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Physiological status of silver carp (*Hypophthalmichthys molitrix*) in the Illinois River: An assessment of fish at the leading edge of the invasion front

Jennifer D. Jeffrey[a,*], Ken M. Jeffries[b], Cory D. Suski[a]

[a] Department of Natural Resources and Environmental Science, University of Illinois at Urbana–Champaign, Urbana, IL, USA
[b] Department of Biology, University of Manitoba, Winnipeg, Manitoba, Canada

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ABSTRACT

Silver carp (*Hypophthalmichthys molitrix*) are invasive to North America, and their range has expanded within the Mississippi River Basin, seemingly unchecked, since their introduction in the late 1970s, with the exception of the upper reaches of the Illinois River. With the imminent threat of their movement into the Great Lakes, the goal of the present study was to assess whether differences in the physiological status between silver carp at the leading edge of their invasion front and core population sites could explain their lack of expansion upstream toward Lake Michigan over the past decade. A transcriptomic approach using RNA sequencing and analysis of plasma variables were used to quantify differences among fish at the leading edge and two downstream core population sites. Leading-edge fish exhibited upregulation of genes associated with xenobiotic defense (e.g., *ATP-binding cassette C1* [*abcc1*], *abcc2*, *abcc6*), decreased cell integrity (i.e., macroautophagy and apoptosis; *autophagy-related protein 9A* [*atg9a*], *caspase 3b* [*casp3b*]), and cholesterol metabolism (e.g., *abca1*, *apolipoprotein A1* [*apoa1*], *sterol O-acyltransferase* [*soat1*]) and downregulation of genes associated with DNA repair (e.g., *tumor suppressor p53-binding protein 1* [*tp53bp1*]) compared to core population sites. Transcriptomic profiles of leading-edge fish were consistent with fish inhabiting a polluted environment and suggest that poorer water quality conditions upstream of the leading edge may represent a non-permanent barrier to silver carp range expansion. The present study provides potential molecular targets for monitoring the physiological status of silver carp over time and in response to future improvements in water quality upstream of their leading edge.

1. Introduction

The range that a species inhabits is driven by a complex interaction of abiotic and biotic conditions as well as the biological capacity of that organism, such that each species has a unique suite of environmental parameters within which it needs to remain for survival and reproduction (i.e., its niche) (Brown et al., 1996; Sexton et al., 2009). At broad spatial scales, the limits of a species range can be set by abiotic factors such as temperature and an individual's thermal tolerance, but, at smaller scales, limits can be heavily influenced by factors such as predation and interspecific competition (Brown et al., 1996). For many species, the range that they inhabit is dynamic and can shift across time, thereby allowing organisms to spread and expand their current range. In particular, owing to a reduced population density and improved access to resources, there can be advantages to the expansion of a species' range. Individuals with improved dispersal ability are often located at the advancing front of a population, thereby generating positive selection pressures that accelerate the spread of a species (Phillips et al., 2010). In essence, the 'leading edge' of a population can experience environmental and evolutionary benefits that accelerate selection on dispersal capabilities, facilitating continued range expansion. Thus, defining both the characteristics of the environment, along with

Abbreviations: ALP, alkaline phosphatase; ANOVA, analysis of variance; *apoa1*, *apolipoprotein A1*; *apoa4*, *apolipoprotein A4*; *abca1*, *ATP-binding cassette A1*; *abcc1*, *ATP-binding cassette C1*; *abcc2*, *ATP-binding cassette C2*; *abcc6*, *ATP-binding cassette C6*; *atg9a*, *autophagy-related protein 9A*; *casp3b*, *caspase 3b*; *topbp1*, *DNA topoisomerase 2 binding protein 1*; FDR, false discovery rate; *gabarapl1*, *gamma-aminobutyric acid receptor-associated protein-like 1*; GO, gene ontology; *g0s2*, *G0/G1 switch protein 2*; *pla2g3*, *group 3 secretory phospholipase A2*; HDL, high-density lipoproteins; *hint2*, *histidine triad nucleotide-binding protein 2*; MDA, malondialdehyde; *acadm*, *medium chain specific acyl-CoA dehydrogenase*; *map1lc3a/b*, *microtubule-associated proteins light chain 3A and B*; *rpa1*, *replication protein A 70 KDa DNA-binding subunit*; *nupr1*, *nuclear transcription factor 1*; RNA-seq, RNA sequencing; *pds5b*, *sister chromatid cohesion protein pds5 homolog B*; SD, standard deviation; *cyp27a1*, *sterol 26-hydroxylase*; *soat1*, *sterol O-acyltransferase*; TAC, total antioxidant capacity; TNF, tumor necrosis factor; *traf1*, *TNF receptor associated factor*; *tp53bp1*, *tumor suppressor p53-binding protein 1*; *ube2e2*, *ubiquitin-conjugating enzyme E2E2*; *wipi1*, *WD repeat domain, phosphoinositide-interacting 1*

* Corresponding author at: Department of Biology, University of Manitoba, Winnipeg, Manitoba, Canada.
E-mail address: jenjeffrey@gmail.com (J.D. Jeffrey).

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how environmental factors interact with an individual's genetics, can be useful in defining factors responsible for range expansion, as well as forces that can prevent further spread of an organism.

Understanding what allows and limits range expansion is particularly important for the management of invasive species. Phenotypic plasticity is thought to play a role in invasive species establishment as well as post-establishment range expansion of successful invaders (Lande, 2015; Wellband and Heath, 2017). Gene expression variation likely facilitates phenotypic responses to environmental change (Aubin-Horth and Renn, 2009) and is the mechanistic basis for different phenotype expression (Wray, 2003). As the initial step in gene expression, gene transcription may evolve through changes in constitutive expression (Whitehead and Crawford, 2006), or be altered in response to environmental cues (Aykanat et al., 2011). As a key regulator of physiological status, gene transcription may provide insights not only into the role of phenotypic plasticity in relation to invasive species range expansion, but also the responses to environmental factors that may limit range expansion. High-throughput RNA sequencing (RNA-seq) approaches have provided a means for assessing broad transcriptional patterns in wild, non-model species. Transcriptomic approaches have been increasingly used in the framework of conservation biology (reviewed by Connon et al., 2018; Oomen and Hutchings, 2017). For example, Miller et al. (2011) used a transcriptomic approach to determine a molecular signature in sockeye salmon (*Oncorhynchus nerka*) with a failed upriver spawning migration that reflected a response to pathogen infection and premature mortality. A transcriptomic approach was also used by Jeffries et al. (2016) to assess how environmentally relevant increases in temperature differentially affect two fishes of conservation concern, the longfin smelt (*Spirinchus thaleichthys*) and the delta smelt (*Hypomesus transpacificus*). Similar transcriptomic approaches could also be used for the purposes of understanding invasion biology, and Wellband and Heath (2017) recently compared transcriptomic plasticity with thermal tolerance and invasion success in two non-native goby species (round goby, *Neogobius melanostomus*; tubenose goby, *Proterorhinus semilunaris*) to the Great Lakes of North America.

Silver carp (*Hypophthalmichthys molitrix*) is an invasive species that has spread widely throughout the central United States of America since their introduction in the early 1970s (Kolar et al., 2007). Silver carp were first introduced to aquaculture ponds to take advantage of their feeding behaviour that allows them to clear the water column of small particles such as algae, which is also what makes them effective at competing for food resources in systems (Kolar et al., 2007). Now widely spread through the Mississippi River Basin, USA, silver carp are an invasion threat to the Great Lakes of North America through systems such as the Illinois River, which gives them access through the Chicago Area Waterway to Lake Michigan (Rasmussen et al., 2011). A number of navigational dams along the Illinois River have not been sufficient to block the upstream movement of silver carp (Lubejko et al., 2017). At present, an electric barrier system in the Chicago Sanitary and Shipping Canal serves to prevent the upstream migration of silver carp; however, there are some concerns over the effectiveness of the barrier due to disruptions such as power outages and the movement of metal-hull barges through the barrier (Parker et al., 2015, 2016). Additional deterrents, such as elevated carbon dioxide levels and sound, are being explored as redundant barrier mechanisms (Vetter et al., 2015; Donaldson et al., 2016). Should silver carp pass through the Chicago Area Waterway and invade the Great Lakes, large regions of eastern North America would become accessible. While the potential impact of a silver carp invasion to the Great Lakes is difficult to predict, negative impacts to specific species may occur, such as a possible collapse of alewife (*Alosa pseudoharengus*) due to competition over food resources, with moderate to large consequences for resident populations of salmonids (Currie et al., 2012; Wittmann et al., 2015; Lauber et al., 2016; Zhang et al., 2016).

Currently, the leading edge of the invasion front is estimated to be approximately 65 km downstream of Lake Michigan (Fig. 1), and, for reasons that remain unclear, the range of silver carp has not substantially advanced toward Lake Michigan in the last decade. The lack of upstream movement by silver carp could be the result of a number of potential factors that include a lack of food resources (i.e., competition) or exposure to sub-optimal water quality (i.e., contaminants or other noxious substances that cause a physiological response) (Brown et al., 1996; Sexton et al., 2009; Duncker et al., 2017). Thus, the aim of the present study was to quantify the physiological status of silver carp populations at the leading edge of their range relative to downstream ('core') populations, in an effort to identify potential factors (e.g., food limitation or increased contaminant exposure) that might be limiting their range. To accomplish this goal, we used an integrative approach that combined the quantification of plasma variables with characterization of the liver transcriptome using RNA-seq. Plasma variables included measures of stress and nutritional status, and the liver was targeted as it is not only a key regulator of a fish's metabolic processes, but also a key target for detoxification of contaminants. Silver carp were sampled at the leading edge of their invasion front and two downstream core populations across two seasons – late summer (September 1–9) and fall (November 7–December 3). Together, this integration of transcriptomic data with plasma variables will allow the quantification of physiological status in silver carp at the leading edge, provide insights into the role that food limitation or environmental contaminants could be playing in preventing upstream movements of carp, and aid in the fight to prevent invasive silver carp from entering the Great Lakes.

2. Materials and methods

2.1. Fish sampling

Male silver carp were collected from three locations along the Illinois River during the late summer (September 1–9) and fall (November 7–December 3) of 2016 (Fig. 1). Fish were collected from the leading edge of the invasion front (Rock Run Rookery, IL, USA) (summer, $n = 2$; fall, $n = 4$) by the Illinois Department of Natural Resources-contracted harvesters using gill and trammel nets of various sizes and depths. Fish were also collected near Morris, IL (Morris East Pit) (summer and fall, $n = 8$) using gill and trammel nets, and near Havana, IL (summer, $n = 6$; fall, $n = 11$) using pulsed direct current electrofishing by the Illinois Natural History Survey. Different methods of sampling were necessary for the collection of fish due to site-specific differences in fish densities. Fish densities are highest in Havana backwaters where electrofishing is most effective for collection. In the upper reaches of the Illinois River at the leading edge of the invading front, fish densities are low, and collection of fish by blocking off areas and pushing fish into gill or trammel nets is more effective. Fish were also collected from additional sites including Dresden Pool, IL (fall, $n = 2$) and Starved Rock, IL (summer and fall, $n = 8$); however, due to low sample size (no fish caught in the summer at Dresden Pool) or extended time held in gill nets (Starved Rock), these fish were included in the reference transcriptome assembly, but not the differential analysis (see Differential analysis of mRNA transcript abundance). As a consequence of sampling fish at the most upstream edge of the population, fish capture was limited at these locations, contributing to the overall small sample size for leading edge fish (i.e., $n = 2$ in the summer), even with similar fishing effort at each site. The capture of females at the leading edge was also limiting (e.g., only a single female silver carp was captured at the leading edge), with the result that only males were included in the analysis.

