.U. C.J.B. and L.B.B. lead the writing of the manuscript, with input from P.J.U.

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REFERENCES

Alvarez, M., Schrey, A. W., & Richards, C. L. (2015). Ten years of transcriptomics in wild populations: What have we learned about their ecology and evolution? *Molecular Ecology*, 24, 710–725. <https://doi.org/10.1111/mec.13055>

- Attard, C., Möller, L., Sasaki, M., Hammer, M. P., Bice, C. M., Brauer, C. J., ... Beheregaray, L. B. (2016). A novel holistic framework for genetic-based captive-breeding and reintroduction programs. *Conservation Biology*, 30, 1060–1069. <https://doi.org/10.1111/cobi.12699>
- Baillon, L., Pierron, F., Coudret, R., Normendeau, E., Caron, A., Peluhet, L., ... Elie, P. (2015). Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels. *Ecotoxicology*, 24, 71–84. <https://doi.org/10.1007/s10646-014-1356-x>
- Bateman, A., Coin, L., Durbin, R., Finn, R. D., Hollich, V., Griffiths-Jones, S., ... Studholme, D. J. (2004). The Pfam protein families database. *Nucleic Acids Research*, 32, D138–D141. <https://doi.org/10.1093/nar/gkh121>
- Bijlsma, R., & Loeschcke, V. (2012). Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications*, 5, 117–129. <https://doi.org/10.1111/j.1752-4571.2011.00214.x>
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Botstein, D., Cherry, J. M., Ashburner, M., Ball, C. A., Blake, J. A., Butler, H., ... Eppig, J. T. (2000). Gene Ontology: Tool for the unification of biology. *Nature Genetics*, 25, 25–29.
- Bozinovic, G., & Oleksiak, M. F. (2011). Genomic approaches with natural fish populations from polluted environments. *Environmental Toxicology and Chemistry*, 30, 283–289. <https://doi.org/10.1002/etc.403>
- Brauer, C. J., Hammer, M. P., & Beheregaray, L. B. (2016). Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. *Molecular Ecology*, 25, 5093–5113. <https://doi.org/10.1111/mec.13830>
- Brown, A. R., Owen, S. F., Peters, J., Zhang, Y., Soffker, M., Paull, G. C., ... Tyler, C. R. (2015). Climate change and pollution speed declines in zebrafish populations. *Proceedings of the National Academy of Sciences*, 112, E1237–E1246. <https://doi.org/10.1073/pnas.1416269112>
- Buckley, D. B., & Klaassen, C. D. (2007). Tissue- and gender-specific mRNA expression of UDP-glucuronosyltransferases (UGTs) in mice. *Drug Metabolism and Disposition*, 35, 121–127.
- Burnett, K. G., Bain, L. J., Baldwin, W. S., Callard, G. V., Cohen, S., Di Giulio, R. T., ... Karchner, S. I. (2007). Fundulus as the premier teleost model in environmental biology: Opportunities for new insights using genomics. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 2, 257–286.
- Charmanier, A., McCleery, R. H., Cole, L. R., Perrins, C., Kruuk, L. E., & Sheldon, B. C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, 320, 800–803. <https://doi.org/10.1126/science.1157174>
- Chevin, L.-M., Gallet, R., Gomulkiewicz, R., Holt, R. D., & Fellous, S. (2013). Phenotypic plasticity in evolutionary rescue experiments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368, 20120089.
- Chevin, L. M., & Lande, R. (2011). Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *Journal of Evolutionary Biology*, 24, 1462–1476. <https://doi.org/10.1111/j.1420-9101.2011.02279.x>
- Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology*, 8, e1000357.
- Chiew, F., Teng, J., Kirono, D., Frost, A. J., Bathols, J. M., Vaze, J., ... Cai, W. J. (2008). *Climate Data for Hydrologic Scenario Modelling Across the Murray-Darling Basin: A Report to the Australian Government from the CSIRO Murray-Darling Basin Sustainable Yields Project* CSIRO Canberra, Australia.
