D12.-14. Expression of target genes in acanthocephalans by RT-qPCR reactions and transcriptome and gene expression profiling in fish intestine with respect to different metal exposure conditions

Irena Vardić Smrzlić, Sara Šariri

THIRD PROJECT MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 19th May 2023

HRZZ Croatian Science Foundation
ANDRIJA STAMPAR TEACHING INSTITUTE OF PUBLIC HEALTH
WELLFISH DIAGNOSTICS
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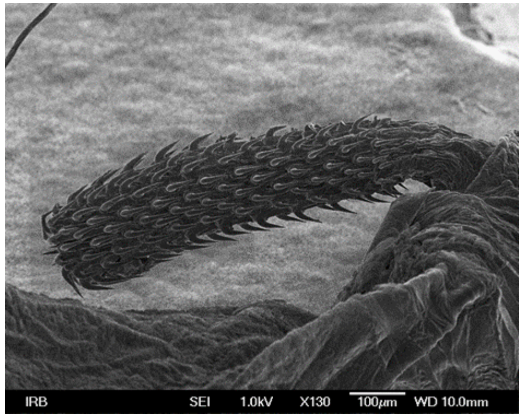
FFoQSI FEED AND FOOD QUALITY, SAFETY AND INNOVATION
BIOTOXMET

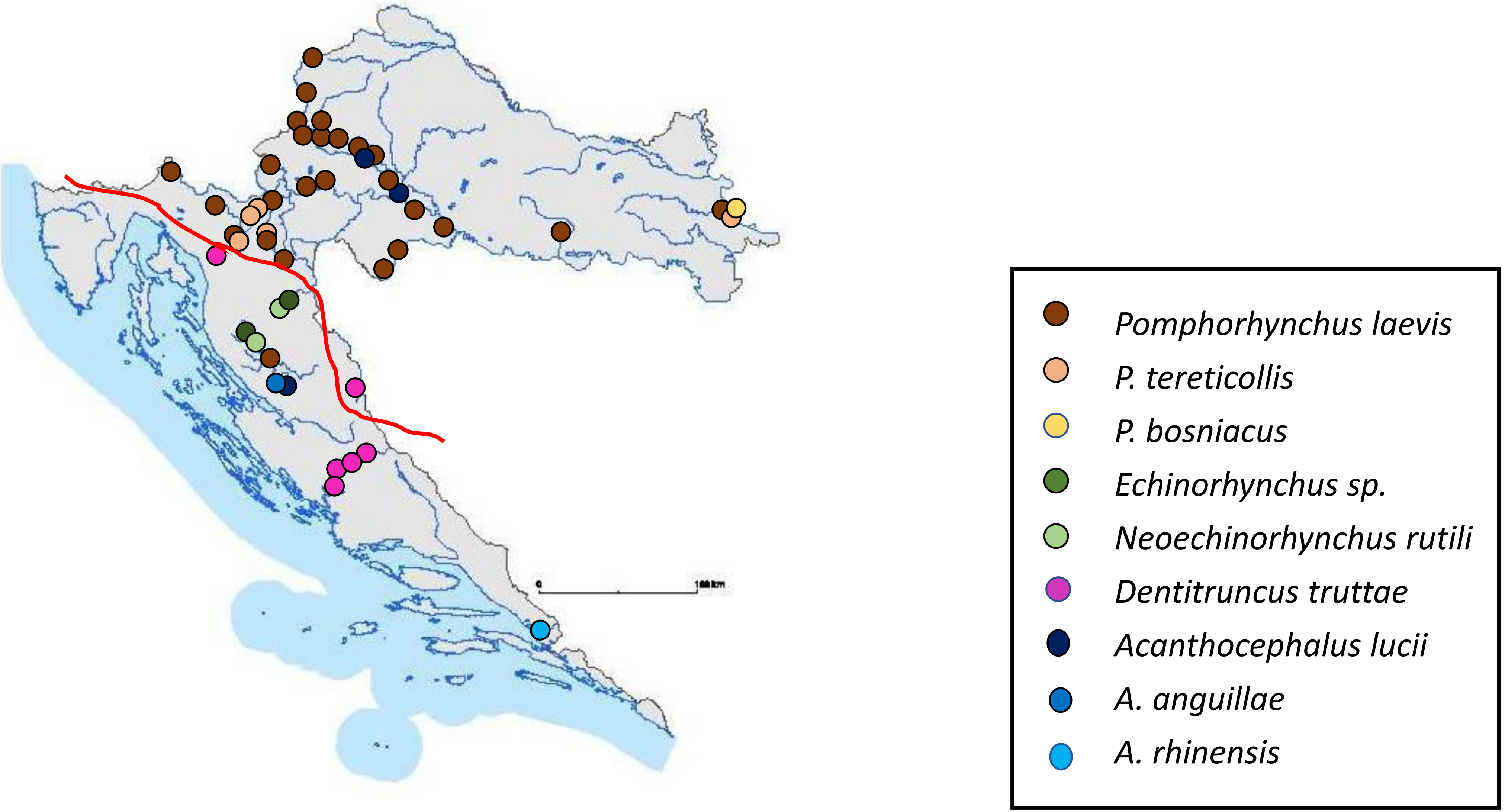
History of fish Acanthocephala research in Croatia

- Long history of Acanthocephala research
- Since 1930s (Babić, 1935)
- Genera: *Pomphorhynchus, Echinorhynchus,*
  Acanthocephalus, Neoechinorhynchus,
  Dentitruncus
- *during the 80s and 90s no research*
- 2021. for the first time *Acanthocephalus rhinensis*



Sampling site	Species	Fish host	Reference
Different former Yugoslavia areas	*Metechinorhynchus truttae*	*Trachyrincus scabrus*	**Babić, 1935**
		Lota lota	
		Esox lucius	
	Acanthocephalus lucii	*Lota lota*	
		Esox lucius	
	Acanthocephalus anguillae	*Barbus barbus*	
	Neoecinorhynchus rutili	*Onchorhynchus mykiss*	
	Pomphorhynchus laevis	*Trachyrincus scabrus*	
		Lota lota	
		Silurus glanis	
		Barbus barbus	
		Abramis brama	
		Acipenser ruthenus	
Prošćansko lake, NP "Plitvička lakes", Croatia	*Metechynorhynchus truttae*	*Salmo trutta*	**Šinžar, 1955**
Lake Kozjak, NP "Plitvička lakes", Croatia	*Acanthocephalus lucii*	*Salmo trutta*	
Bohinjsko lake and tributary Savica, Slovenia	*Neoechinorhynchus rutili*	*Salmo trutta*	**Šinžar, 1956**
		Onchorhynchus laevis	
Una River, Bosnia and Herzegovina	*Dentitruncus trutae*	*Salmo trutta*	
Savinja River, Slovenia	*Pomphorhynchus laevis*	*Salmo trutta*	**Brglez, 1962**
		Thymallus thymallus	
	Metechinorhynchus truttae	*Salmo trutta*	
	Neoechinorhynchus rutili	*Salmo trutta*	