Following capture, blood samples were taken from silver carp using a heparinized (Sigma-Aldrich, St. Louis, MO, USA) 1 ml syringe with a 25 or 38 mm, 22 G needle (BD, Fisher Scientific, Hanover Park, IL, USA) by caudal puncture. Blood samples were kept in an ice bath prior to centrifugation at 2000 $\times g$ for 3 min. Plasma samples were flash frozen in liquid nitrogen and subsequently stored at −80 °C. Following blood

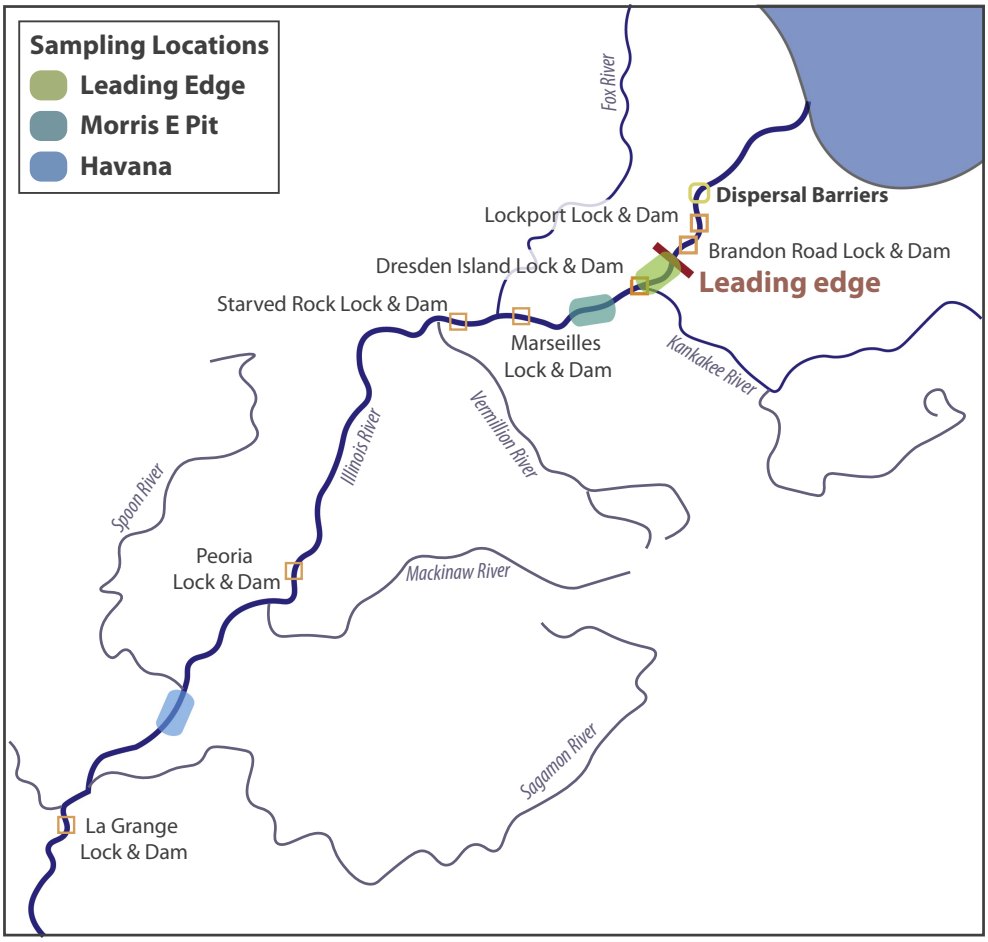

Fig. 1. Silver carp (*Hypophthalmichthys molitrix*) sampling locations along the Illinois River. Male silver carp were captured at the leading edge of the invasion front and at two locations in the core population, Morris and Havana.

sampling, fish were euthanized by severing the spinal cord. Fish were also sampled for liver, which was placed in RNA*later* Stabilization Solution (Fisher Scientific), and stored overnight at 4 °C prior to storage at −20 °C. This study was conducted in accordance with the University of Illinois Institutional Animal Care and Use Committee (protocol #16095).

2.2. Plasma analysis

Plasma variables were measured to quantify nutritional status, oxidative stress, and general stress. Nutritional status was assessed by measuring plasma alkaline phosphatase (ALP) activity, an enzyme involved in processing energy substrates in the liver (Congleton and Wagner, 2006), and cholesterol, using commercially available kits (QuantiChrom™ Alkaline Phosphatase Assay Kit, DALP-250; EnzyChrom™ Cholesterol Assay Kit, ECCH-100; BioAssay Systems, Haywood, CA, USA). Oxidative stress was measured by assessing aspects of oxidative damage (e.g., malondialdehyde; MDA) and total antioxidant capacity (TAC), using commercially available kits (Antioxidant Assay Kit, 709001; TBARS, TCA Method, Assay Kit, 700870; Cayman Chemicals, Ann Arbor, MI, USA). Plasma cortisol levels were measured to assess general stress using an enzyme-linked immunosorbent assay (402710; Neogen, Lexington, KY, USA).

Plasma variables were analyzed using a two-way analysis of variance (ANOVA) with location, season, and the interaction of location and season as the fixed factors. If either the interaction, or individual main effects were significant, Tukey-Kramer honestly significant difference post hoc test was used, using the "multcomp" package (Hothorn et al., 2008) in R (R Core Team, 2017). Assumptions of normality were assessed with a Shapiro-Wilk normality test, homogeneity of variances were assessed using a Levene's test, and model outputs were evaluated with visual analysis of fitted residuals using a normal probability plot (Zuur et al., 2010). If either the assumption of normality or the homogeneity of variance was violated, data were transformed with a rank or square root transformation (Conover and Iman, 1981), and run with the same parametric model provided that the assumptions were met after transformation. Statistical analyses were performed using R v3.4.3 and the level of significance (α) was 0.05. Data are presented as means ± standard deviation (SD).

2.3. RNA extraction and cDNA library sequencing

Total RNA was extracted from 30 to 40 mg of liver (n = 32 total; n = 4 for all sites and seasons with the exception of the leading edge in the summer and Dresden in the fall where n = 2; Table S1) using TRIzol Reagent (Fisher Scientific) according to the manufacturer's protocol. Tissues were homogenized using a BeadBug Microtube homogenizer (Denville Scientific, Holliston, MA, USA). Total RNA was purified using a phenol-chloroform extraction followed by a precipitation with ethanol and sodium acetate. Extracted RNA was quantified using a Qubit 3.0 Fluorometer (Fisher Scientific), purity was assessed using a NanoDrop One spectrophotometer (Fisher Scientific), and integrity was checked by visualizing the RNA on a 1% agarose gel. cDNA library construction and sequencing were performed by the DNA Services Lab at the Roy J. Carver Biotechnology Center at the University of Illinois Urbana-Champaign (https://biotech.illinois.edu/htdna). Quality of

RNA was assessed on a bioanalyzer prior to cDNA library construction using Illumina's TruSeq Stranded mRNAseq Sample Prep Kit (Illumina, San Diego, CA, USA). All 32 cDNA libraries were individually barcoded and then pooled together for sequencing (paired-end) across four lanes on a HiSeq 4000 (Illumina). Fastq files were generated and demultiplexed with the bcl2fastq v2.17.1.14 Conversion Software (Illumina) resulting in 3.04 billion total reads and 47.6 ± 6.1 (mean ± SD) million paired-end reads per sample (Table S1). Raw reads were submitted to the NCBI SRA database (accession **PRJNA510853**).

2.4. Transcriptome analysis and annotation

Raw reads were trimmed and filtered for adapters and low quality sequences using Trimmomatic v0.33 with the parameters: ILLUMINACLIP: trimmomatic-0.33/adapters/TruSeq3-PE-2.fa:2:30:10 LEADING:28 TRAILING:28 MINLEN:30 (Zuur et al., 2010), resulting in the removal of < 0.04% of total reads. Trinity v2.4.0 (Trinity.pl) was used to generate a *de novo* transcriptome using quality-trimmed reads from the 32 individuals using the default *in silico* read normalization and paired-end settings (Grabherr et al., 2011; Haas et al., 2013). Basic assembly statistics were performed within Trinity using TrinityStats.pl. Following transcript abundance estimation using Salmon (Patro et al., 2017; see below), transcript contig E-statistics were performed using contig_ExN50_statistic.pl within Trinity. To assess transcriptome completeness, the transcriptome was subjected to BUSCO analysis (Simao et al., 2015; Waterhouse et al., 2018) using the Actinopterygii and vertebrata databases.

Transcriptome annotation was performed by HPC-Bio at the University of Illinois Urbana-Champaign (https://hpcbio.illinois.edu/) using Trinotate (Bryant et al., 2017). Additional annotation using BLASTX and BLASTP were performed against the *Danio rerio* database. In total, 265,589 transcripts and 55,399 genes were annotated.

2.5. Transcript abundance estimation

Salmon v0.7.2 software (Patro et al., 2017) was used to estimate mRNA transcript abundance values for transcripts within Trinity using the align_and_estimate_abundance.pl script and the default quasi-mapping-based mode. The R/Bioconductor package "tximport" (Soneson et al., 2016) was used to generate an estimated counts matrix using gene-level summarization and counts from abundances that were scaled using the average transcript length, averaged over samples, and library size (i.e., argument countsFromAbundance = lengthScaledTPM).

2.6. Differential analysis of mRNA transcript abundance

Differential mRNA transcript abundance was assessed for a subset of the silver carp included in the reference transcriptome assembly. As no fish from Dresden pool were collected in the summer, this sampling location was excluded from the differential analysis. In addition, fish sampled at Starved Rock in the summer remained in nets for an extended period of time relative to other sampling seasons and locations, resulting in a large number of genes with differential mRNA transcript levels being detected in these fish (evident in a preliminary analysis). As additional sampling/handling stress may have contributed to the transcriptomic patterns observed in fish from Starved Rock, silver carp from this location were excluded from further analysis. Therefore, differential analysis of mRNA transcript abundance was assessed for fish from the leading edge of the invasion front, compared to Morris and Havana, representing two locations in the downstream core population of the silver carp.