- Cole, T., Hammer, M., Unmack, P., Teske, P. R., Brauer, C. J., Adams, M., & Beheregaray, L. B. (2016). Range-wide fragmentation in a threatened fish associated with post-European settlement modification in the Murray-Darling Basin, Australia. *Conservation Genetics*, 17, 1377–1391. <https://doi.org/10.1007/s10592-016-0868-8>

- Dayan, D. I., Crawford, D. L., & Oleksiak, M. F. (2015). Phenotypic plasticity in gene expression contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. *Molecular Ecology*, 24, 3345–3359. <https://doi.org/10.1111/mec.13188>
- DeBiasse, M. B., & Kelly, M. W. (2016). Plastic and evolved responses to global change: what can we learn from comparative transcriptomics? *Journal of Heredity*, 107, 71–81. <https://doi.org/10.1093/jhered/esv073>
- Dunn, C. W., Luo, X., & Wu, Z. (2013). Phylogenetic analysis of gene expression. *Integrative and Comparative Biology*, 53, 847–856. <https://doi.org/10.1093/icb/ict068>
- Eaton, D. A. (2014). PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, 30, 1844–1849. <https://doi.org/10.1093/bioinformatics/btu121>
- Fischer, J., & Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: A synthesis. *Global Ecology and Biogeography*, 16, 265–280. <https://doi.org/10.1111/j.1466-8238.2007.00287.x>
- Fraser, D. J., Debes, P. V., Bernatchez, L., & Hutchings, J. A. (2014). Population size, habitat fragmentation, and the nature of adaptive variation in a stream fish. *Proceedings of the Royal Society of London B: Biological Sciences*, 281, <https://doi.org/10.1098/rspb.2014.0370>
- Gavin, M., Brooke, D., & Howe, P. (1996). *Environmental hazard assessment: Naphthalene*. Great Britain: Department of the Environment, Chemical and Biotechnology Division.
- Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, 525, 372–375.
- Gibson, G., & Weir, B. (2005). The quantitative genetics of transcription. *Trends in Genetics*, 21, 616–623. <https://doi.org/10.1016/j.tig.2005.08.010>
- Gienapp, P., Teplitsky, C., Alho, J. S., Mills, J. A., & Merila, J. (2008). Climate change and evolution: Disentangling environmental and genetic responses. *Molecular Ecology*, 17, 167–178. <https://doi.org/10.1111/j.1365-294X.2007.03413.x>
- Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., ... Chen, Z. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, 29, 644–652. <https://doi.org/10.1038/nbt.1883>
- Hammer, M. P., Bice, C. M., Hall, A., Frears, A., Watt, A., Whiterod, N. S., ... Zampatti, B. P. (2013). Freshwater fish conservation in the face of critical water shortages in the southern Murray-Darling Basin, Australia. *Marine and Freshwater Research*, 64, 807–821. <https://doi.org/10.1071/MF12258>
- Harrisson, K. A., Pavlova, A., Telonis-Scott, M., & Sunnucks, P. (2014). Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications*, 7, 1008–1025. <https://doi.org/10.1111/eva.12149>
- Hermes-Lima, M., & Zenteno-Savin, T. (2002). Animal response to drastic changes in oxygen availability and physiological oxidative stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 133, 537–556.
- Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, 470, 479–485. <https://doi.org/10.1038/nature09670>
- Hoffmann, A. A., Sgrò, C. M., & Kristensen, T. N. (2017). Revisiting adaptive potential, population size, and conservation. *Trends in Ecology & Evolution*, 32, 506–517.
- Houle, D., Govindaraju, D. R., & Omholt, S. (2010). Phenomics: The next challenge. *Nature Reviews Genetics*, 11, 855–866. <https://doi.org/10.1038/nrg2897>
- Hudson, R. R., Kreitman, M., & Aguadé, M. (1987). A test of neutral molecular evolution based on nucleotide data. *Genetics*, 116, 153–159.
- Khaitovich, P., Weiss, G., Lachmann, M., Hellmann, I., Enard, W., Muetzel, B., & Pääbo, S. (2004). A neutral model of transcriptome evolution. *PLoS Biology*, 2, e132. <https://doi.org/10.1371/journal.pbio.0020132>
- Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences*, 104, 8897–8901. <https://doi.org/10.1073/pnas.0609568104>
- Koskinen, M. T., Haugen, T. O., & Primmer, C. R. (2002). Contemporary fisherian life-history evolution in small salmonid populations. *Nature*, 419, 826–830. <https://doi.org/10.1038/nature01029>
- Krogh, A., Larsson, B., Von Heijne, G., & Sonnhammer, E. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *Journal of Molecular Biology*, 305, 567–580. <https://doi.org/10.1006/jmbi.2000.4315>
- Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, 10, 1.