Map of Acanthocephala species in Croatia

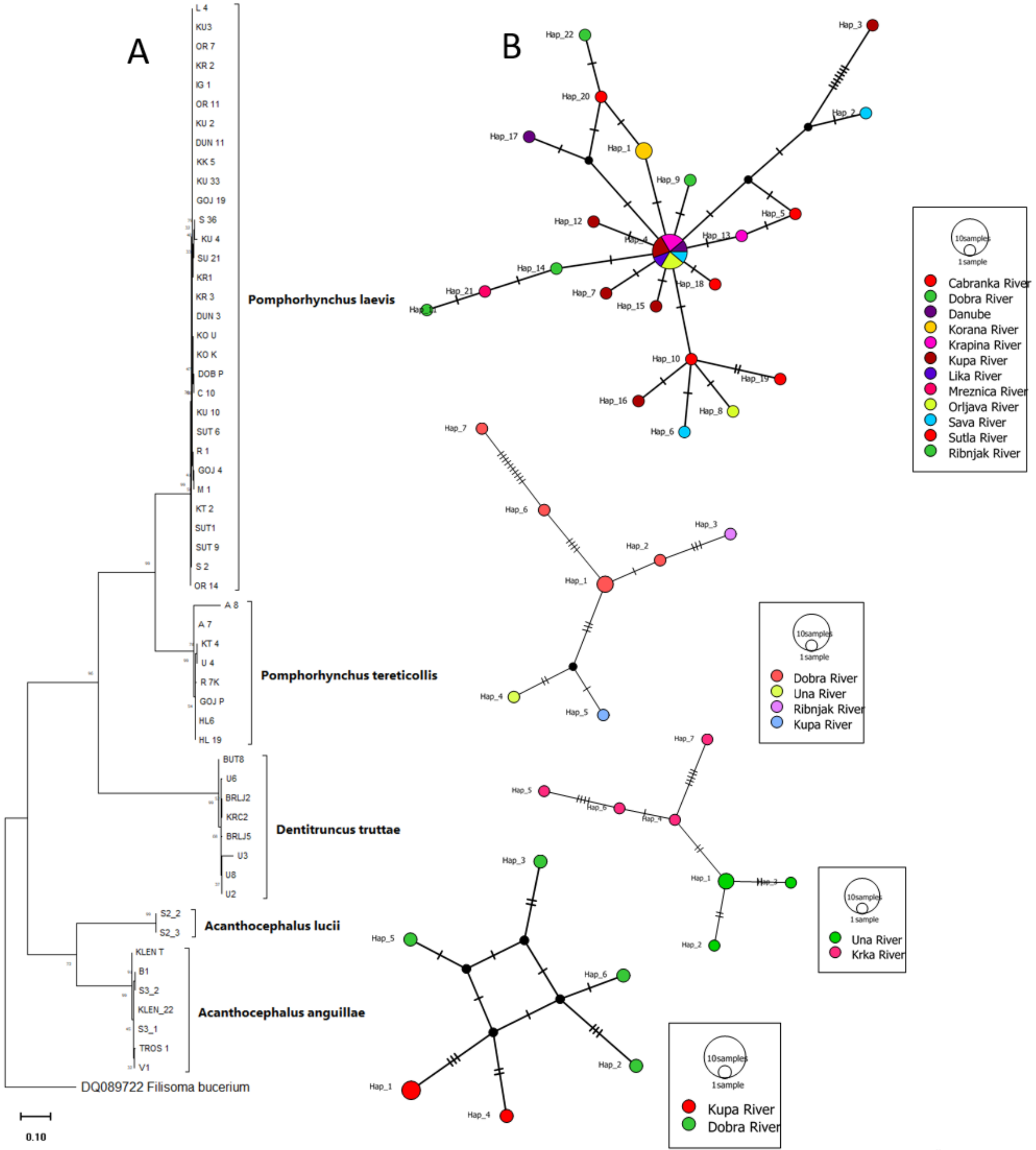


Haplotype network of Acanthocephala in Croatia

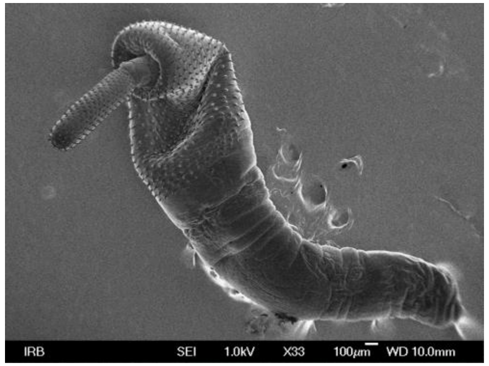
Nucleotide diversity of ITS and COI region in analysed acanthocephalan species. Number of haplotypes (H), number of polymorphic sites (S), nucleotide diversity (% π) and haplotype diversity (Hd) are given.

Species	**ITS**					**COI**				
	N	H	S	% π	% Hd	N	H	S	% π	% Hd
P. laevis	23	6	9	0.230	63.2	31	22	28	0.446	92.0
P. tereticollis	10	3	2	0.111	60.0	8	7	22	1.156	96.4
D. truttae	5	4	7	0.654	90.0	8	7	14	0.732	96.4
A. anguillae	4	3	3	0.250	83.3	7	6	14	0.892	95.2

Vardić Smrzlić I., Čolić B., Kapetanović D., Šariri S., Mijošek T. Filipović Marijić, V. Phylogeny and genetic variability of Rotifer's closest relatives Acanthocephala: an example from Croatia, *Hydrobiologia, submitted*



Dentitruncus truttae Sinzar, 1955



Dentitruncus truttae, SEM

Distribution

WoRMS - World Register of Marine Species - Dentitruncus truttae Sinzar, 1955

Biology

Intermediate hosts	*Gammarus balcanicus*
	G. italicus
	Echinogammarus roco
	E. tibaldi
Hosts	brown trout (*Salmo trutta*)
	rainbow trout (*Oncorhynchus mykiss*)
	adriatic trout (*Salmo obtusirostris*)
	grayling (*Thymallys thymallus*)
	powan (*Coregonus clupeoides*)
	european eel (*Anguillae anguillae*)

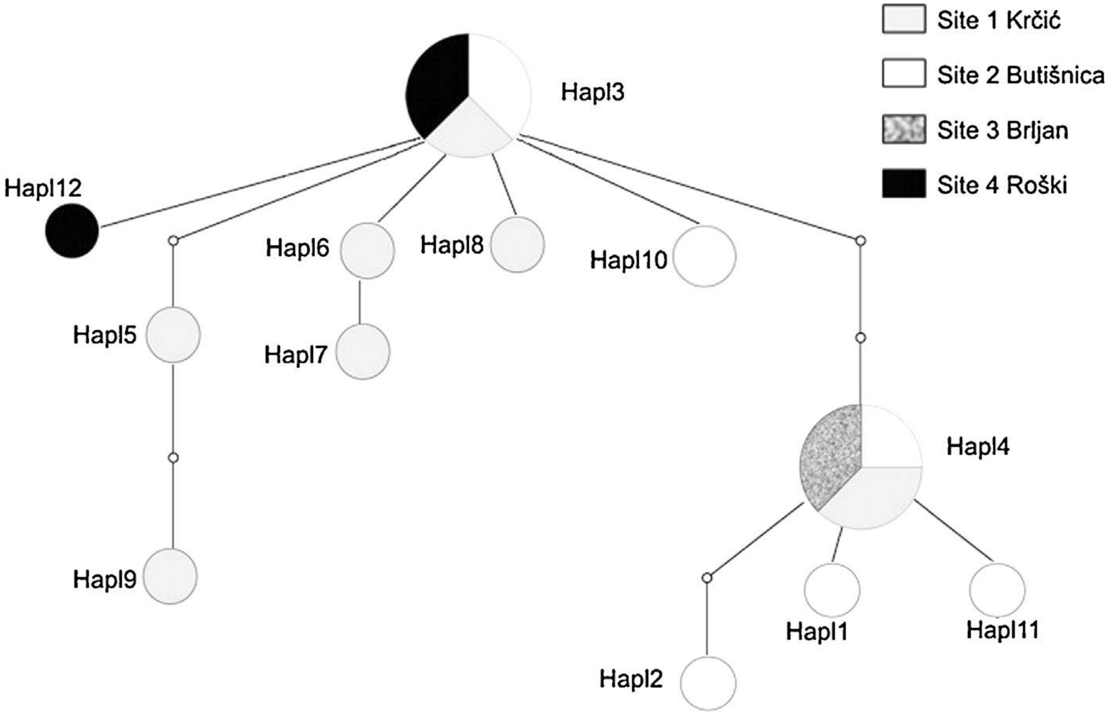
Genus Dentitruncus

***D. truttae* –** *partial 18S rRNA region: no intra- or interpopulation differences*

***D. truttae* –** *partial* COI gene

Locality	N	H	S	% π	% Hd
Krcic	10	7	9	0.490	91.1
Butisnica	10	6	8	0.429	88.9
Brljan	3	1	0	0	0
Roski	4	2	1	0.076	50.0
Total	**27**	**12**	**15**	**0.416**	**83.8**