Differential mRNA transcript abundance of genes was assessed using "edgeR" (Robinson et al., 2010). Only genes with at least one count per million reads across four samples were retained for further analysis (32,517 genes; ~12.5% of genes and > 98% of reads per sample). To normalize across samples, an effective library size was calculated using "calcNormFactors" within edgeR that computes scale factors using a trimmed mean of M-values between each pair of samples (Robinson and Oshlack, 2010). A general linear model was run in edgeR with sampling site and season as factors. Quasi-likelihood *F*-tests were used to conduct hypothesis tests as they provide a more robust and reliable error rate control when the number of replicates is small (Lun et al., 2016). *A priori* contrasts were designed to compare sampling sites within a season. Genes were considered to have differential mRNA transcript abundance at a Benjamini-Hochberg corrected False Discovery Rate (FDR) < 0.05. Only those genes with a differential mRNA transcript abundance between the leading edge and the two core population sites (Morris and Havana), within each season, were considered for further analysis and interpretation. In this way, any gene responses associated with the differences in sampling technique (i.e., gill netting at the leading edge and Morris vs. electrofishing at Havana) should have been excluded, and the focus was instead put on differences between fish at the leading edge compared to the core population sites. A complete list of genes with differential mRNA transcript abundance at FDR < 0.05 are provided in the supplementary material (Table S2).

Gene set enrichment analysis was performed using EnrichR (Chen et al., 2013) for genes with mRNA levels that were up or downregulated in either the summer or fall at the leading edge compared to the core population. Only gene ontology (GO) terms that had a minimum of four genes were considered significantly enriched in the gene list at an adjusted p-value < 0.05. EnrichR only considers one copy of a gene for the analysis, thus multiple copies of a gene represented by more than one transcript would have been disregarded from the analysis. Results of the gene set enrichment analysis were visualized with REVIGO (Supek et al., 2011).

3. Results

3.1. Plasma variables

Across both seasons, plasma ALP activity levels were significantly elevated in silver carp sampled from the leading edge compared to fish collected downstream in Havana, and also were higher for silver carp sampled in summer compared to fall (Tables 1 and 2). Plasma cholesterol concentrations did not vary across sample location, but there was a significant seasonal effect with cholesterol levels being significantly higher in fall relative to summer (Tables 1 and 2).

For both MDA and TAC, there was a significant effect of location, with oxidative damage (MDA) being highest in silver carp sampled at the two upstream locations relative to the downstream Havana location, and antioxidant capacity (TAC) being elevated in fish at the leading edge relative to fish sampled from Havana (Tables 1 and 2). Silver carp sampled from Morris showed TAC levels intermediate to the upstream and downstream locations (Tables 1 and 2). In addition, there was a significant main effect of season, with plasma levels of MDA and TAC being elevated in fall compared to summer (Tables 1 and 2).

Plasma cortisol levels appeared to be related to the method of sampling in the summer but not in fall, and a significant interaction between location and season was detected (Tables 1 and 2). In the summer, silver carp captured using gill netting at the leading edge and Morris had cortisol levels more than three-times higher than those of fish captured using electrofishing (Havana). In the fall, cortisol levels were not different between fish captured using different sampling techniques, but cortisol levels were lower in fish from Morris in the fall compared to the summer.

3.2. De novo assembled silver carp transcriptome

Over 3 billion 100-base paired-end reads from 32 RNA-seq samples were assembled using Trinity. The assembly consisted of 548,389 transcript contigs clustered into 259,827 "gene" groupings with a median transcript length of 421 bases. Most of the transcriptome

Table 1
Plasma variables for silver carp (*Hypophthalmichthys molitrix*) at the leading edge of the invasion front and at two locations in the core of the population (Morris and Havana) across two seasons.

	Summer			Fall		
	Leading edge	Morris	Havana	Leading edge	Morris	Havana
ALP (U/l)*	30.5 ± 15.8^{A}	15.5 ± 4.7^{AB}	9.8 ± 2.3^{B}	7.5 ± 1.3^{A}	6.1 ± 3.4^{AB}	5.3 ± 1.3^{B}
Cholesterol (mg/dl)*	199.7 ± 1.0	230.9 ± 75.8	152.7 ± 52.7	195.6 ± 19.7	263.0 ± 100.8	240.5 ± 92.6
TAC (mM Trolox)*	0.42 ± 0.08^{A}	0.35 ± 0.07^{AB}	0.35 ± 0.08^{B}	0.58 ± 0.03^{A}	0.47 ± 0.07^{AB}	0.46 ± 0.10^{B}
MDA (μM)*	11.5 ± 1.1^{A}	11.8 ± 2.2^{A}	4.3 ± 2.0^{B}	11.3 ± 0.7^{A}	14.3 ± 1.0^{A}	8.9 ± 3.3^{B}
Cortisol (ng/ml)	127.9 ± 49.4^{a}	157.5 ± 20.6^{a}	20.4 ± 24.8^{b}	101.3 ± 41.0	91.7 ± 38.5**	67.9 ± 64.9

ALP, alkaline phosphatase; TAC, total antioxidant capacity; MDA, Malondialdehyde.
Values are represented as means ± SD; N = 2–3 for leading edge, N = 7–8 for Morris, N = 6–11 for Havana.
Locations that do not share a letter are significantly different from one another; uppercase letters represent a significant main effect of location and lowercase letters represent a significant effect of location within a season (i.e., significant interaction); two-way ANOVA, see Table 2.
* Represents a significant main effect of season for an indicated variable.
** Represents a significant effect of season within a location.

Table 2
Two-way analysis of variance for plasma variables in silver carp (*Hypophthalmichthys molitrix*) sampled along the Illinois River.

Variable	Main effects	Degrees of freedom	Sum of squares	*F* value	*p*
ALP	Location	2	568.5	7.167	**0.003**
	Season	1	2392.2	60.312	< **0.001**
	Location × Season	2	27.7	0.35	0.708
Cholesterol	Location	2	0.342	1.377	0.267
	Season	1	0.519	4.18	**0.049**
	Location × Season	2	0.332	1.334	0.278
TAC	Location	2	0.04639	3.652	**0.037**
	Season	1	0.12915	20.334	< **0.001**
	Location × Season	2	0.00329	0.259	0.773
MDA	Location	2	265.87	23.701	< **0.001**
	Season	1	81.75	14.575	< **0.001**
	Location × Season	2	22.53	2.008	0.151
Cortisol	Location	2	1380.6	10.217	< **0.001**
	Season	1	134.9	1.997	0.167
	Location × Season	2	892	6.602	**0.004**

Significant *p* values are represented as bolded text.
ALP, alkaline phosphatase; TAC, total antioxidant capacity; MDA, Malondialdehyde.

corresponded to lowly expressed transcript contigs, as 90% of the total transcriptome was represented by 6348 transcripts with an E90N50 of 1566 bases. Using BUSCO analysis to assess transcriptome completeness, the transcriptome contained near-complete gene sequence information for > 80% of genes (Actinopterygii, C:81.2% [S:25.2%, D:56.0%], F:7.9%, M:10.9%, n:4584; vertebrata, C:87.2% [S:24.1%, D:63.1%], F:8.9%, M:3.9%, n:2586; where C, S, D, F, and M represent complete, complete single-copy, complete duplicated, fragmented, and missing proportions, respectively, and n is the number of genes).

3.3. Differential analysis of mRNA transcript abundance

In the summer, there were 156 genes with differential mRNA transcript abundance between the leading edge and the core sites of the silver carp population in the Illinois River (Morris and Havana) at an FDR < 0.05 (Tables 3 and S2). Of these genes, 85 were upregulated and 71 were downregulated in fish at the leading edge compared with the two core population sites (Fig. 2A). In contrast, there were only 19 genes with differential mRNA transcript abundance between the leading edge and the core sites of the silver carp population at an FDR < 0.05 in the fall (Tables 3 and S2). Two of these genes in the fall were upregulated and 17 genes were downregulated in fish at the leading edge compared with the core population (Fig. 2B). There were no genes with differential mRNA transcript abundance in common between the summer and fall at an FDR < 0.05 for the leading edge fish compared with the core population sites.

Enrichment analysis revealed GO terms that were significantly enriched for gene lists for the summer, but not the fall. For genes with mRNA levels that were upregulated in fish at the leading edge compared to the core population in the summer, a total of 101 GO terms were significantly enriched, including 59 biological processes, 29 molecular functions, and 13 cellular components. Using Revigo to visualize the significantly enriched GO terms, biological processes were grouped into four categories, cholesterol efflux which included several transmembrane transport processes, cholesterol metabolism, cholesterol homeostasis, and macroautophagy (Fig. 3A). Upregulated molecular functions in the summer consisted mainly of GO terms involved in ATPase activity coupled to transmembrane movement of substances, and cholesterol binding (Fig. 3B). Significantly enriched cellular components that were upregulated in the summer included GO terms pertaining to autophagosomes (Fig. 3C). In total, 21 genes were associated with the enriched GO terms for upregulated processes in the summer (Fig. 4).

For genes with mRNA levels that were downregulated in the summer in fish at the leading edge compared to the core population, a total of 46 GO terms were significantly enriched. The 19 significantly enriched biological processes included processes related to DNA repair (Fig. 5A). The 27 downregulated molecular functions consisted mainly of GO terms involved in RNA polymerase II transcription coactivator activity, and triplex DNA binding (Fig. 5B). In total, 16 genes were associated with enriched GO terms for downregulated processes in the summer (Fig. 6).

4. Discussion

Invasive silver carp in the Illinois River constitute a major threat to both the ecosystems they currently inhabit, as well as to those that they may invade, such as the Great Lakes of North America. Interestingly, the leading edge of the silver carp invasion front has not significantly advanced closer to Chicago in over a decade (ACRCC, 2017). In the present study, we used an integrative approach to test the hypotheses that food limitation or increased contaminant exposure might contribute to the lack of upstream migration of silver carp, by examining their physiological status. Overall, we found little evidence to suggest that the nutritional status of silver carp was negatively impacted at upstream compared to downstream locations (e.g., plasma ALP and cholesterol levels were not reduced in fish at the leading edge). In contrast, liver transcriptomic patterns indicated that silver carp at the leading edge exhibited increased mRNA transcript levels of genes

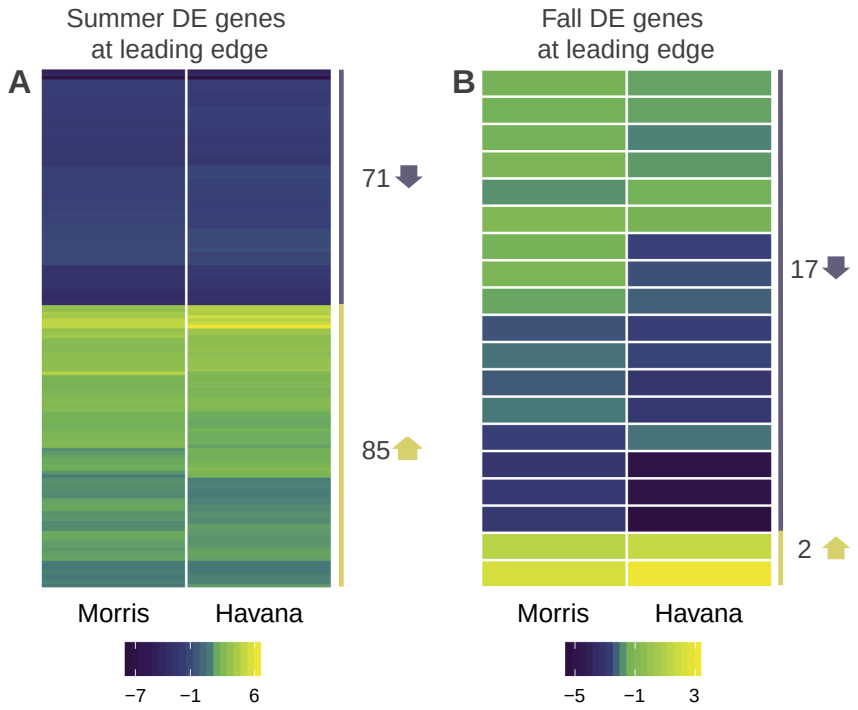

Fig. 2. Heatmap of genes with differential mRNA transcript levels in silver carp (*Hypophthalmichthys molitrix*) at the leading edge compared to the core population sites (Morris and Havana) in the summer (A) and fall (B). Genes were considered DE at an FDR < 0.05.

