SUPPLEMENTARY INFORMATION **Page number**

Table S1. Index of samples for transcriptome sequencing

Treatment	Tissue
Freshwater Male	Kidney
	Liver
	Gill
Freshwater Female	Kidney
	Liver
	Gill
Seawater Male	Kidney
	Liver
	Gill
Seawater Female	Kidney
	Liver
	Gill
10% Seawater Male	Kidney
	Liver
	Gill
10% Seawater Female	Kidney
	Liver
	Gill
Freshwater to seawater (30 min)	Kidney
	Liver
	Gill
Freshwater to seawater (1 hr)	Kidney
	Liver
	Gill
Freshwater to seawater (3 hr)	Kidney
	Liver
	Gill
Seawater to freshwater (30 min)	Kidney
	Liver
	Gill
Seawater to freshwater (1 hr)	Kidney
	Liver
	Gill
Seawater to freshwater (3 hr)	Kidney
	Liver
	Gill
Embryo (8 day)	----
Embryo (14 day)	----
*Arsenic (8000 µg/L)	Gill
	Liver
*Arsenic (12,000 µg/L)	Gill
	Liver
*Cadmium (500 µg/L)	Gill
	Liver

Table S1. Continued

*Cadmium (5000 µg/L)	Gill
	Liver
*Lead (500 µg/L)	Gill
	Liver
*Lead (5000 µg/L)	Gill
	Liver
*Copper (4.8 µg/L)	Gill
	Liver
*Copper (480 µg/L)	Gill
	Liver
*Lead (500 µg/L) + Copper (4.8 µg/L)	Liver
*Arsenic (12,000 µg/L) + Copper (480 µg/L)	Liver
*Cadmium (5000 µg/L) + Copper (480 µg/L)	Liver

*Fish were exposed to metals for 24 h in seawater

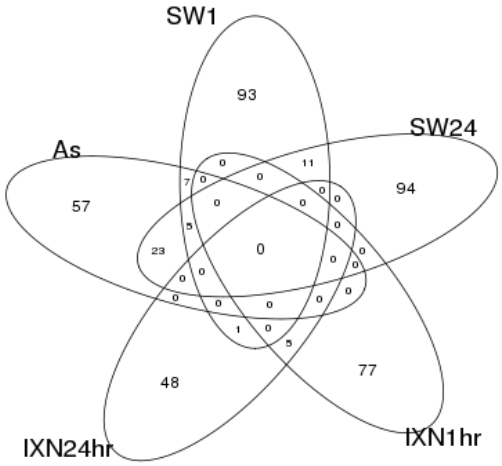
Table S2. ***Fundulus heteroclitus transcriptome assembly statistics***

Input sequences	
Number of sequence reads, raw	1,340,048
Number of sequence reads, trimmed	1,302,633
Number of bases, raw	273,469,835
Number of bases, trimmed	229,144,736
Output Sequences	
Unique Contigs (all)	38,673
Total bases (bp)	18,682,616
Coverage	9.8X
Average size (bp)	483
N50	646
Unique Contigs (large, >500bp)	11,873
Total bases (bp)	11,287,711
Average size (bp)	950
N50	1,028
Unique Contigs (major, >1000bp)	4,113
Total bases (bp)	5,879,893
Average size (bp)	1,429
N50	1,426
Singleton ESTs	169,915

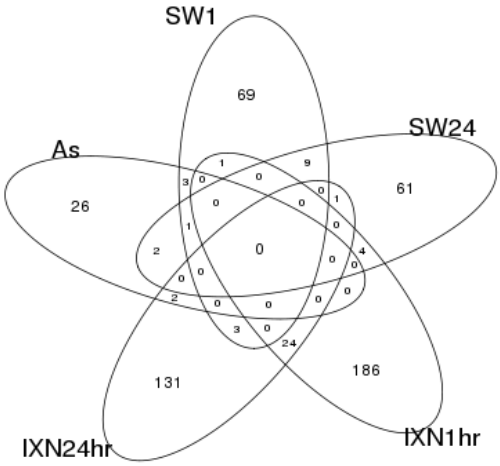
Table S3. ***Quantitative PCR primer pairs***