Region	H	S	%GC	% π	% Hd
ITS1	3	2	46.5	0.166	37.8
ITS2	10	14	49.5	2.095	100



Vardić Smrzlić et al, 2013

EXPOSURE EXPERIMENTS


live individuals from the Krka River


RPMI medium + antibiotics at 16 ± 1 °C, 3-5 days

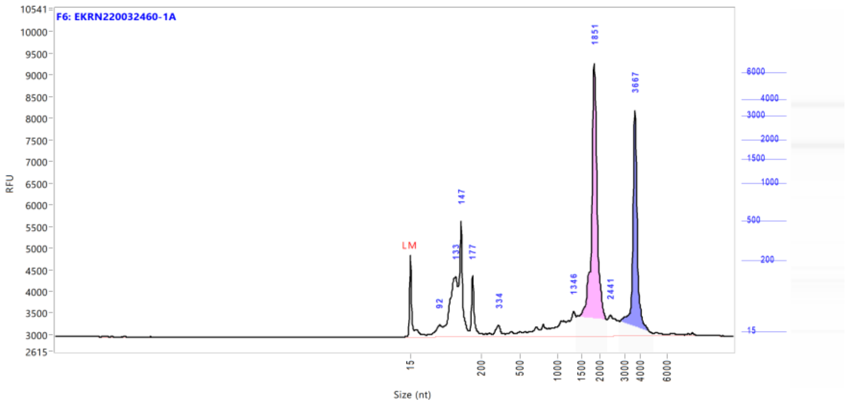
Group 1
Control group

Group 2
Treatment 2mg/ml Cd2+

Group 3
Treatment 10mg/ml Cd2+

Isolation of RNA of appropriate concentration and quality isolated from acanthocephalans

No.	Sample Name	Nucleic Acid ID	Concentration (ng/ul)	Volume(ul)	Total amount(ug)	RIN	Sample QC Results
1	C7A_cont	EKRN220032460-1A	62.74	31	1.94496	8.3	Pass
2	C7B_cont	EKRN220032461-1A	47.59	23	1.09466	8.1	Pass
3	KONN4	FKRN210226091-1A	17	81.00	1.37700	6.70	Pass
4	C7B_2Cd	EKRN220032464-1A	66.61	24	1.59860	8	Pass
5	C7A_2Cd	EKRN220032462-1A	31.66	31	0.98157	6.9	Pass
6	T157_2mgCd	FKRN210226093-1A	217	36.00	7.81200	7.30	Pass
7	C7A_10Cd	EKRN220032463-1A	49.52	30	1.48568	6.5	Pass
8	C7B_10Cd	EKRN220032465-1A	61.00	27	1.64713	8.7	Pass
9	T155_10mgCd	FKRN210226092-1A	232	36.00	8.35200	8.30	Pass



Genus Dentitruncus

D. truttae - transcriptomics

- A total of 342,3 million clean reads were obtained by sequencing eight libraries (Table 1).
- Approximately, 39.8 to 46.1 million clean reads were obtained from each library

Table 1. Summary statistics of the transcriptome assembly for *D. truttae*

Transcriptome feature	Values
Raw reads	347,357,875
Clean reads	342,293,411
Number of transcripts	133990
Number of unigenes	52585
Average length (bp) transcripts	1026
Average length (bp) unigenes	912
N50 transcripts	1407
N50 unigene	1250
GC content	45,2%

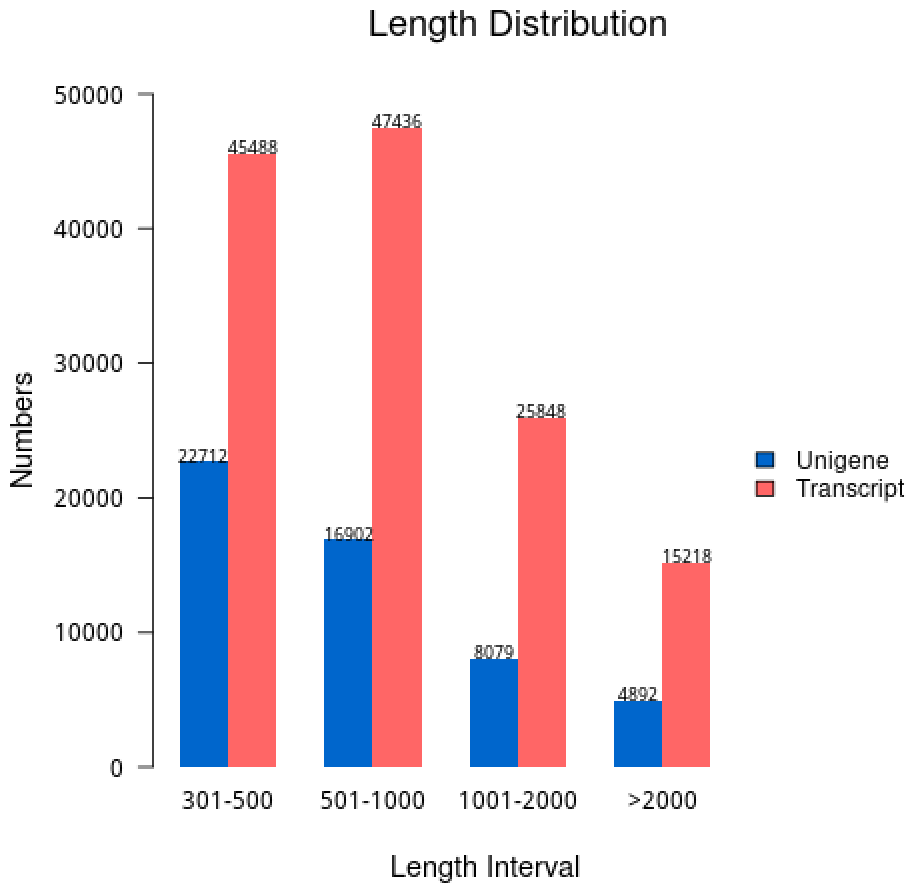
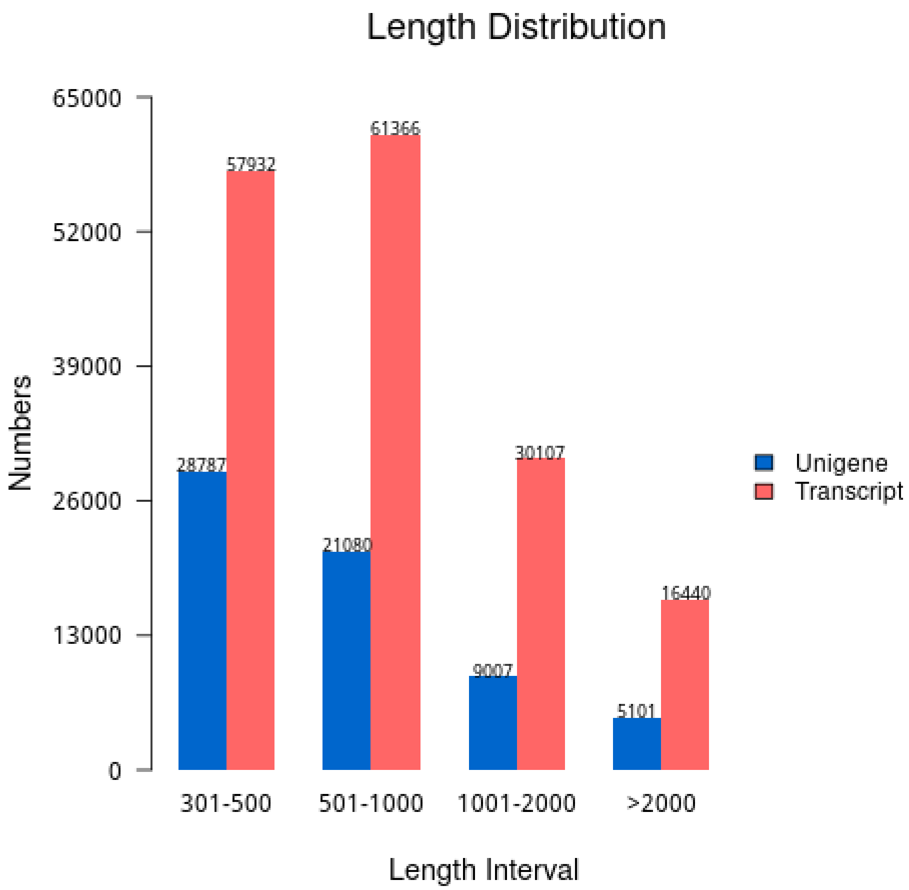