associated with xenobiotic defense and decreased cell integrity (i.e., macroautophagy and apoptosis), a genotoxic response (i.e., decrease in mRNA transcript levels of genes associated with DNA repair), as well as alterations in cholesterol metabolism, all of which are consistent with fish inhabiting a polluted environment. Similarly, responses in the mRNA transcript levels of genes associated with DNA repair mechanisms, macroautophagy, and apoptosis are consistent with whole-transcriptome responses to pyrethroid pesticides in delta smelt (Jeffries et al., 2015). Indeed, contaminant sampling of 639 constituents within the Illinois River across three location above and four locations below the leading edge of the invasion front (near river mile 278) by the United States Geological Survey in 2015, demonstrated that the number and concentration of several contaminants were greater upstream of the invading front relative to downstream (Duncker et al., 2017). For example, Duncker et al. (2017) found that the total concentration of pharmaceuticals, other wastewater indicators, and volatile organic compounds decreased from 9251to 417 ng/L, 4092 to 309 ng/L, and 9626 to 340 ng/L, respectively, in June of 2015 from a site just above

Table 3.
Top genes with differential mRNA transcript abundance for silver carp (*Hypophthalmichthys molitrix*) at the leading edge of their invasion front relative to the core population sites in the Illinois River.

Season	Up or down	Gene name	Protein name	Function	LE–M FC	LE–H FC
Summer	Up	*irx3a*	Iroquois 3 homeobox protein	Transcription factor	25.71	33.35
		g0s2	G0/G1 switch 2	Lipid metabolism; Apoptosis and inflammation	25.52	22.26
		pla2g3	Group 3 secretory phospholipase A2	Lipid metabolism; inflammatory response; oxidative stress	25.42	79.56
		apoa4b.2	Apolipoprotein A-IV b	Lipid transport	24.05	5.69
		apoa4b.1	Apolipoprotein A-IV b	Lipid transport	13.76	8.37
	Down	*chrm3a*	Muscarinic acetylcholine receptor	G protein-coupled receptor that mediates various cellular responses	-32.55	-24.21
		nfil3-5	Nuclear factor, interleukin 3-regulated, member 5	Transcriptional repressor; circadian regulation of gene expression	-12.22	-9.04
		glcea	Glucuronic acid epimerase a	Heparan sulfate/heparin biosynthesis	-9.11	-9.67
		jupb	Junction plakoglobin b	Cell adhesion	-7.74	-4.29
		bub1b	Mitotic checkpoint serine/ threonine-protein kinase BUB1 beta	Cell cycle; Apoptosis of polyploid cells	-6.62	-5.27
Fall	Up	*enpp5*	Ectonucleotide pyrophosphatase/ phosphodiesterase family member 5	Type-I transmembrane glycoprotein	4.52	9.02
		ranbp10	Ran-binding protein 10	Microtubule cytoskeleton organization	2.28	2.95
	Down	*ttr*	Transthyretin	Thyroid hormone transport	-7.58	-43.09
		rcn3	Reticulocalbin 3, EF-hand calcium-binding domain	Calcium-binding protein	-6.26	-4.19
		hpx	Hemopexin	Heme transport	-3.46	-2.74
		pemt	Phosphatidylethanolamine *N*-methyltransferase	Lipid metabolism	-3.03	-4.79
		pebp1	Phosphatidylethanolamine binding protein	Modulation of signaling pathways	-2.74	-3.12

False discovery rate < 0.05; see Table S2 for a complete list of genes with differential mRNA transcript levels
LE–M, leading edge vs. Morris; LE–H, leading edge vs. Havana; FC, fold-change

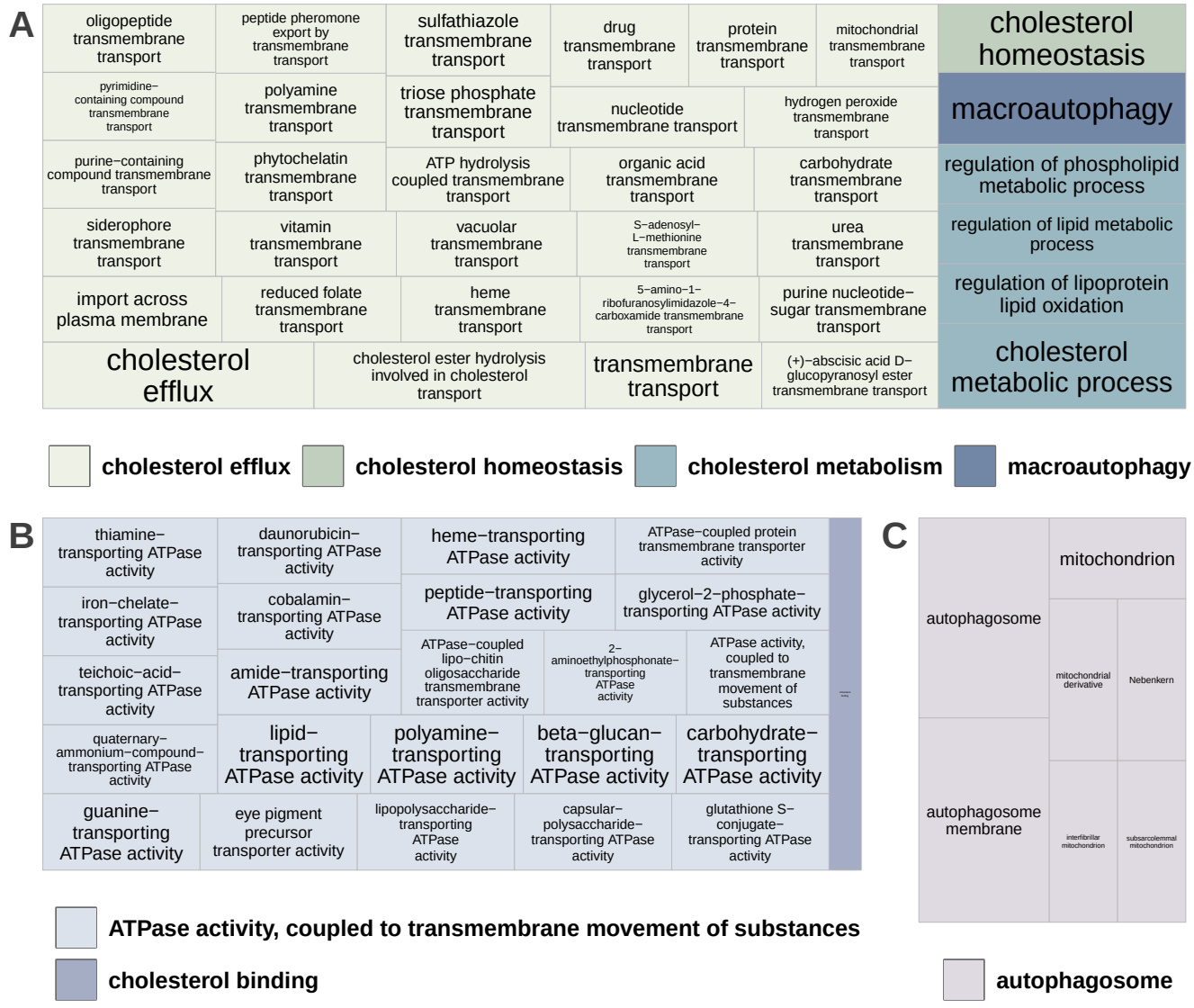

Fig. 3. Significantly enriched biological processes (A), molecular functions (B), and cellular components (C) for genes with mRNA transcript levels upregulated at the leading edge of the silver carp (*Hypophthalmichthys molitrix*) invasion front compared to the core population sites in the Illinois River in the summer. Genes with differential mRNA transcript levels at an FDR < 0.05 were included in the enrichment analysis and only gene ontology (GO) terms with a minimum of 4 genes and an adjusted p-value < 0.05 were considered significantly enriched. Enriched GO terms were visualized using Revigo.

the leading edge of the invasion front at river mile 286 (downstream of the Brandon Road Lock and Dam) compared to a site near Marseilles, IL (river mile 243). Notably, the effects observed in fish at the leading edge in the present study were more pronounced in the summer compared to the fall, suggesting a seasonal effect on responses of silver carp to environmental effects.

Silver carp at the leading edge of the invasion front showed enrichment of processes associated with increased active transport of substances in the liver relative to both sampling locations closer to the core of their distribution. Of the genes with upregulated mRNA transcript abundance in the liver of fish at the leading edge compared to the core population, were three members of the ATP-binding cassette proteins, *ATP-binding cassette C1* (*abcc1*), *abcc2*, and *abcc6*, commonly referred to as ABC transporters. ABC transporters are highly conserved transmembrane active transport proteins (Dean and Annilo, 2005), and some members of this superfamily confer multixenobiotic-resistance to aquatic organisms living in polluted environments (Kurelec, 1992; Epel, 1998). Enhanced expression of ABC transporters may allow organisms to survive in a habitat, despite high pollution levels. For instance, killifish found in tar ponds show coordinated cellular detoxification mechanisms that include some xenobiotic ABC transporters (e.g., *abcc2*) (Paetzold et al., 2009). Additionally, common carp (*Cyprinus carpio*) exposure to environmentally-relevant levels of clofibric acid, the active metabolite of the lipid lowering fibrate clofibrate, resulted in elevated levels of *abcc2* (Corcoran et al., 2015). The increased mRNA transcript abundance of xenobiotic defense genes in some species or populations may point to a selective advantage for occupying a more polluted environment (Paetzold et al., 2009).