- Lázaro-Nogal, A., Matesanz, S., Godoy, A., Pérez-Trautman, F., Gianoli, E., & Valladares, F. (2015). Environmental heterogeneity leads to higher plasticity in dry-edge populations of a semi-arid Chilean shrub: Insights into climate change responses. *Journal of Ecology*, 103, 338–350. <https://doi.org/10.1111/1365-2745.12372>
- Leder, E. H., McCairns, R. S., Leinonen, T., Cano, J. M., Viitaniemi, H. M., Nikinmaa, M., ... Merilä, J. (2015). The evolution and adaptive potential of transcriptional variation in sticklebacks—signatures of selection and widespread heritability. *Molecular Biology and Evolution*, 32, 674–689. <https://doi.org/10.1093/molbev/msu328>
- Li, B., & Dewey, C. N. (2011). RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*, 12, 1.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 1.
- McCairns, R. J. S., Smith, S., Sasaki, M., Bernatchez, L., & Beheregaray, L. B. (2016). The adaptive potential of subtropical rainbowfish in the face of climate change: Heritability and heritable plasticity for the expression of candidate genes. *Evolutionary Applications*, 9, 531–545. <https://doi.org/10.1111/eva.12363>
- McKenzie-Smith, F., Tiller, D., & Allen, D. (1994). Organochlorine pesticide residues in water and sediments from the Ovens and King rivers, north-east Victoria, Australia. *Archives of Environmental Contamination and Toxicology*, 26, 483–490. <https://doi.org/10.1007/BF00214151>
- Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7, 1–14. <https://doi.org/10.1111/eva.12137>
- Mommsen, T. P. (2004). Salmon spawning migration and muscle protein metabolism: The August Krogh principle at work. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 139, 383–400. <https://doi.org/10.1016/j.cbpc.2004.09.018>
- Morronegiello, J. R., Bond, N. R., Crook, D. A., & Wong, B. B. M. (2010). Nuptial coloration varies with ambient light environment in a freshwater fish. *Journal of Evolutionary Biology*, 23, 2718–2725. <https://doi.org/10.1111/j.1420-9101.2010.02149.x>
- Morronegiello, J. R., Bond, N. R., Crook, D. A., & Wong, B. (2011). Eucalyptus leachate inhibits reproduction in a freshwater fish. *Freshwater Biology*, 56, 1736–1745. <https://doi.org/10.1111/j.1365-2427.2011.02605.x>
- Morronegiello, J. R., Bond, N. R., Crook, D. A., & Wong, B. B. M. (2012). Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. *Journal of Animal Ecology*, 81, 806–817. <https://doi.org/10.1111/j.1365-2656.2012.01961.x>
- Morronegiello, J. R., Bond, N. R., Crook, D. A., & Wong, B. (2013). Intraspecific variation in the growth and survival of juvenile fish exposed to *Eucalyptus* leachate. *Ecology and Evolution*, 3, 3855–3867. <https://doi.org/10.1002/ece3.757>

- Nilsson-Örtman, V., Stoks, R., De Block, M., & Johansson, F. (2012). Generalists and specialists along a latitudinal transect: Patterns of thermal adaptation in six species of damselflies. *Ecology*, 93, 1340–1352. <https://doi.org/10.1890/11-1910.1>
- Oksanen, J., Blanchet, F. G., Kindt, R., Oksanen, M. J., & Suggests, M. A. S. (2015). Package 'vegan'. *Community ecology package, version*, 2.3–0.
- Oleksiak, M. F. (2008). Changes in gene expression due to chronic exposure to environmental pollutants. *Aquatic Toxicology*, 90, 161–171. <https://doi.org/10.1016/j.aquatox.2008.08.010>
- Oleksiak, M. F., Churchill, G. A., & Crawford, D. L. (2002). Variation in gene expression within and among natural populations. *Nature Genetics*, 32, 261–266. <https://doi.org/10.1038/ng983>
- Oleksiak, M. F., Roach, J. L., & Crawford, D. L. (2005). Natural variation in cardiac metabolism and gene expression in *Fundulus heteroclitus*. *Nature Genetics*, 37, 67–72.