Figure 1. Length distribution of *D. truttae* unigenes

EXPOSURE EXPERIMENTS

Transcriptome feature	**Values**
Raw reads	69129998,11
Clean reads	68120231,56
Number of transcripts	165845
Number of unigenes	63975
Average length (bp) transcripts	638
Average length (bp) unigenes	539
N50 transcripts	1276
N50 unigene	1106
GC content	45,6%



Functional annotation

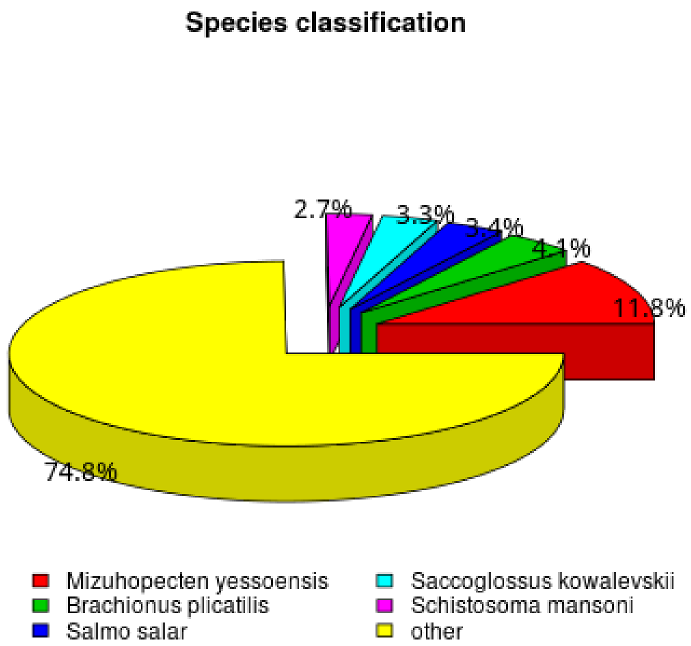
Environment

Statistical Items	Number of Unigenes	Percent (%)
Total Unigenes	52585	100
Annotated in SwissProt	6087	11.57
Annotated in PFAM	13796	26.23
Annotated in NT	1701	3.23
Annotated in NR	14947	28.42
Annotated in KOG	3834	7.29
Annotated in KO	4432	8.42
Annotated in GO	13790	26.22
Annotated in at least one Database	21806	41.46
Annotated in all Databases	621	1.18

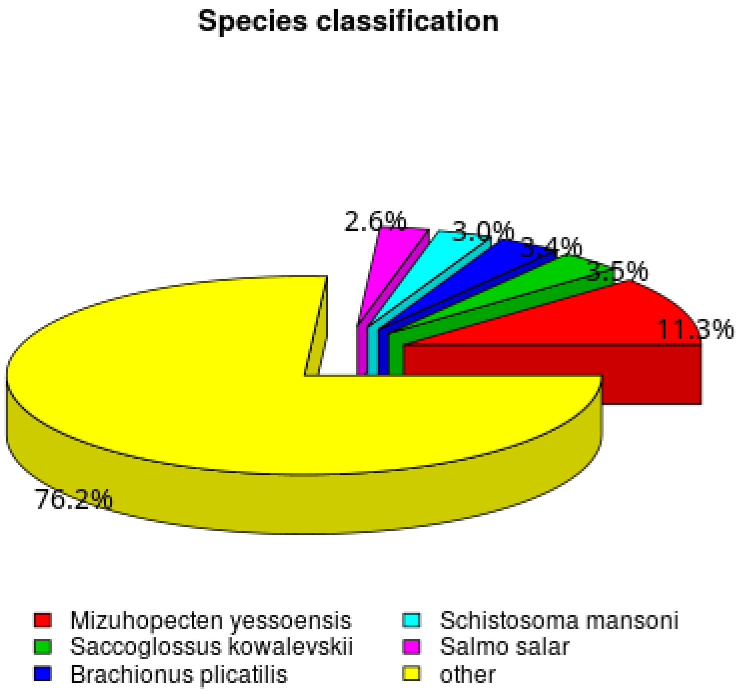
Exposure

Statistical Items	Number of Unigenes	Percent (%)
Total Unigenes	63975	100
Annotated in SwissProt	7442	11.63
Annotated in PFAM	16422	25.66
Annotated in NT	1534	2.40
Annotated in NR	17702	27.67
Annotated in KOG	4691	7.33
Annotated in KO	5256	8.21
Annotated in GO	16422	25.66
Annotated in at least one Database	25785	40.3
Annotated in all Databases	806	1.26

Environmental



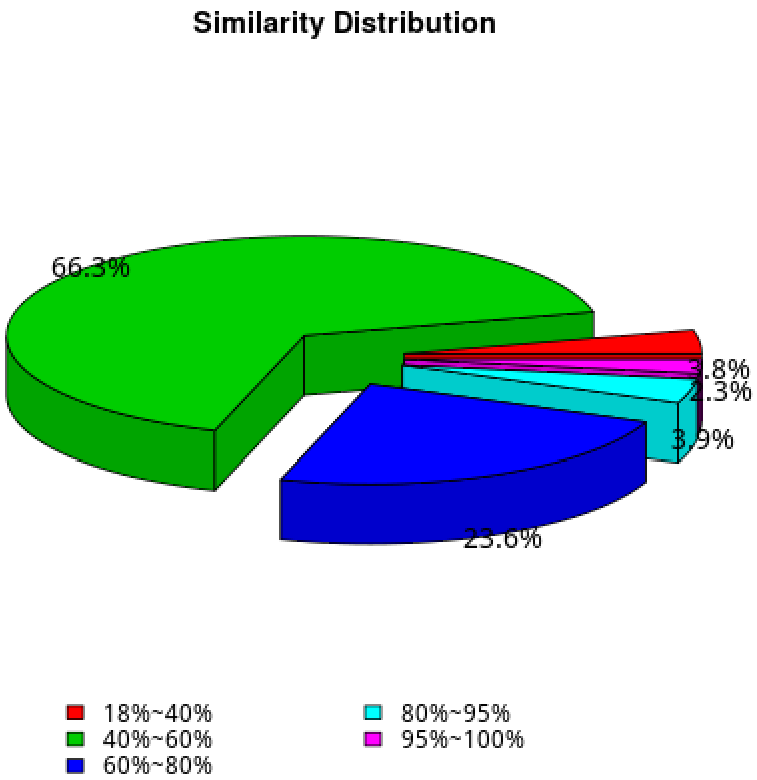
Exposure



Only 3.4% or 2.6% of the unigenes matched with sequences in the transcriptome database of *Salmo salar*. Similarly, Mauer at el. (2020) showed 4% of contigs from *Pomphorhynchus laevis* matched with sequences in the genome and transcriptome database of its fish host (*Cyprinus carpio*).