Contig	Gene	Sense	Antisense	Tm
2378	ATPIF1	AGCCCTTACAAAGAGGTCAGTAGA	GAACGCCTACAAAGAGAGATTGAC	60/61
8426	ANGPTL4	TGAACTGAGACTTCATTTTTCTCG	CCCAGAGAACTGGGTCTTTAACTA	60/60
4632	CH25H	AACAATGAAGGAACAAAAAGAAGG	TGTACATGGGATTACACATTCTCC	60/60
6109	CLDN8	CTTTTTATGTGCAAAATTGGTTGA	AACAAACAAATGTCACAAGCTGAT	60/60
6683	GSTO1	AGGATTCGAAGCTGAGTTAAAAGA	AACATCTCAAGCCTCTCAAAGAAT	60/60
35232	TOMM40	AGTTGACAAACTTATGCTGCTGAG	TACGTTGGAAGTAAACAGACTGGA	60/60
23405	Acetyl-CoA	TGTGGTGCATTAAATTATTTCCAG	GTTACGCAAAAGTACCGATGAAG	60/60

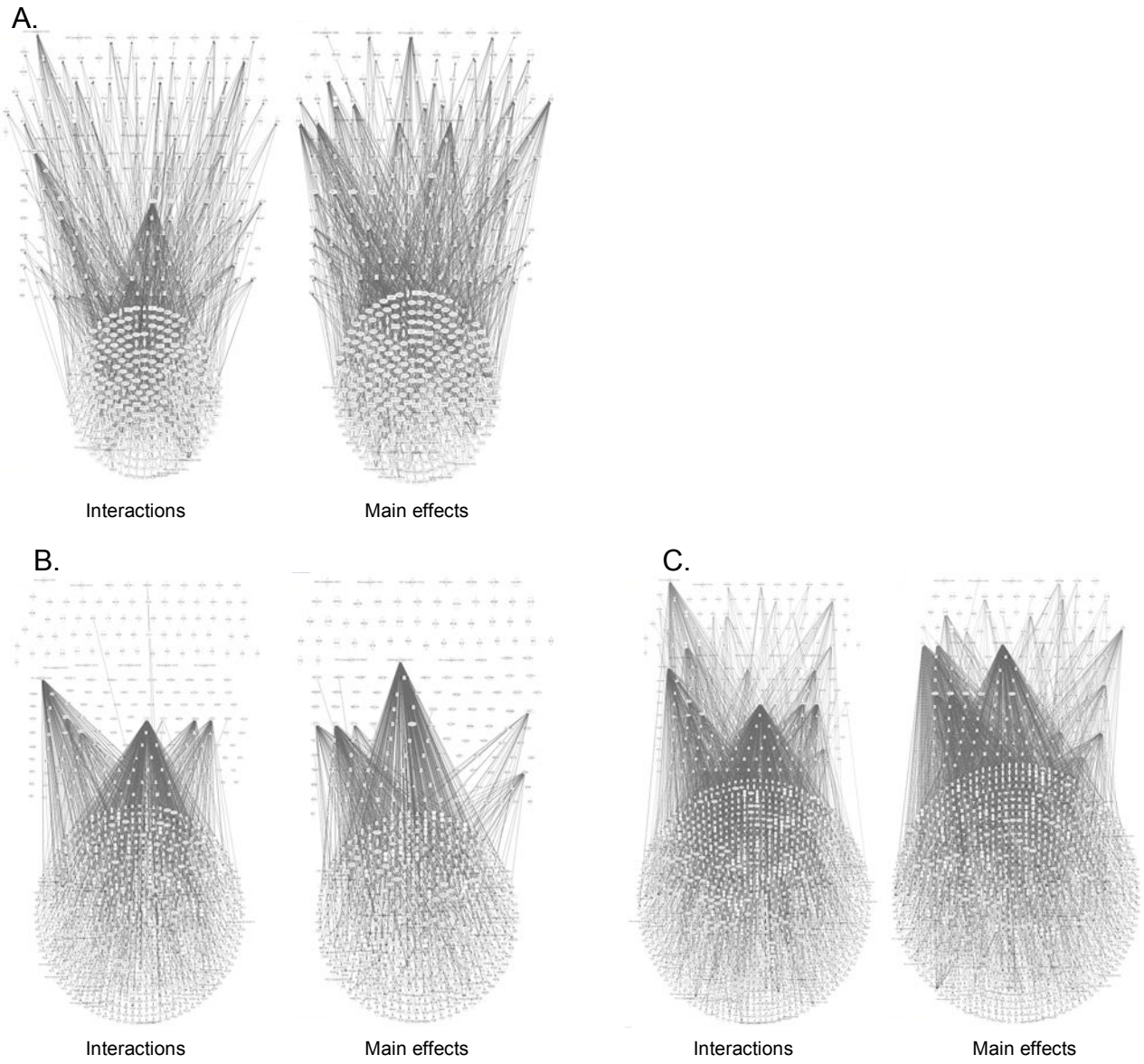
A. up-regulated



B. down-regulated

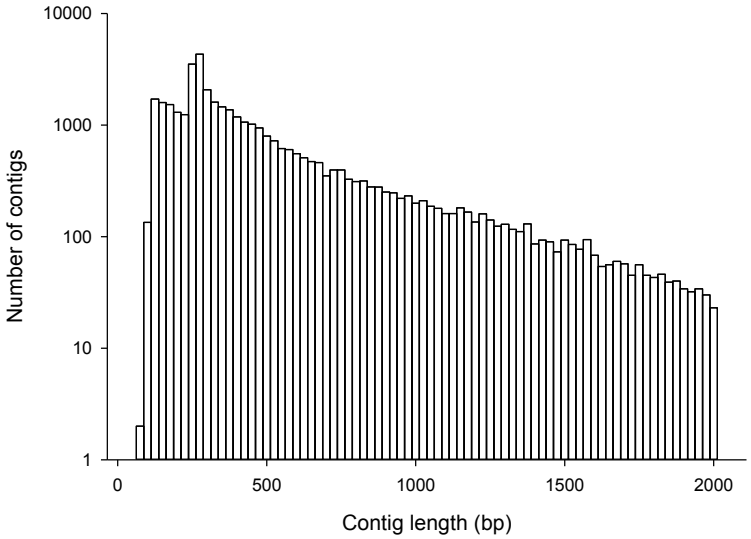


Supplementary Figure S1. Venn diagram of differentially expressed genes. Transcriptomic response of *F. heteroclitus* gills (n=4, independent male fish per treatment) to arsenic during seawater acclimation reveals salinity dependent, arsenic mediated gene expression. Quantile normalized log2 expression values were analyzed using a simple two-factor linear mode, implemented in R that included two categorical variables (presence of arsenic and time spent in seawater), as well as, interactions between levels of the two categorical variables. Probes with p value <0.05 and fold change ≥ 2 for at least one effect were deemed significantly affected by treatment. Numbers represent differentially (A) up-regulated and (B) down-regulated genes for the following treatments: arsenic (As), 1hr in seawater (SW1), 24hr in seawater (SW24), interactions between arsenic and SW1 (IXN1hr) and arsenic and SW24 (IXN24hr). Interaction effects are not predicted by simple addition of effects attributable to arsenic or seawater alone, and all were antagonistic (i.e., less than additive).