- Paschke, M., Bernasconi, G., Schmid, B., & Williams, R. (2003). Population size and identity influence the reaction norm of the rare, endemic plant *Cochlearia bavarica* across a gradient of environmental stress. *Evolution*, 57, 496–508. <https://doi.org/10.1111/j.0014-3820.2003.tb01541.x>
- Pavey, S. A., Bernatchez, L., Aubin-Horth, N., & Landry, C. R. (2012). What is needed for next-generation ecological and evolutionary genomics? *Trends in Ecology & Evolution*, 27, 673–678. <https://doi.org/10.1016/j.tree.2012.07.014>
- Petersen, T. N., Brunak, S., von Heijne, G., & Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nature Methods*, 8, 785–786. <https://doi.org/10.1038/nmeth.1701>
- Pettigrove, V. (1989). Larval mouthpart deformities in *Procladius paludicola* Skuse (Diptera: Chironomidae) from the Murray and Darling rivers, Australia. *Hydrobiologia*, 179, 111–117. <https://doi.org/10.1007/BF00007598>
- van de Pol, M., Osmond, H. L., & Cockburn, A. (2012). Fluctuations in population composition dampen the impact of phenotypic plasticity on trait dynamics in superb fairy-wrens. *Journal of Animal Ecology*, 81, 411–422.
- Pollino, C. A., Georgiades, E., & Holdway, D. A. (2009). Physiological changes in reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to naphthalene. *Ecotoxicology and Environmental Safety*, 72, 1265–1270. <https://doi.org/10.1016/j.ecoenv.2009.01.012>
- Pollino, C. A., & Holdway, D. A. (2002). Toxicity testing of crude oil and related compounds using early life stages of the crimson-spotted Rainbowfish (*Melanotaenia fluviatilis*). *Ecotoxicology and Environmental Safety*, 52, 180–189. <https://doi.org/10.1006/eesa.2002.2190>
- Primmer, C. R., Papakostas, S., Leder, E. H., Davis, M. J., & Ragan, M. A. (2013). Annotated genes and nonannotated genomes: Cross-species use of Gene Ontology in ecology and evolution research. *Molecular Ecology*, 22, 3216–3241. <https://doi.org/10.1111/mec.12309>
- Pujolar, J. M., Marino, I. A., Milan, M., Coppe, A., Maes, G. E., Capoccioni, F., ... Cramb, G. (2012). Surviving in a toxic world: Transcriptomics and gene expression profiling in response to environmental pollution in the critically endangered European eel. *BMC Genomics*, 13, 1.
- Rabergh, C., Airaksinen, S., Soitamo, A., Björklund, H., Johansson, T., Nikinmaa, M., & Sistonon, L. (2000). Tissue-specific expression of zebrafish (*Danio rerio*) heat shock factor 1 mRNAs in response to heat stress. *Journal of Experimental Biology*, 203, 1817–1824.
- Réale, D., McAdam, A. G., Boutin, S., & Berteaux, D. (2003). Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London B: Biological Sciences*, 270, 591–596. <https://doi.org/10.1098/rspb.2002.2224>
- Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26, 139–140. <https://doi.org/10.1093/bioinformatics/btp616>
- Robinson, J. A., Ortega-Del Vecchyo, D., Fan, Z., Kim, B. Y., Marsden, C. D., Lohmueller, K. E., & Wayne, R. K. (2016). Genomic flatlining in the endangered island fox. *Current Biology*, 26, 1183–1189. <https://doi.org/10.1016/j.cub.2016.02.062>
- Rohlf, R. V., & Nielsen, R. (2015). Phylogenetic ANOVA: The expression variance and evolution model for quantitative trait evolution. *Systematic Biology*, 64, 695–708. <https://doi.org/10.1093/sysbio/syv042>
- Romero, I. G., Ruvinsky, I., & Gilad, Y. (2012). Comparative studies of gene expression and the evolution of gene regulation. *Nature Reviews Genetics*, 13, 505–516. <https://doi.org/10.1038/nrg3229>
- Ruvinsky, I., Oates, A. C., Silver, L. M., & Ho, R. K. (2000). The evolution of paired appendages in vertebrates: T-box genes in the zebrafish. *Development Genes and Evolution*, 210, 82–91. <https://doi.org/10.1007/s004270050014>
- Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, 4, 326–337. <https://doi.org/10.1111/j.1752-4571.2010.00157.x>
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31, btv351.
- Slaninova, A., Smutna, M., Modra, H., & Svobodova, Z. (2009). A review: Oxidative stress in fish induced by pesticides. *Neuroendocrinology Letters*, 30, 2.