Contamination with host DNA should not play an important role in the *D. truttae* transcriptome.

Environmental



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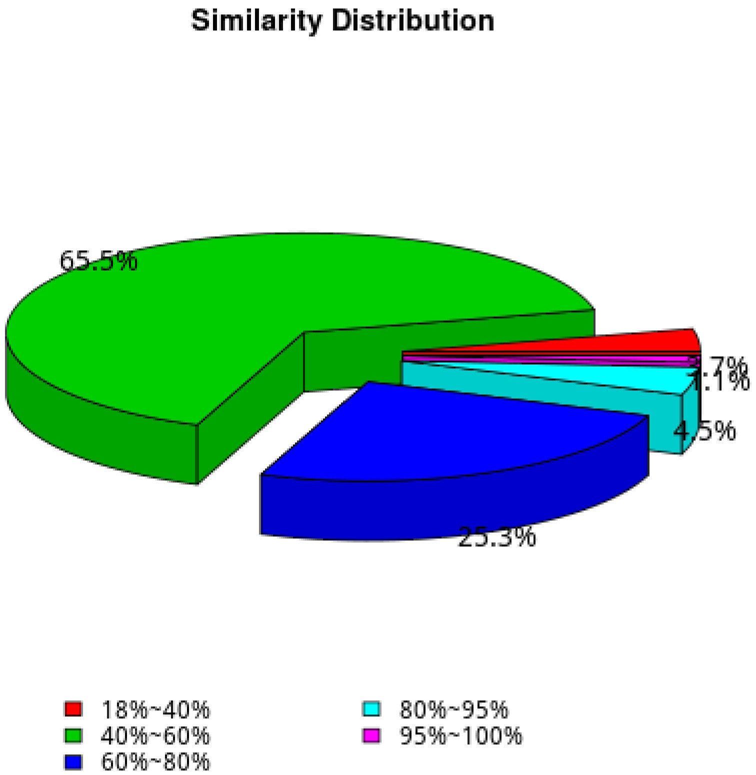
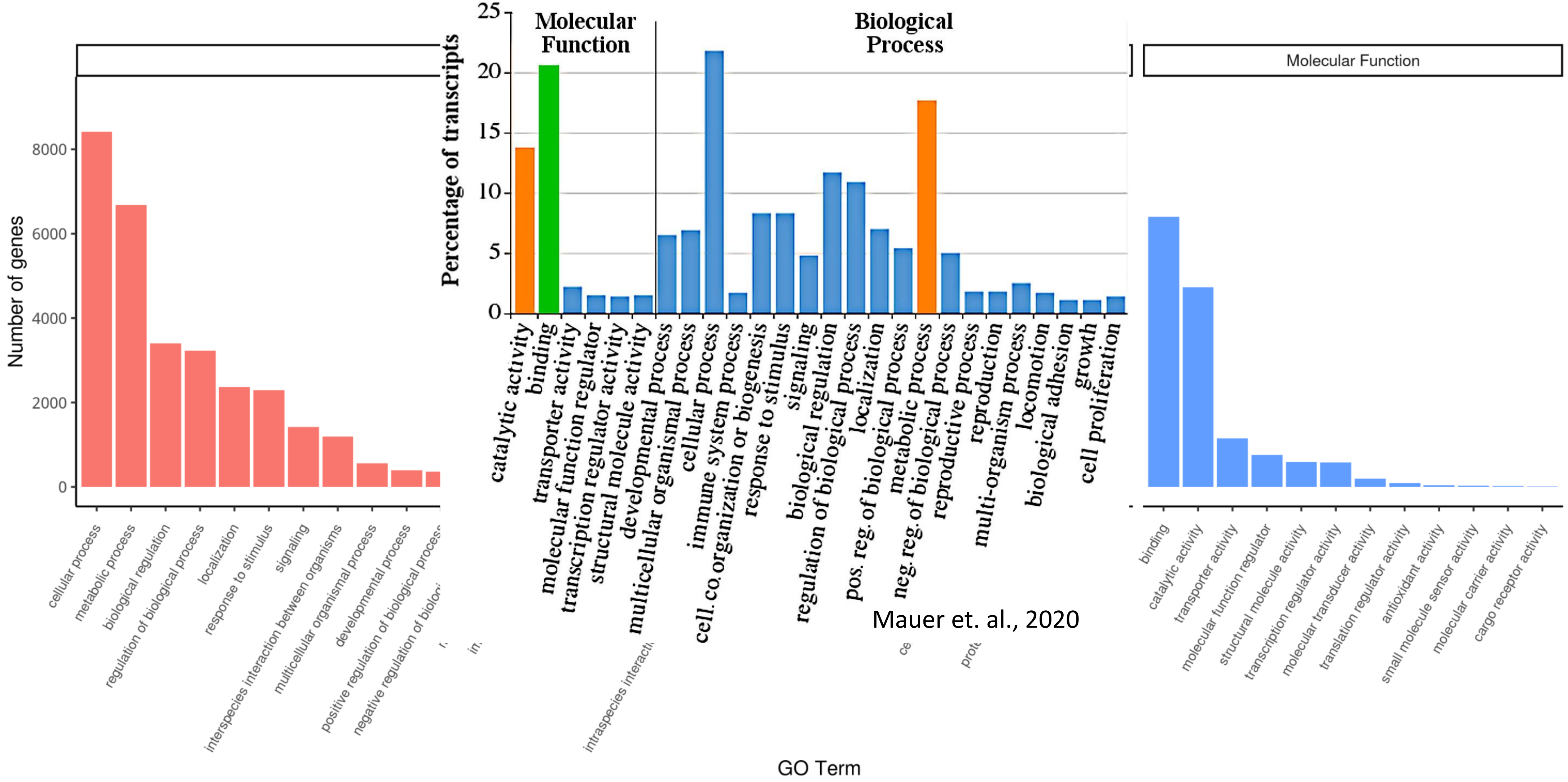
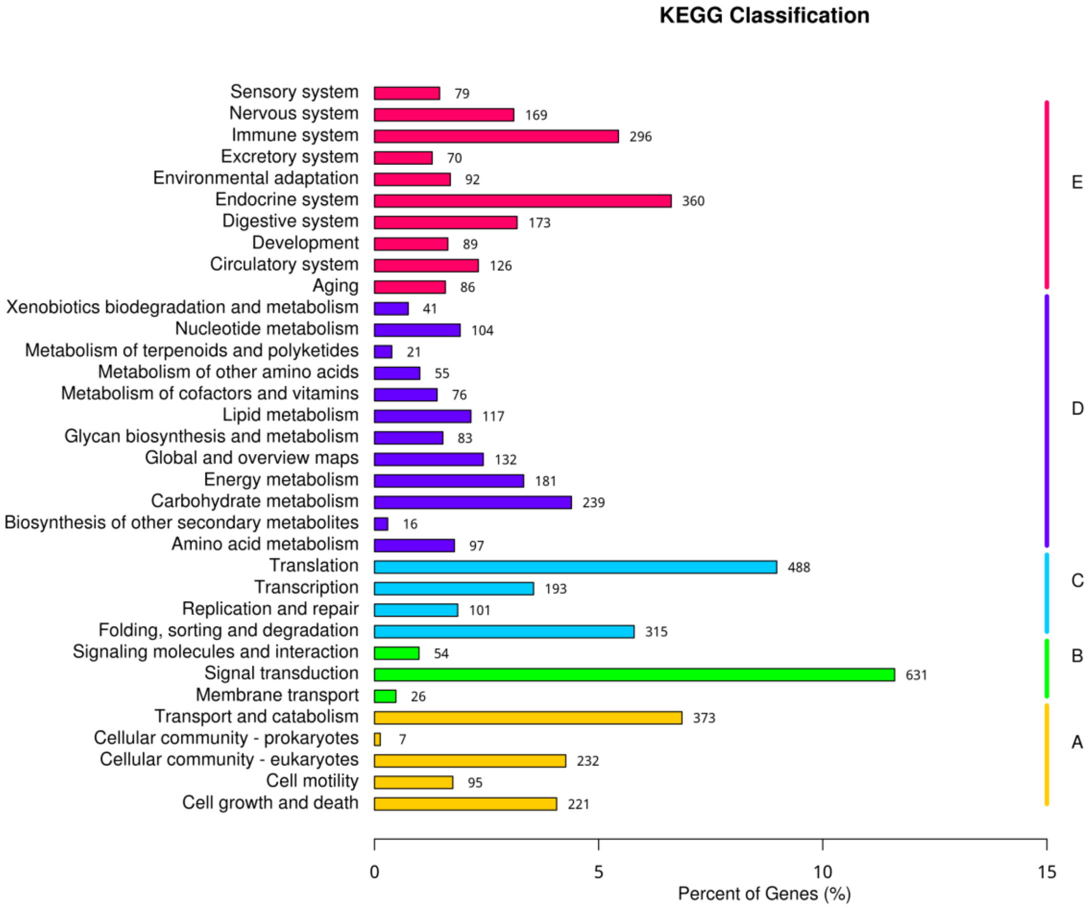