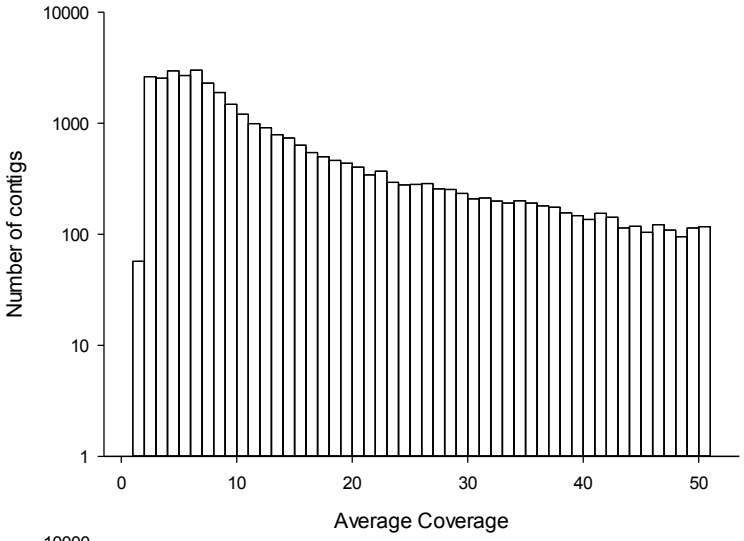


Supplementary Figure S2. Network analysis for (A) upstream direct, (B) downstream direct, and (C) downstream direct and indirect gene regulatory networks. Gene regulatory networks were constructed using Integrated Pathway Analysis software (IPA, Ingenuity Systems) between the uniquely interaction gene sets and the uniquely main effect gene sets, and their known trans-regulating molecules (circles). Relationships are indicated with lines and visual inspection suggests that the interaction gene sets form less complex networks with upstream trans-regulating molecules than main effects gene sets (A), but there are less differences in the complexity of networks formed by the interaction and main effects gene sets and their downstream targets (B, C) for which no statistical differences were observed.

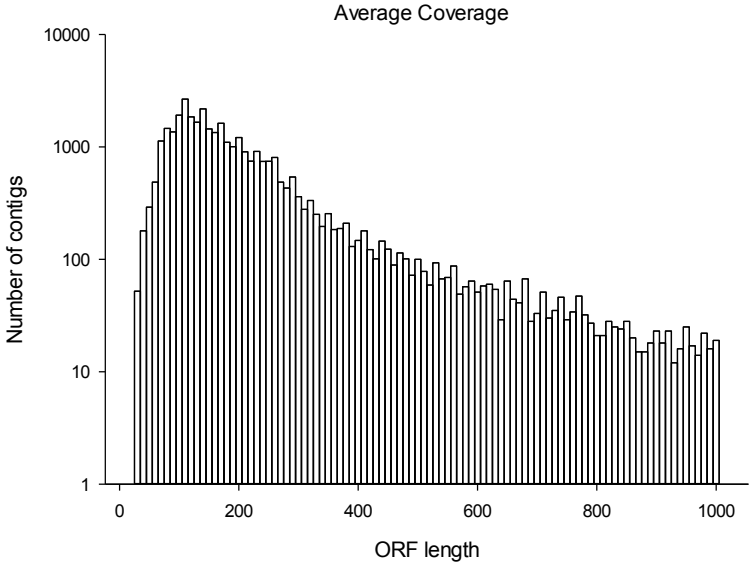
A.