The active transport action of ABC transporters in response to contaminant exposure results in an increased energy demand that is responsible, at least in part, for an increase in metabolic rate (Bains and Kennedy, 2005; Hildebrand et al., 2009). Consistent with an increased energy demand, upregulation of *medium chain specific acyl-CoA dehydrogenase* (*acadm*) mRNA transcript levels, an enzyme that catalyzes the first step in mitochondrial medium-chain fatty acid β-oxidation, was found in silver carp at the leading edge relative to both downstream locations. Additionally, leading-edge fish exhibited elevated plasma ALP levels compared to the Havana fish, an enzyme that is involved in

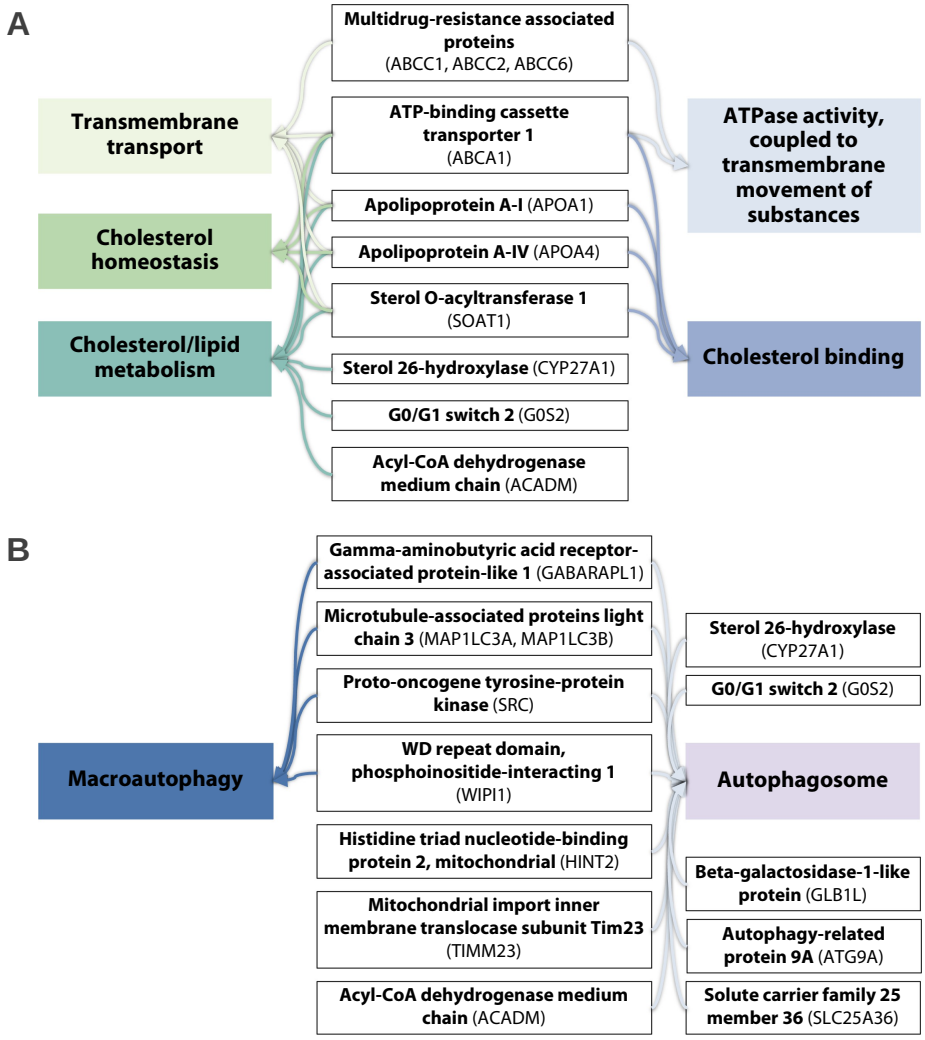

Fig. 4. Genes with upregulated mRNA transcript levels associated with significantly enriched gene ontology (GO) categories at the leading edge of the silver carp (*Hypophthalmichthys molitrix*) invasion front compared to the core population sites in the Illinois River in the summer. Enriched GO terms were summarized based on visualizations produced by Revigo (Fig. 3) and represent genes associated with active transmembrane transport of substances and cholesterol metabolism (A) as well as macroautophagy (B).

substrate processing in the liver (Congleton and Wagner, 2006). Elevated levels of *acadm* mRNA and plasma ALP are consistent with the increased energetic demand required to support active transmembrane processes in the liver. Together, increased mRNA transcript abundance of genes associated with the xenobiotic response, as well as processes to support these active transporters, suggests that silver carp at the leading edge of their invasion front may be eliciting mechanisms to cope with increased exposure to contaminants.

Biological processes and cellular components associated with macroautophagy and autophagosomes were enriched in the liver of silver carp at the leading edge of the invasion front. Macroautophagy is an autophagic process in which substrates such as damaged organelles, cytosolic proteins, and invasive microbes, are sequestered in cytosolic double-membrane vesicles called autophagosomes (Feng et al., 2014). Autophagosomes merge with lysosomes allowing for the degradation of these cellular components, which are then released back into the cytosol to recycle molecular constituents and generate energy to maintain cell viability under unfavorable conditions and to protect cells during various stressful conditions (Feng et al., 2014). The mRNA transcript levels of several genes associated with macroautophagy were upregulated in leading-edge silver carp including autophagy-related proteins (*autophagy-related protein 9A*, *atg9a*; *gamma-aminobutyric acid receptor-associated protein-like 1*, *gabarapl1*; *microtubule-associated proteins light chain 3A and B*, *map1lc3a/b*), as well as other autophagy-related genes (*WD repeat domain, phosphoinositide-interacting 1*, *wipi1*; for a full list see Fig. 4B). Upregulation of genes associated with macroautophagy suggests an increased need for energy resources and/or breakdown of damaged cellular components. For instance, macroautophagy was elevated in fish during periods of nutritional starvation as an adaptive mechanism to increase protein degradation and mobilize energy resources (Yabu et al., 2012). Exposure to polluted chemicals, such as metals and organic xenobiotics, induces cell injury and results in a pathological change that frequently involves autophagy and lysosomal degradation (reviewed by Moore et al., 2008). The results of the present study suggest that silver carp at the leading edge are potentially experiencing environmental stressors that result in cellular damage or increased energy demand, resulting in the elevated mRNA transcript abundance of genes associated with macroautophagy.

Consistent with the increase in macroautophagic processes, silver carp at their leading edge also demonstrated an increase in the mRNA transcript abundance of genes associated with apoptosis. The gene with the second most upregulated mRNA transcript level in silver carp at the leading edge was *G0/G1 switch protein 2* (*g0s2*) (Table 3), a gene that strongly promotes apoptosis by binding to bcl-2 and inhibiting its anti-apoptotic activity, through tumor necrosis factor (TNF) signaling and nuclear factor-κB activity (Welch et al., 2009). *G0/G1 switch protein 2*

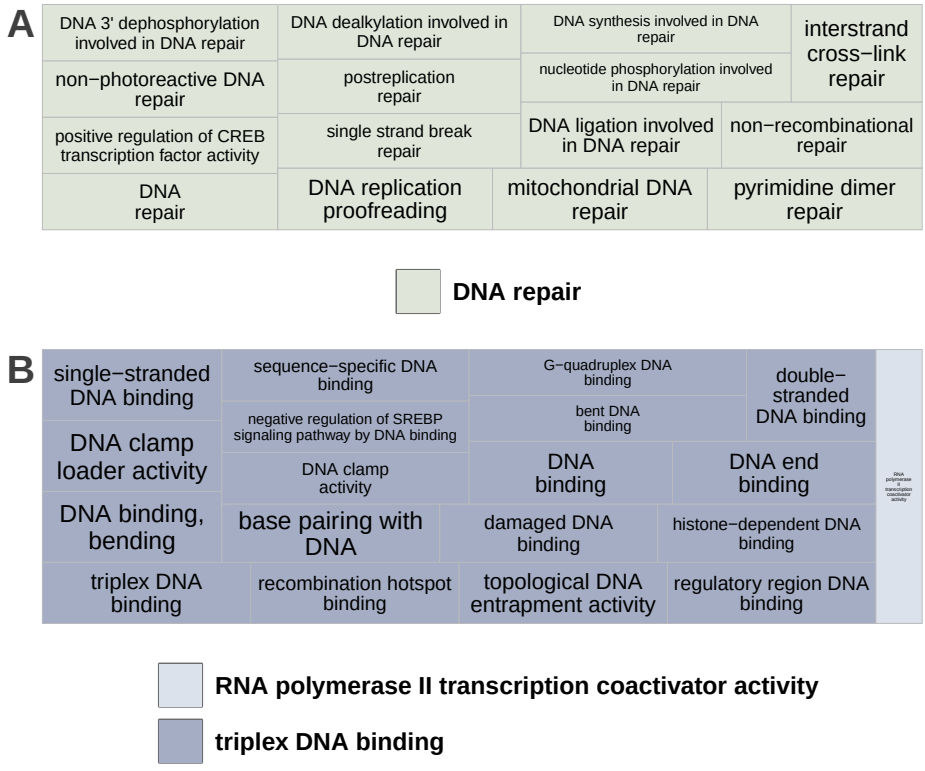

Fig. 5. Significantly enriched biological processes (A), and molecular functions (B) for genes with mRNA transcript levels downregulated at the leading edge of the silver carp (*Hypophthalmichthys molitrix*) invasion front compared to the core population sites in the Illinois River in the summer. Genes with differential mRNA transcript levels at an FDR < 0.05 were included in the enrichment analysis and only gene ontology (GO) terms with a minimum of 4 genes and an adjusted *p*-value < 0.05 were considered significantly enriched. Enriched GO terms were visualized using Revigo.

also acts as a negative regulator of adipose triglyceride lipase enzyme levels and its overexpression can result in accumulation of triglycerides in the liver (Heckmann et al., 2013). An upregulation in the mRNA transcript abundance of *caspase 3b* (*casp3b*) was also detected in leading edge fish, a gene that plays an important role in the intrinsic apoptosis pathway (McIlwain et al., 2013). *Histidine triad nucleotide-binding protein 2* (*hint2*) mRNA transcript abundance was upregulated in fish at the leading edge, and its overexpression has been linked to increased apoptosis in pancreatic cancer cells (Chen et al., 2017). Both *casp3* and *hint1*, which are also involved in regulating apoptosis, have been shown to respond to exposure to the pyrethroid pesticide permethrin (Jeffries et al., 2015). Interestingly, *TNF receptor associated factor* (*traf1*) mRNA transcript abundance was highest in fish at the leading edge, a gene that may play a negative role in TNF-signaling in teleosts, and thus an anti-apoptotic effect, although the main function of *traf1* in fish remains to be investigated (Zhu et al., 2013). Together, upregulation of the mRNA transcript abundance of factors associated with apoptosis, suggests increased exposure to environmental stressors of fish at the leading edge of their invasion front.