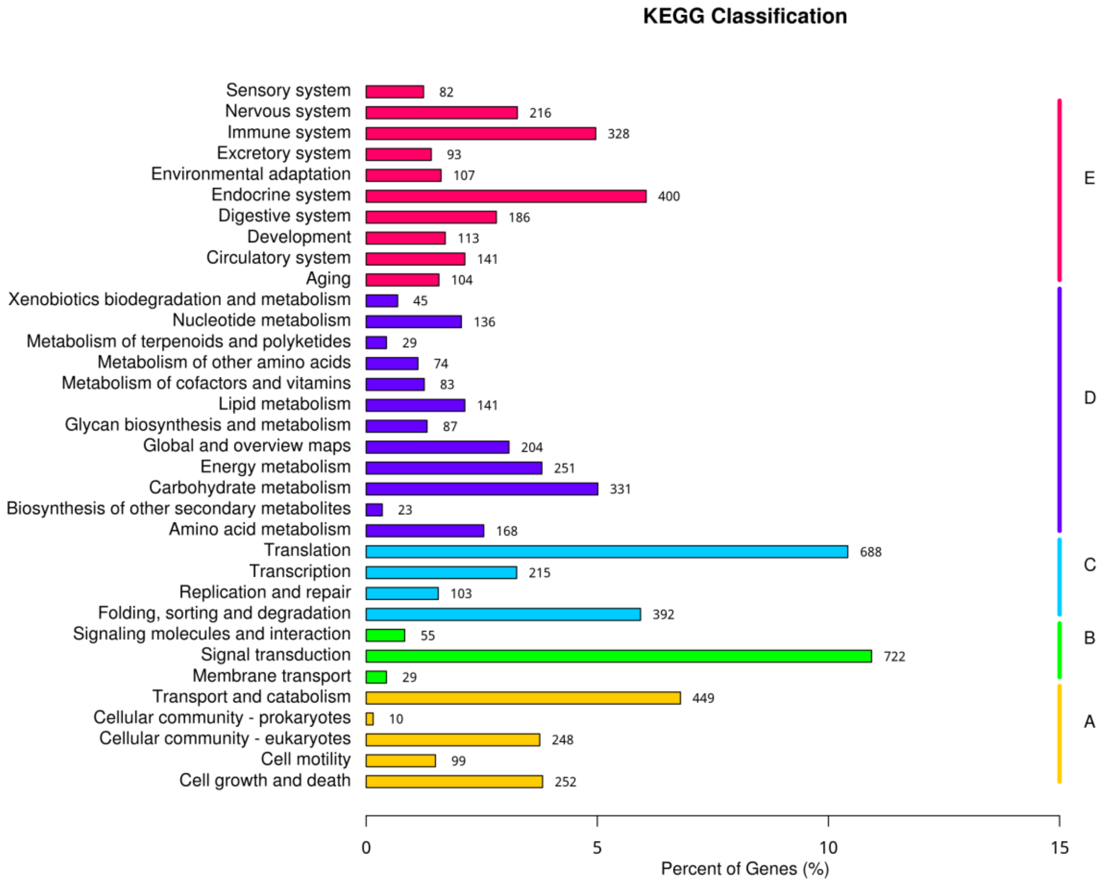


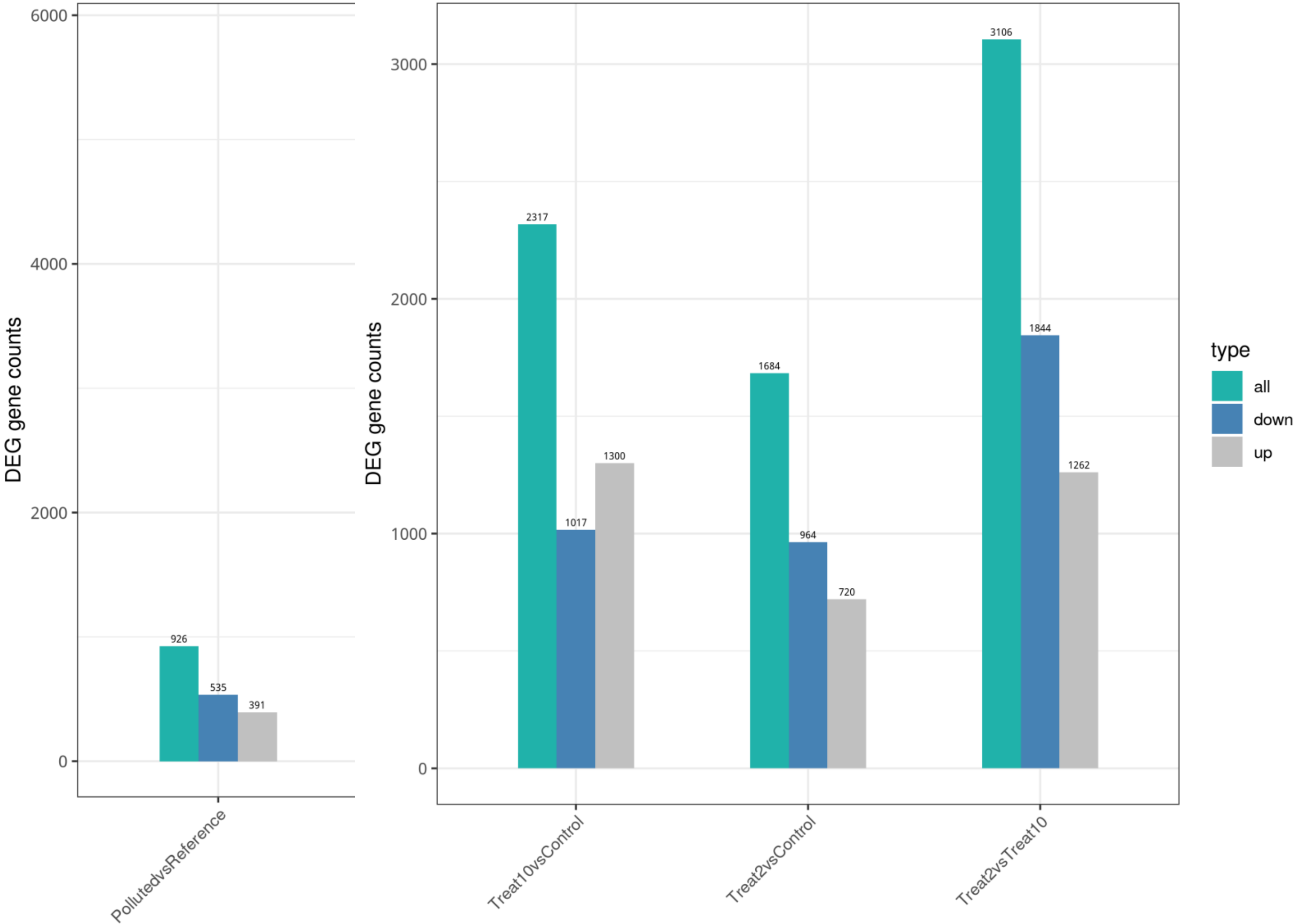
Mauer et. al., 2020

Environmental



Exposure





6000
4000
2000
0
DEG gene counts
926
535
391
PollutedvsReference
3000
2000
1000
0
DEG gene counts
2317
1017
1300
1684
964
720
3106
1844
1262
Treat10vsControl
Treat2vsControl
Treat2vsTreat10
compare group
type
all
down
up

Environmental

Log2Fold Change	KOG description
77.508	Cytochrome oxidase subunit III and related proteins
68.795	-
66.901	Cytochrome c oxidase subunit II and related proteins
62.484	Cytochrome c oxidase subunit I
60.446	Cytochrome b
56.967	ATP Synthase F0 subunit 6 and related proteins
50.379	NADH dehydrogenase, subunit 4
48.626	NADH dehydrogenase subunits 2,5, and related proteins

Exposure, 2 mg Cd^{2+}

Log2Fold Change	KOG description
97.944	-
59.306	-
55.397	-
55.019	-
54.699	Ctr copper transport family
54.507	Gammaherpesvirus membrane protein
54.399	-
53.412	-

Exposure 10 mg^{2+}

Log2Fold Change	KOG description
99.332	Barrier to autointegration factor
97.071	-
94.507	-
89.716	-
89.406	-
84.199	-
79.333	Phosphatase activity
71.026	Pneumovirus glycoprotein

Lower level of exposure exposure

PF17068//PF1211	Required for respiratory growth protein 8 mitochondrial//Protein of unknown function (DUF3586)