- Smith, S., Bernatchez, L., & Beheregaray, L. B. (2013). RNA-seq analysis reveals extensive transcriptional plasticity to temperature stress in a freshwater fish species. *BMC Genomics*, 14, 375. <https://doi.org/10.1186/1471-2164-14-375>
- Smith, L., & Oseid, D. (1972). Effects of hydrogen sulfide on fish eggs and fry. *Water Research*, 6, 711–720. [https://doi.org/10.1016/0043-1354\(72\)90186-8](https://doi.org/10.1016/0043-1354(72)90186-8)
- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Tao, T. (2014). *Standalone BLAST setup for Unix*. Bethesda (MD): National Center for Biotechnology Information BLAST® Help [Internet].
- Uebbing, S., Künstner, A., Mäkinen, H., Backström, N., Bolivar, P., Burri, R., ... Flicek, P. (2016). Divergence in gene expression within and between two closely related flycatcher species. *Molecular Ecology*, 25, 2015–2028. <https://doi.org/10.1111/mec.13596>
- UniProt Consortium (2015). UniProt: A hub for protein information. *Nucleic Acids Research*, 43, 204–212.
- Unmack, P. J., Hammer, M. P., Adams, M., & Dowling, T. E. (2011). A phylogenetic analysis of pygmy perches (Teleostei: Percichthyidae) with an assessment of the major historical influences on aquatic biogeography in Southern Australia. *Systematic Biology*, 60, 797–812. <https://doi.org/10.1093/sysbio/syr042>
- Vajda, A. M., Kumar, A., Woods, M., Williams, M., Doan, H., Tolsher, P., ... Barber, L. B. (2015). Integrated assessment of wastewater treatment plant effluent estrogenicity in the Upper Murray River, Australia, using the native Murray rainbowfish (*Melanotaenia fluviatilis*). *Environmental Toxicology and Chemistry*, 34, 1078–1087. <https://doi.org/10.1002/etc.2895>
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzon, M., ... Nicotra, A. B. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, 17, 1351–1364. <https://doi.org/10.1111/ele.12348>
- Wang, P. A., Stenvik, J., Larsen, R., Mæhre, H., & Olsen, R. L. (2007). Cathepsin D from Atlantic cod (*Gadus morhua* L.) liver. Isolation and comparative studies. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 147, 504–511. <https://doi.org/10.1016/j.cbpb.2007.03.004>
- Webster, M. S., Colton, M. A., Darling, E. S., Armstrong, J., Pinsky, M. L., Knowlton, N., & Schindler, D. E. (2017). Who should pick the winners of climate change? *Trends in Ecology & Evolution*, 32, 167–173.

- Whitehead, A. (2012). Comparative genomics in ecological physiology: Toward a more nuanced understanding of acclimation and adaptation. *Journal of Experimental Biology*, 215, 884–891. <https://doi.org/10.1242/jeb.058735>
- Whitehead, A., Galvez, F., Zhang, S., Williams, L. M., & Oleksiak, M. F. (2011). Functional genomics of physiological plasticity and local adaptation in killifish. *Journal of Heredity*, 102, 499–511. <https://doi.org/10.1093/jhered/esq077>
- Whitehead, A., Triant, D. A., Champlin, D., & Nacci, D. (2010). Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. *Molecular Ecology*, 19, 5186–5203. <https://doi.org/10.1111/j.1365-294X.2010.04829.x>
- Wood, J. L. A., & Fraser, D. J. (2015). Similar plastic responses to elevated temperature among different-sized brook trout populations. *Ecology*, 96, 1010–1019. <https://doi.org/10.1890/14-1378.1>
- Wood, J. L. A., Tezel, D., Joyal, D., & Fraser, D. J. (2015). Population size is weakly related to quantitative genetic variation and trait differentiation in a stream fish. *Evolution*, 69, 2303–2318. <https://doi.org/10.1111/evo.12733>
- Wood, J. L. A., Yates, M. C., & Fraser, D. J. (2016). Are heritability and selection related to population size in nature? Meta-analysis and conservation implications. *Evolutionary Applications*, 9, 640–657. <https://doi.org/10.1111/eva.12375>
- Yates, A., Akanni, W., Amode, M. R., Barrell, D., Billis, K., Carvalho-Silva, D., ... Girón, C. G. (2016). Ensembl 2016. *Nucleic Acids Research*, 44, D710–D716. <https://doi.org/10.1093/nar/gkv1157>
- Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biology*, 11, 1.

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