The abundance of mRNA transcripts associated with mechanisms of DNA repair were downregulated in silver carp at the leading edge of their invasion front compared to individuals sampled from both downstream locations. The mRNA transcript levels of several genes, including *tumor suppressor p53-binding protein 1* (*tp53bp1*), *sister chromatid cohesion protein pds5 homolog B* (*pds5b*), *replication protein A 70 KDa DNA-binding subunit* (*rpa1*), *DNA topoisomerase 2 binding protein 1* (*topbp1*), and *ubiquitin-conjugating enzyme E2E2* (*ube2e2*), were lower in leading-edge fish compared with the core populations. A decrease in mRNA transcript abundance of key genes associated with the DNA damage response could decrease DNA repair, leading to decreased DNA integrity and chromosomal abnormalities. For instance, *pds5b* is involved in the cohesion of chromosomes during cell division and

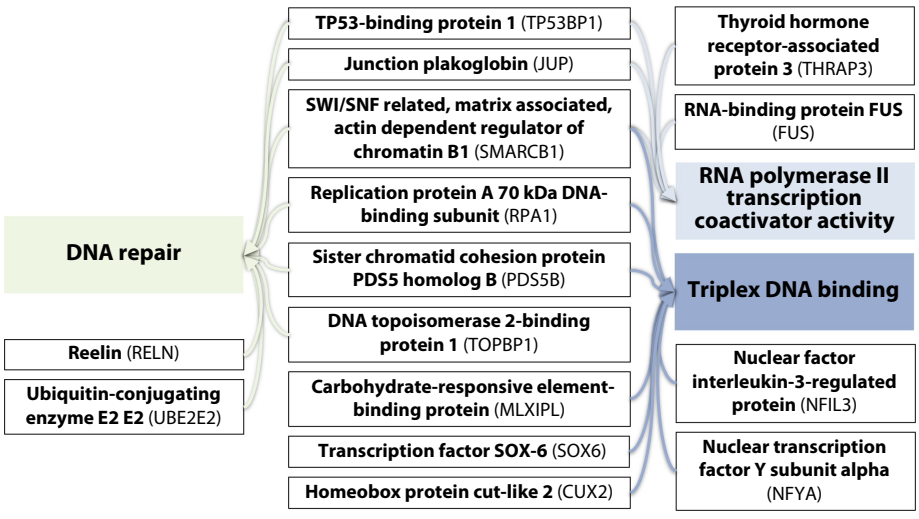

Fig. 6. Genes with downregulated mRNA transcript levels associated with significantly enriched gene ontology (GO) categories at the leading edge of the silver carp (*Hypophthalmichthys molitrix*) invasion front compared to the core population sites in the Illinois River in the summer. Enriched GO terms were summarized based on visualizations produced by Revigo (Fig. 5) and represent genes associated with DNA repair.

facilitates homologous recombination-mediated DNA repair (Carretero et al., 2013). As part of the heterotrimeric RPA complex, *rpa1* binds and stabilizes single-stranded DNA intermediates that form during DNA replication or DNA stress, and plays an essential role in several DNA repair pathways (Maréchal and Zou, 2015). The double-stranded break repair protein *tp53bp1* also plays an important role in various DNA repair pathways and a decrease in *tp53bp1* can result in translocation and other genome rearrangements that diminish cell viability and increase the chance of tumorigenic changes (reviewed by Zimmermann and de Lange, 2014). The effectiveness of DNA repair by *tp53bp1* is also affected by its association with *topbp1* (Sokka et al., 2010) and *ube2e2*, the latter contributes to ubiquitination and degradation of oncogenes (Mizukami et al., 2017). A downregulation of the mRNA transcript abundance of several key genes in the DNA damage response might suggest a genotoxic response to increased environmental contaminants at the leading edge. An increase in DNA damage could result in increased cell turnover and is consistent with the observed upregulation of genes associated macroautophagy and apoptosis.

Cholesterol transport and metabolism was also enriched in fish at the leading edge of their invasion front. High-density lipoproteins (HDL) play important roles in antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-senescence activities (Cho, 2009), as well as the reverse transport of free, unesterified cholesterol to the liver for catabolism and elimination (Phillips, 2014). *Apolipoproteins A-1* (*apoa1*) and *A-4* (*apoa4*), as well as *ATP-binding cassette A1* (*abca1*) are key components in the production of HDL (Yoo et al., 2015), and their mRNA transcript levels were upregulated in leading edge fish. Consistent with this response, *sterol O-acyltransferase* (*soat1*) mRNA transcript abundance, an enzyme that forms cholesterol esters from free cholesterol and is involved in lipoprotein formation (Suckling and Stange, 1985), was upregulated in silver carp at the leading edge. *Sterol 26-hydroxylase* (*cyp27a1*) mRNA transcript abundance, an enzyme involved in bile acid formation and a major route for cholesterol metabolism and elimination (Hagey et al., 2010), was also elevated in fish at the leading edge. Sensitivity of hepatic energy and cholesterol metabolism to aquatic contaminant exposure has been found in previous studies (e.g., Cocci et al., 2015; Corcoran et al., 2015; Teles et al., 2016). Although cholesterol metabolism processes appeared to be altered in the transcriptomes of leading edge fish, total plasma cholesterol was not different among sampling sites. Although plasma cholesterol levels were not significantly affected, our transcriptomic results suggest potential alterations in cholesterol and lipoprotein metabolism in silver carp at the leading edge of their invasion front, which may be a consequence of poorer water quality at this site.

Plasma TAC and MDA levels were elevated in sites closer to the leading edge compared to Havana (Table 1). Modest elevations in TAC were detected in leading edge fish across both seasons compared with fish from Havana, suggesting a greater capacity or need to respond to damaging free radicals. An indicator of oxidative damage, MDA, was also elevated in fish nearer to the leading edge compared with Havana, suggesting elevated oxidative damage in these groups. However, as fish from both the leading edge and Morris were captured differently than fish from Havana (i.e., gill netting vs. electrofishing, respectively), capture stress may have contributed to the observed differences in MDA levels. Additionally, plasma cortisol levels were consistently elevated in leading edge and Morris fish compared to Havana fish, indicating that capture stress was likely a factor. Although there was not a strong transcriptional response in the liver to indicate an oxidative or general stress response (i.e., enrichment of terms in the functional analysis), the mRNA transcript abundance of two stress-related genes was upregulated in fish at the leading edge, *group 3 secretory phospholipase A2* (*pla2g3*) and *nuclear transcription factor 1* (*nupr1*) (Table 3). With roles in antioxidant defense, *pla2g3* mRNA levels were also found to be elevated in the liver of polar cod (*Boreogadus saida*) exposed to benzo(a) pyrene (Song et al., 2019). The transcription factor *nupr1* regulates the expression of genes related to cell growth and apoptosis and is commonly activated during stress (Sopinka et al., 2016). Rainbow trout (*Oncorhynchus mykiss*) exposed to a 3 h, but not a 0.5 h, low-water stressor exhibited an upregulation of liver *nupr1* mRNA levels that was sustained 21 h post-stressor (Momoda et al., 2007). Further, sockeye salmon and pink salmon exposed to an acute chase and handling stressor showed no change in *nupr1* mRNA levels over a 24 h period (Donaldson et al., 2014). These studies suggest that *nupr1* may be less sensitive to short-term handling stressors and an appropriate longer-term indicator of stress. Overall, the results of the present study suggest that fish at the leading edge may be experiencing increased stress and oxidative damage and may upregulate their antioxidant capacity in response; however, handling and capture stress may have played a role in mediating some of these responses.

Transcriptional responses to environmental conditions in silver carp showed seasonal variation across the summer and fall sampling periods. The transcriptional responses representing likely exposure to elevated contaminant levels at the leading edge were only observed in silver carp collected in the summer, and these responses were not evident in fish sampled in the late fall. Previous studies suggest that contaminant levels in the water and the uptake of these contaminants by fish can vary seasonally (e.g., Edwards et al., 2001; Mzimela et al., 2003; Pereira et al., 2010b), as can the biomarker responses (e.g., oxidative stress markers) to contaminant exposure (e.g., Gorbi et al., 2005; Padmini et al., 2009; Pereira et al., 2010a, 2010b; Tsangaris et al., 2011). These differences in biomarker responses across seasons may be a result of seasonal changes in biotic and abiotic factors such as metabolism, feeding status, reproductive status, and temperature, which have been shown to affect basal levels of many biomarkers and their responses to pollutants (e.g., Collier et al., 1995; Eggens et al., 1996; Ronisz and Larsson, 1999; Gorbi et al., 2005). Interestingly, a study by Coulter et al. (2016) found that invasive silver carp in the Wabash River, Indiana, USA, increased the number of downstream movements in the fall (September and October) compared to the spring (March and April) and summer (May to August) months, likely for overwintering and in response to seasonal changes in habitat requirements (e.g., temperature). Thus, responses to contaminants may play a lesser role in mediating silver carp upstream migration during the cooler months. Additional studies examining potential seasonal variation in silver carp transcriptional responses, in combination with fish and water contaminant levels at the leading edge of their invasion front are warranted.

5. Conclusions

Overall, the liver transcriptomic profiles of silver carp from the present study suggest that degraded water quality and/or the presence of contaminants in the Chicago Area Waterway may play a role in limiting range expansion of silver carp in the direction of Chicago, and ultimately the Great Lakes. We saw little evidence to suggest nutritional deficiencies in silver carp at the upstream leading edge relative to downstream locations, suggesting that limited access to food or poor condition are likely not preventing upstream movement. Thus, the range expansion of silver carp at the leading edge may be limited by more contaminated areas upstream. Because a number of other fish species do reside within these upstream regions of the Chicago Area Waterway (Metropolitan Water Reclamation District of Greater Chicago, 2013), our data suggest that the sensitivity of silver carp to these contaminants may be more exaggerated than in other species. Additional laboratory studies should be conducted to test this hypothesis and potentially better define whether there is a behavioral avoidance response of silver carp to water from the Chicago Area Waterway. Additionally, further investigation is merited to examine possible seasonal variation in transcriptional responses of silver carp at a finer scale, as the results of the present study suggest that either contaminant levels or the responses to contaminants in the environment at the leading edge may vary with season. A number of remediation efforts are in place to improve water quality in the Chicago Area Waterway

including reductions in the discharge of untreated storm water and sewage discharge (Metropolitan Water Reclamation District of Greater Chicago, 2016, 2017). The goal of these improvements may have the unintended consequence of improving water quality that facilitates the upstream movement of silver carp. Overall, the use of transcriptomics to assess a subgroup of the silver carp population at the leading edge compared to the core population in the Illinois River provides several potential markers (e.g., ABC transporters) for assessing a larger group of the silver carp population at the leading edge, and monitoring the status of the silver carp population over time, or as environmental conditions may shift. From a management standpoint, increasing our understanding of the physiological status of silver carp at the leading edge of the invasion front, provides managers with useful information regarding the potential for silver carp movement in the future.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbd.2019.100614.

Declaration of Competing Interest

There are no conflicts of interest to declare regarding the study "Physiological status of silver carp (*Hypophthalmichthys molitrix*) in the Illinois River: An assessment of fish at the leading edge of the invasion front".

Acknowledgments

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References

ACRCC, 2017. Interm Summary Report: Asian Carp Monitoring and Response Plan. (Asian Carp Regional Coordinating Committee).

Aubin-Horth, N., Renn, S.C.P., 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol. Ecol. 18, 3763–3780. https://doi.org/10.1111/j.1365-294X.2009.04313.x.

Aykanat, T., Thrower, F.P., Heath, D.D., 2011. Rapid evolution of osmoregulatory function by modification of gene transcription in steelhead trout. Genetica 139, 233–242. https://doi.org/10.1007/s10709-010-9540-2.

Bains, O.S., Kennedy, C.J., 2005. Alterations in respiration rate of isolated rainbow trout hepatocytes exposed to the P-glycoprotein substrate rhodamine 123. Toxicology 214, 87–98. https://doi.org/10.1016/j.tox.2005.06.002.

Brown, J.H., Stevens, G.C., Kaufman, D.M., 1996. The geographic range: size, shape, boundaries, and internal structure. Annu. Rev. Ecol. Syst. 27, 597–623. https://doi.org/10.1146/annurev.ecolsys.27.1.597.