PF03776	Septum formation topological specificity factor MinE
PF12678//PF0007	RING-H2 zinc finger domain//Zinc finger, C3HC4 type (RING finger)
PF15320	mRNA cap methylation, RNMT-activating mini protein

PF09271	LAG1, DNA binding
PF01478	Type IV leader peptidase family
PF01222	Ergosterol biosynthesis ERG4/ERG24 family
PF09478//PF0050/	Carbohydrate binding domain CBM49//Ion transport protein//Malonate transporter MadL subunit//ARID/BRIGHT DNA binding domain
PF03219	TLC ATP/ADP transporter

Higher level of exposure

PF06888	Putative Phosphatase
PF00802	Pneumovirus attachment glycoprotein G
PF00648	Calpain family cysteine protease
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PF12678//PF04147//PF13841	RING-H2 zinc finger domain//Nop14-like family//Beta defensin
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PF07714//PF00069	Protein tyrosine and serine/threonine kinase//Protein kinase domain
PF04145//PF08918	Ctr copper transporter family//PhoQ Sensor
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PF03727//PF00349	Hexokinase//Hexokinase
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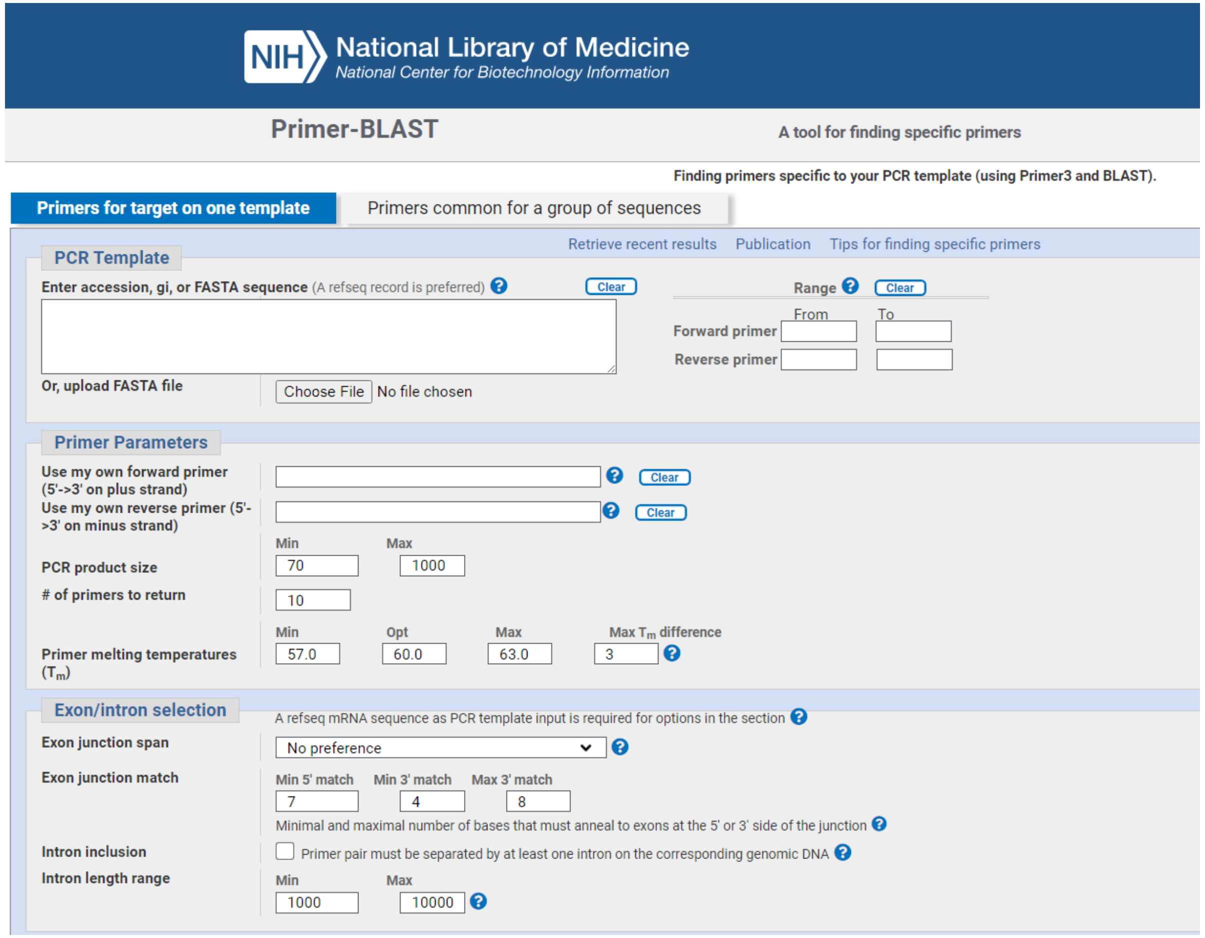
--	--
PF00349//PF03727	Hexokinase//Hexokinase