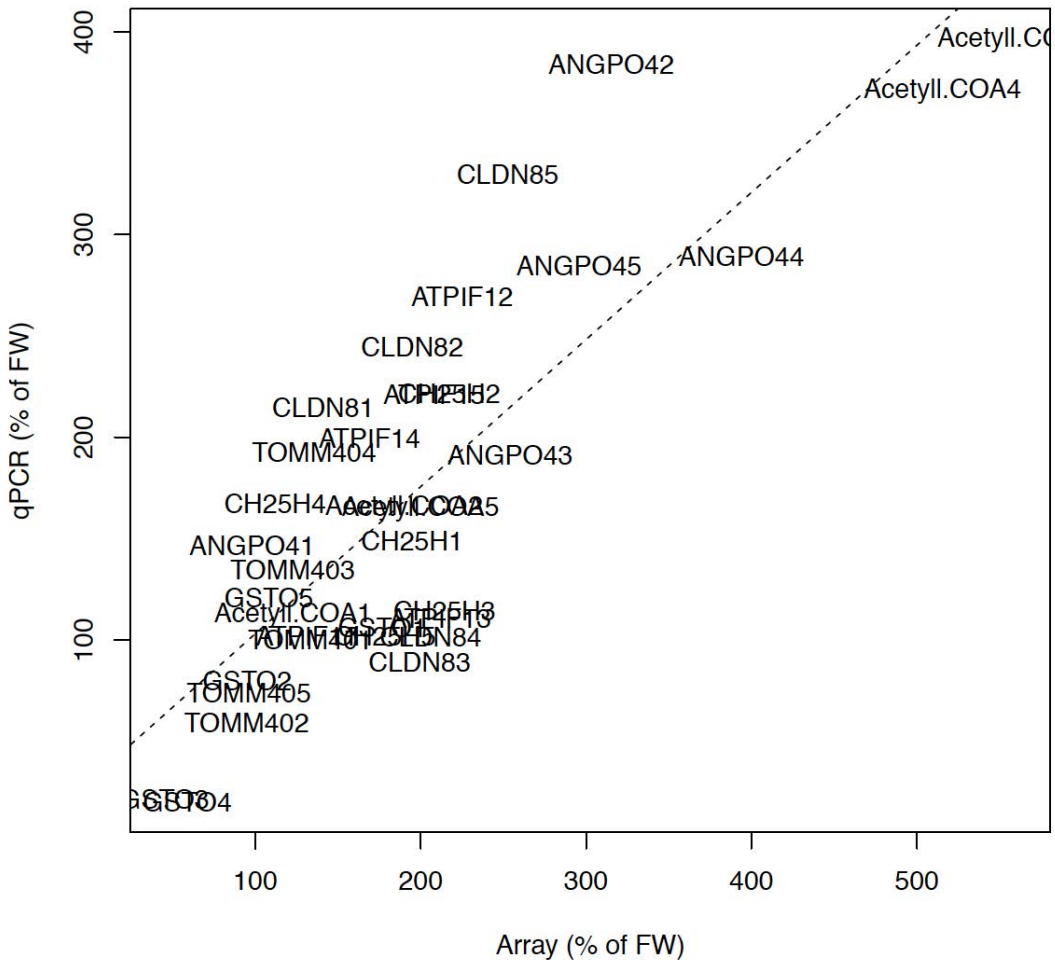
B.



C.



Supplementary Figure S3. Characteristics of assembled Fundulus heteroclitus contigs. A) Length and B) coverage and C) size of open reading frames (ORFs) of contigs. Note the logarithmic vertical axes. Summary statistics are provided in Table S2.



Supplementary Figure S4. Validation of microarray results. We independently validated microarray results in a separate set of experiments in which different fish (n=6 fish per treatment) were exposed to the same treatment conditions and gene expression of seven target genes assessed via qPCR. The seven genes, Acetyl COA, CLDN8, GSTO1, TOMM40, ANGPTL4, ATPIF1, CH25H, were selected because they were well annotated, well expressed and showed significant regulation in at least one treatment. For each gene, we examined the treatment means expressed as a percentage of the freshwater treatment in both the microarray and qPCR experiments. This yielded 35 data pairs (qPCR:microarray, 5 treatment values excluding freshwater for each of 7 genes), which had an overall Pearson's correlation of 0.83 ($p < 1.08e-09$) indicating the gene expression patterns observed in the microarray experiment were recapitulated in the qPCR studies.