Bryant, D.M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M.B., Payzin-Dogru, D., Lee, T.J., Leigh, N.D., Kuo, T.-H., Davis, F.G., Bateman, J., Bryant, S., Guzikowski, A.R., Tsai, S.L., Coyne, S., Ye, W.W., Freeman, R.M., Peshkin, L., Tabin, C.J., Regev, A., Haas, B.J., Whited, J.L., 2017. A tissue-mapped axolotl *de novo* transcriptome enables identification of limb regeneration factors. Cell Rep. 18, 762–776. https://doi.org/10.1016/j.celrep.2016.12.063.

Carretero, M., Ruiz-Torres, M., Rodríguez-Corsino, M., Barthelemy, I., Losada, A., 2013. Pds5B is required for cohesion establishment and Aurora B accumulation at centromeres. EMBO J. 32, 2938–2949. https://doi.org/10.1038/emboj.2013.230.

Chen, E.Y., Tan, C.M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G.V., Clark, N.R., May'ayan, A., 2013. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 14, 128.

Chen, L., Sun, Q., Zhou, D., Song, W., Yang, Q., Ju, B., Zhang, L., Xie, H., Zhou, L., Hu, Z., Yao, H., Zheng, S., Wang, W., 2017. HINT2 triggers mitochondrial Ca^{2+} influx by regulating the mitochondrial Ca^{2+} uniporter (MCU) complex and enhances gemcitabine apoptotic effect in pancreatic cancer. Cancer Lett. 411, 106–116. https://doi.org/10.1016/j.canlet.2017.09.020.

Cho, K.-H., 2009. Biomedicinal implications of high-density lipoprotein: its composition, structure, functions, and clinical applications. BMB Rep. 42, 393–400.

Cocci, P., Mosconi, G., Arukwe, A., Mozzicafreddo, M., Angeletti, M., Aretusi, G., Palermo, F.A., 2015. Effects of diisodecyl phthalate on PPAR:RXR-dependent gene expression pathways in sea bream hepatocytes. Chem. Res. Toxicol. 28, 935–947. https://doi.org/10.1021/tx500529x.

Collier, T.K., Anulacion, B.F., Stein, J.E., Varanasi, U., Goksøyr, A., 1995. A field evaluation of cytochrome P4501A as a biomarker of contaminant exposure in three species of flatfish. Environ. Toxicol. Chem. 14, 143–152. https://doi.org/10.1002/etc.5620140116.

Congleton, J.L., Wagner, T., 2006. Blood-chemistry indicators of nutritional status in juvenile salmonids. J. Fish Biol. 69, 473–490. https://doi.org/10.1111/j.1095-8649.2006.01114.x.

Connon, R.E., Jeffries, K.M., Komoroske, L.M., Todgham, A.E., Fangue, N.A., 2018. The utility of transcriptomics in fish conservation. J. Exp. Biol. 221, jeb148833. https://doi.org/10.1242/jeb.148833.

Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35, 124–129. https://doi.org/10.2307/2683975.

Corcoran, J., Winter, M.J., Lange, A., Cumming, R., Owen, S.F., Tyler, C.R., 2015. Effects of the lipid regulating drug clofibric acid on PPARα-regulated gene transcript levels in common carp (*Cyprinus carpio*) at pharmacological and environmental exposure levels. Aquat. Toxicol. 161, 127–137. https://doi.org/10.1016/j.aquatox.2015.01.033.

Coulter, A.A., Bailey, E.J., Keller, D., Goforth, R.R., 2016. Invasive Silver Carp movement patterns in the predominantly free-flowing Wabash River (Indiana, USA). Biol. Invasions 18, 471–485. https://doi.org/10.1007/s10530-015-1020-2.

Currie, W.J.S., Cuddington, K.M.D., Stewart, T.J., Zhang, H., Koops, M.A., 2012. Modelling Spread, Establishment and Impact of Bighead and Silver Carps in the Great Lakes, DFO Canadian Science Advisory Secretariat Research Document 2011/113.

Dean, M., Annilo, T., 2005. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Ann. Rev. Genom. Hum. G. 6, 123–142. https://doi.org/10.1146/annurev.genom.6.080604.162122.

Donaldson, M.R., Hinch, S.G., Jeffries, K.M., Patterson, D.A., Cooke, S.J., Farrell, A.P., Miller, K.M., 2014. Species- and sex-specific responses and recovery of wild, mature pacific salmon to an exhaustive exercise and air exposure stressor. Comp. Biochem. Physiol. A 173, 7–16. https://doi.org/10.1016/j.cbpa.2014.02.019.

Donaldson, M.R., Amberg, J., Adhikari, S., Cupp, A., Jensen, N., Romine, J., Wright, A., Gaikowski, M., Suski, C.D., 2016. Carbon dioxide as a tool to deter the movement of invasive bigheaded carps. Trans. Am. Fish. Soc. 145, 657–670. https://doi.org/10.1080/00028487.2016.1143397.

Duncker, J.J., Battaglin, W.A., Terrio, P.J., Barber, L.B., 2017. Laboratory Results for Anthropogenic Bioactive Chemicals in the Illinois Waterway Upstream and Downstream of the Bigheaded Carp Population Front (2015). U.S. Geological Survey data release. https://doi.org/10.5066/F77S7M7Z.

Edwards, J.W., Edyvane, K.S., Boxall, V.A., Hamann, M., Soole, K.L., 2001. Metal levels in seston and marine fish flesh near industrial and metropolitan centres in South Australia. Mar. Pollut. Bull. 42, 389–396. https://doi.org/10.1016/S0025-326X(00)00168-5.

Eggens, M.L., Opperhuizen, A., Boon, J.P., 1996. Temporal variation of CYP1A indices, PCB and 1-OH pyrene concentration in flounder, *Platichthys flesus*, from the Dutch Wadden Sea. Chemosphere 33, 1579–1596. https://doi.org/10.1016/0045-6535(96)00277-9.

Epel, D., 1998. Use of multidrug transporters as first lines of defense against toxins in aquatic organisms. Comp. Biochem. Physiol. A 120, 23–28. https://doi.org/10.1016/S1095-6433(98)10005-3.

Feng, Y., He, D., Yao, Z., Klionsky, D.J., 2014. The machinery of macroautophagy. Cell Res. 24, 24–41. https://doi.org/10.1038/cr.2013.168.

Gorbi, S., Baldini, C., Regoli, F., 2005. Seasonal variability of metallothioneins, cytochrome P450, bile metabolites and oxyradical metabolism in the European eel *Anguilla anguilla* L. (Anguillidae) and striped mullet *Mugil cephalus* L. (Mugilidae). Arch. Environ. Contam. Toxicol. 49, 62–70. https://doi.org/10.1007/s00244-004-0150-9.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., Macmanes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., Leduc, R.D., Friedman, N., Regev, A., 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8, 1494–1512. https://doi.org/10.1038/nprot.2013.084.

Hagey, L.R., Møller, P.R., Hofmann, A.F., Krasowski, M.D., 2010. Diversity of bile salts in fish and amphibians: evolution of a complex biochemical pathway. Physiol. Biochem. Zool. 83, 308–321. https://doi.org/10.1086/649966.

Heckmann, B.L., Zhang, X., Xie, X., Liu, J., 2013. The G0/G1 switch gene 2 (G0S2): regulating metabolism and beyond. Biochim. Biophys. Acta 1831, 276–281. https://doi.org/10.1016/j.bbalip.2012.09.016.

Hildebrand, J.L., Bains, O.S., Lee, D.S.H., Kennedy, C.J., 2009. Functional and energetic characterization of P-gp-mediated doxorubicin transport in rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Comp. Biochem. Physiol. C 149, 65–72. https://doi.org/10.1016/j.cbpc.2008.07.003.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. https://doi.org/10.1002/bimj.200810425.

Jeffries, K., Komoroske, L., Truong, J., Werner, I., Hasenbein, M., Hasenbein, S., Fangue, N., Connon, R., 2015. The transcriptome-wide effects of exposure to a pyrethroid pesticide on the critically endangered delta smelt *Hypomesus transpacificus*. Endanger. Species Res. 28, 43–60. https://doi.org/10.3354/esr00679.

Jeffries, K.M., Connon, R.E., Davis, B.E., Komoroske, L.M., Britton, M.T., Sommer, T., Todgham, A.E., Fangue, N.A., 2016. Effects of high temperatures on threatened estuarine fishes during periods of extreme drought. J. Exp. Biol. 219, 1705–1716. https://doi.org/10.1242/jeb.134528.

Kolar, C.S., Chapman, D.C., Courtenay Jr., W.R., Housel, C.M., Williams, J.D., Jennings,

D.P., 2007. Bigheaded Carps: A Biological Synopsis and Environmental Risk Assessment. (American Fisheries Society).
Kurelec, B., 1992. The multixenobiotic resistance mechanism in aquatic organisms. Crit. Rev. Toxicol. 22, 23–43. https://doi.org/10.3109/10408449209145320.
Lande, R., 2015. Evolution of phenotypic plasticity in colonizing species. Mol. Ecol. 24, 2038–2045. https://doi.org/10.1111/mec.13037.
Lauber, T.B., Stedman, R.C., Connelly, N.A., Rudstam, L.G., Ready, R.C., Poe, G.L., Bunnell, D.B., Höök, T.O., Koops, M.A., Ludsin, S.A., Rutherford, E.S., 2016. Using scenarios to assess possible future impacts of invasive species in the Laurentian Great Lakes. N. Am. J. Fish. Manage. 36, 1292–1307. https://doi.org/10.1080/02755947.2016.1214647.
Lubejko, M.V., Whitledge, G.W., Coulter, A.A., Brey, M.K., Oliver, D.C., Garvey, J.E., 2017. Evaluating upstream passage and timing of approach by adult bigheaded carps at a gated dam on the Illinois River. River Res. Appl. 33, 1268–1278. https://doi.org/10.1002/rra.3180.
Lun, A.T., Chen, Y., Smyth, G.K., 2016. It's DE-licious: a recipe for differential expression analyses of RNA-seq experiments using quasi-likelihood methods in edgeR. In: Mathé, E., Davis, S. (Eds.), Methods in Molecular Biology. Springer, New York, pp. 391–416.
Maréchal, A., Zou, L., 2015. RPA-coated single-stranded DNA as a platform for post-translational modifications in the DNA damage response. Cell Res. 25, 9–23. https://doi.org/10.1038/cr.2014.147.
McIlwain, D.R., Berger, T., Mak, T.W., 2013. Caspase functions in cell death and disease. CSH Perspect. Biol. 5, a008656. https://doi.org/10.1101/cshperspect.a008656.
Metropolitan Water Reclamation District of Greater Chicago, 2013. Metropolitan Water Reclamation District of Greater Chicago (No. 13–34).
Metropolitan Water Reclamation District of Greater Chicago, 2016. Stormwater management program.
Metropolitan Water Reclamation District of Greater Chicago, 2017. TARP Status Report as of December 31, 2017.
Miller, K.M., Li, S., Kaukinen, K.H., Ginther, N., Hammill, E., Curtis, J.M.R., Patterson, D.A., Sierocinski, T., Donnison, L., Pavlidis, P., Hinch, S.G., Hruska, K.A., Cooke, S.J., English, K.K., Farrell, A.P., 2011. Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science 331, 214–217. https://doi.org/10.1126/science.1196901.
Mizukami, S., Watanabe, Y., Saegusa, Y., Nakajima, K., Ito, Y., Masubuchi, Y., Yoshida, T., Shibutani, M., 2017. Downregulation of UBE2E2 in rat liver cells after hepatocarcinogen treatment facilitates cell proliferation and slowing down of DNA damage response in GST-P-expressing preneoplastic lesions. Toxicol. Appl. Pharmacol. 334, 207–216. https://doi.org/10.1016/j.taap.2017.09.005.
Momoda, T., Schwindt, A., Feist, G., Gerwick, L., Bayne, C., Schreck, C., 2007. Gene expression in the liver of rainbow trout, *Oncorhynchus mykiss*, during the stress response. Comp. Biochem. Physiol. D 2, 303–315. https://doi.org/10.1016/j.cbd.2007.06.002.
Moore, M.N., Koehler, A., Lowe, D., Viarengo, A., 2008. Lysosomes and autophagy in aquatic animals. In: Methods in Enzymology. Elsevier, pp. 581–620. https://doi.org/10.1016/S0076-6879(08)03233-3.
Mzimela, H.M., Wepener, V., Cyrus, D.P., 2003. Seasonal variation of selected metals in sediments, water and tissues of the groovy mullet, *Liza dumerelii* (Mugilidae) from the Mhlathuze Estuary, South Africa. Mar. Pollut. Bull. 46, 659–664. https://doi.org/10.1016/S0025-326X(03)00088-2.
Oomen, R.A., Hutchings, J.A., 2017. Transcriptomic responses to environmental change in fishes: insights from RNA sequencing. FACETS 2, 610–641. https://doi.org/10.1139/facets-2017-0015.
Padmini, E., Usha Rani, M., Vijaya Geetha, B., 2009. Studies on antioxidant status in *Mugil cephalus* in response to heavy metal pollution at Ennore estuary. Environ. Monit. Assess. 155, 215–225. https://doi.org/10.1007/s10661-008-0430-3.
Paetzold, S.C., Ross, N.W., Richards, R.C., Jones, M., Hellou, J., Bard, S.M., 2009. Up-regulation of hepatic ABCC2, ABCG2, CYP1A1 and GST in multixenobiotic-resistant killifish (*Fundulus heteroclitus*) from the Sydney Tar Ponds, Nova Scotia. Canada. Mar. Environ. Res. 68, 37–47. https://doi.org/10.1016/j.marenvres.2009.04.002.
Parker, A.D., Glover, D.C., Finney, S.T., Rogers, P.B., Stewart, J.G., Simmonds, R.L., 2015. Direct observations of fish incapacitation rates at a large electrical fish barrier in the Chicago Sanitary and Ship Canal. J. Great Lakes Res. 41, 396–404. https://doi.org/10.1016/j.jglr.2015.03.004.
Parker, A.D., Glover, D.C., Finney, S.T., Rogers, P.B., Stewart, J.G., Simmonds, R.L., 2016. Fish distribution, abundance, and behavioral interactions within a large electric dispersal barrier designed to prevent Asian carp movement. Can. J. Fish. Aquat. Sci. 73, 1060–1071. https://doi.org/10.1139/cjfas-2015-0309.
Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., Kingsford, C., 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14, 417–419. https://doi.org/10.1038/nmeth.4197.
Pereira, P., de Pablo, H., Pacheco, M., Vale, C., 2010a. The relevance of temporal and organ specific factors on metals accumulation and biochemical effects in feral fish (*Liza aurata*) under a moderate contamination scenario. Ecotoxicol. Environ. Saf. 73, 805–816. https://doi.org/10.1016/j.ecoenv.2010.02.020.
Pereira, P., de Pablo, H., Vale, C., Pacheco, M., 2010b. Combined use of environmental data and biomarkers in fish (*Liza aurata*) inhabiting a eutrophic and metal-contaminated coastal system – gills reflect environmental contamination. Mar. Environ. Res. 69, 53–62. https://doi.org/10.1016/j.marenvres.2009.08.003.
Phillips, M.C., 2014. Molecular mechanisms of cellular cholesterol efflux. J. Biol. Chem. 289, 24020–24029. https://doi.org/10.1074/jbc.R114.583658.
Phillips, B.L., Brown, G.P., Shine, R., 2010. Evolutionarily accelerated invasions: the rate of dispersal evolves upwards during the range advance of cane toads. J. Evol. Biol. 23, 2595–2601. https://doi.org/10.1111/j.1420-9101.2010.02118.x.
R Core Team, 2017. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Viena, Austria.
Rasmussen, J.L., Regier, H.A., Sparks, R.E., Taylor, W.W., 2011. Dividing the waters: the case for hydrologic separation of the North American Great Lakes and Mississippi River Basins. J. Great Lakes Res. 37, 588–592. https://doi.org/10.1016/j.jglr.2011.05.015.
Robinson, M.D., Oshlack, A., 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11, R25. https://doi.org/10.1186/gb-2010-11-3-r25.
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.
Ronisz, D., Larsson, D.G.J., 1999. Seasonal variations in the activities of selected hepatic biotransformation and antioxidant enzymes in eelpout 9.
Sexton, J.P., McIntyre, P.J., Angert, A.L., Rice, K.J., 2009. Evolution and ecology of species range limits. Ann. Rev. Ecol. Evol. S. 40, 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317.
Simao, 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, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351.
Sokka, M., Parkkinen, S., Pospiech, H., Syväoja, J.E., 2010. Function of TopBP1 in genome stability. In: Nasheuer, H.-P. (Ed.), Genome Stability and Human Diseases. Springer Netherlands, Dordrecht, pp. 119–141. https://doi.org/10.1007/978-90-481-3471-7_7.
Soneson, C., Love, M.I., Robinson, M.D., 2016. Differential Analyses for RNA-seq: Transcript-level Estimates Improve Gene-level Inferences [Version 2; Referees: 2 Approved]. 4. F1000Research, pp. 1521. https://doi.org/10.12688/f1000research.7563.2.
Song, Y., Nahrgang, J., Tollefsen, K.E., 2019. Transcriptomic analysis reveals dose-dependent modes of action of benzo(a)pyrene in polar cod (*Boreogadus saida*). Sci. Total Environ. 653, 176–189. https://doi.org/10.1016/j.scitotenv.2018.10.261.
Sopinka, N.M., Donaldson, M.R., O'Connor, C.M., Suski, C.D., Cooke, S.J., 2016. Stress indicators in fish. In: Fish Physiology. Elsevier, pp. 405–462. https://doi.org/10.1016/B978-0-12-802728-8.00011-4.
Suckling, K.E., Stange, E.F., 1985. Role of acyl-CoA: cholesterol acyltransferase in cellular cholesterol metabolism. J. Lipid Res. 26, 647–671.
Supek, F., Bosnjak, M., Skunca, N., Smuc, T., 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One 6, e21800. https://doi.org/10.1371/journal.pone.0021800.
Teles, M., Fierro-Castro, C., Na-Phatthalung, P., Tvarijonaviciute, A., Soares, A.M.V.M., Tort, L., Oliveira, M., 2016. Evaluation of gemfibrozil effects on a marine fish (*Sparus aurata*) combining gene expression with conventional endocrine and biochemical endpoints. J. Hazard. Mater. 318, 600–607. https://doi.org/10.1016/j.jhazmat.2016.07.044.
Tsangaris, C., Vergolyas, M., Fountoulaki, E., Nizheradze, K., 2011. Oxidative stress and genotoxicity biomarker responses in grey mullet (*Mugil cephalus*) from a polluted environment in Saronikos Gulf. Greece. Arch. Environ. Contam. Toxicol. 61, 482–490. https://doi.org/10.1007/s00244-010-9629-8.
Vetter, B.J., Cupp, A.R., Fredricks, K.T., Gaikowski, M.P., Mensinger, A.F., 2015. Acoustical deterrence of Silver Carp (*Hypophthalmichthys molitrix*). Biol. Invasions 17, 3383–3392. https://doi.org/10.1007/s10530-015-0964-6.
Waterhouse, R.M., Seppey, M., Simão, F.A., Manni, M., Ioannidis, P., Klioutchnikov, G., Kriventseva, E.V., Zdobnov, E.M., 2018. BUSCO applications from quality assessments to gene prediction and phylogenomics. Mol. Biol. Evol. 35, 543–548. https://doi.org/10.1093/molbev/msx319.
Welch, C., Santra, M.K., El-Assaad, W., Zhu, X., Huber, W.E., Keys, R.A., Teodoro, J.G., Green, M.R., 2009. Identification of a protein, G0S2, that lacks Bcl-2 homology domains and iInteracts with and antagonizes Bcl-2. Cancer Res. 69, 6782–6789. https://doi.org/10.1158/0008-5472.CAN-09-0128.
Wellband, K.W., Heath, D.D., 2017. Plasticity in gene transcription explains the differential performance of two invasive fish species. Evol. Appl. 10, 563–576. https://doi.org/10.1111/eva.12463.
Whitehead, A., Crawford, D.L., 2006. Neutral and adaptive variation in gene expression. P. Natl. Acad. Sci. USA 103, 5425–5430. https://doi.org/10.1073/pnas.0507648103.
Wittmann, M.E., Cooke, R.M., Rothlisberger, J.D., Rutherford, E.S., Zhang, H., Mason, D.M., Lodge, D.M., 2015. Use of structured expert judgment to forecast invasions by bighead and silver carp in Lake Erie. Conserv. Biol. 29, 187–197. https://doi.org/10.1111/cobi.12369.
Wray, G.A., 2003. The evolution of transcriptional regulation in eukaryotes. Mol. Biol. Evol. 20, 1377–1419. https://doi.org/10.1093/molbev/msg140.
Yabu, T., Imamura, S., Mizusawa, N., Touhata, K., Yamashita, M., 2012. Induction of autophagy by amino acid starvation in fish cells. Mar. Biotechnol. 14, 491–501. https://doi.org/10.1007/s10126-012-9432-9.
Yoo, J.-A., Lee, E.-Y., Park, J.Y., Lee, S.-T., Ham, S., Cho, K.-H., 2015. Different functional and structural characteristics between ApoA-I and ApoA-4 in lipid-free and reconstituted HDL state: ApoA-4 showed less anti-atherogenic activity. Mol. Cells 38, 573–579. https://doi.org/10.14348/molcells.2015.0052.
Zhang, H., Rutherford, E.S., Mason, D.M., Breck, J.T., Wittmann, M.E., Cooke, R.M., Lodge, D.M., Rothlisberger, J.D., Zhu, X., Johnson, T.B., 2016. Forecasting the impacts of silver and bighead carp on the Lake Erie food web. Trans. Am. Fish. Soc. 145, 136–162. https://doi.org/10.1080/00028487.2015.1069211.
Zhu, L., Nie, L., Zhu, G., Xiang, L., Shao, J., 2013. Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. Dev. Comp. Immunol. 39, 39–62. https://doi.org/10.1016/j.dci.2012.04.001.
Zimmermann, M., de Lange, T., 2014. 53BP1: pro choice in DNA repair. Trends Cell Biol. 24, 108–117. https://doi.org/10.1016/j.tcb.2013.09.003.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.