Quantitative RT-PCR, RNA extraction

Locality	Sample	RNA concentration(ng/µL)	OD 260/280	OD 260/230
KRS	AC 2 P2	274,97	2,20	2,40
KRS	AC 3 P2	220,95	2,21	2,44
KRK	AC 3 P1	125,71	2,11	2,24
KRK	AC 4 P1	303,05	2,16	2,39
KRK	AC 1 P1	123,63	2,11	2,14
KRK	AC 5 P1	176,17	2,14	2,24
KRS	AC 6 P2	138,34	2,11	2,25
KRK	AC 7 P1	183,48	2,10	2,15
KRK	AC 8 P1	121,68	2,14	2,1
KRS	AC 1 P2	95,99	2,11	2,25
KRS	AC 4 P2	190,42	2,09	2,04
KRS	AC 5 P2	111,44	2,08	2,02
KRS	AC 7 P2	185,31	2,12	2,34
KRS	AC 8 P2	129,12	2,11	2,32

Quantitative RT-PCR, primer synthesis

Primer 3 software (NCBI)



PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred)

Range: From / To

Forward primer

Reverse primer

Or, upload FASTA file: No file chosen

Primer Parameters

Use my own forward primer (5'->3' on plus strand)

Use my own reverse primer (5'->3' on minus strand)

PCR product size: Min 70, Max 1000

# of primers to return: 10

Primer melting temperatures (T_m): Min 57.0, Opt 60.0, Max 63.0, Max T_m difference 3

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span: No preference

Exon junction match: Min 5' match 7, Min 3' match 4, Max 3' match 8

Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction

Intron inclusion: ☐ Primer pair must be separated by at least one intron on the corresponding genomic DNA

Intron length range: Min 1000, Max 10000

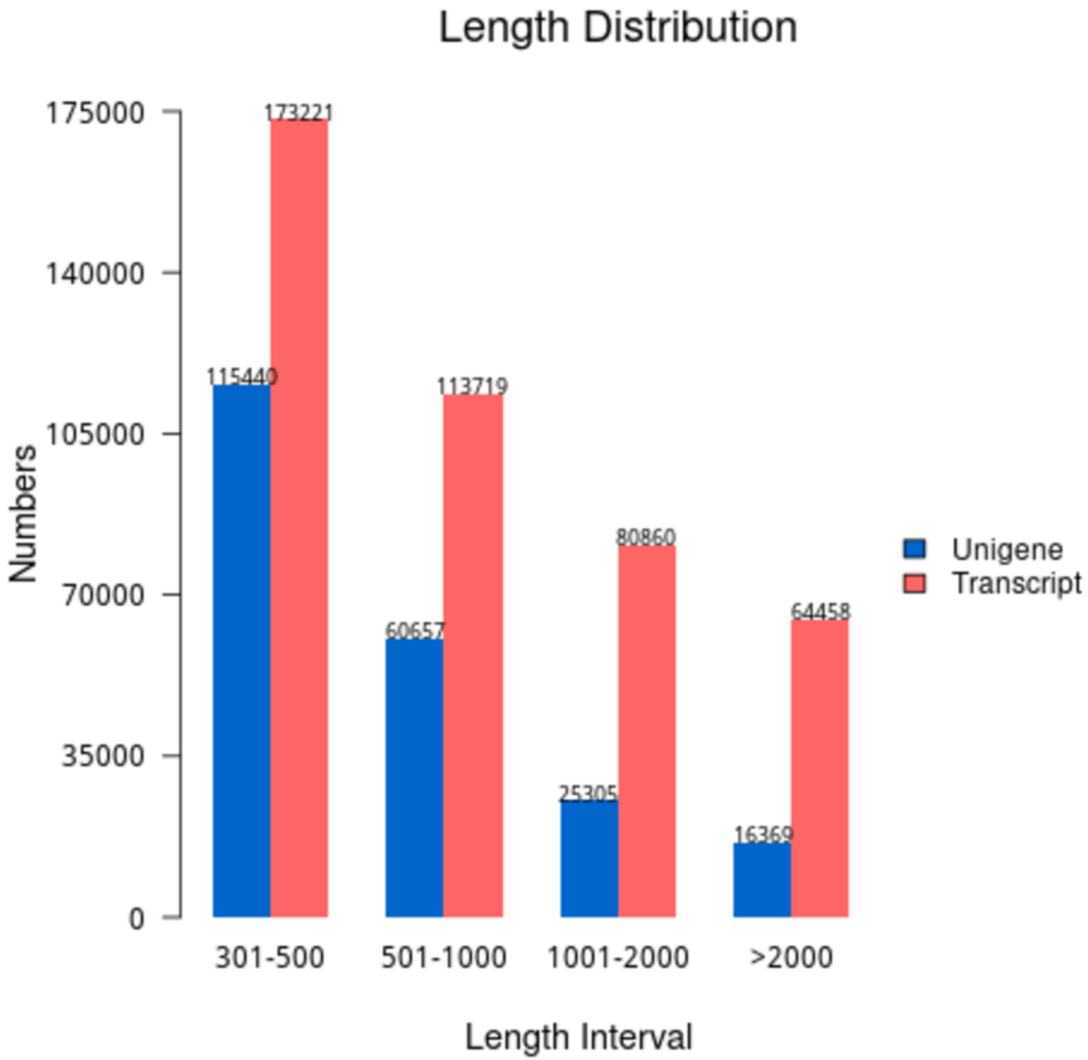
Reference Genes

Type	Sequence name	Sequence
DNA	MD_f1	GAGGGCACTTGGGTGTCCAT
DNA	MD_r1	GCTCCTCTGCTGTCAAGTCCA
DNA	MD_f7	ACTGTTCAACAACGTGGTGC
DNA	MD_r7	GGGGTTCCAAGGACCCAATCA
DNA	MD_f8	GCGGATGATGCATGGTTGAAGG
DNA	MD_r8	TGGGGTTCCAAGGACCCAATC
DNA	MD_f9	CCCTGTCACTTGTCGCAATGG
DNA	MD_r9	TGAGGCATGAGTAAGCCAAGGT
DNA	MD_f10	TGGAACCCCAGAGGGCACTT
DNA	MD_r10	TCTTCCACTCTCCATTGCGACA
DNA	Actin1_f1	GCAGTGTCAAGTCGCCGATG
DNA	Actin1_r1	GACAGGCGTAGCTTCGAGGT
DNA	Actin1_f2	AGATCATGACCGAGCGTGGG
DNA	Actin1_r2	AAGCGCTCGTTACCGACTGT
DNA	Actin1_f3	CCTGTATGCTTCGGGCAGGA
DNA	Actin1_r3	TTCACGCTCAGCCGTCGTAA
DNA	Actin1_f8	GCCGTTATGGTTGGCATGGG
DNA	Actin1_r8	AGGATCCCACGCTTGCTCTG
DNA	Actin1_f10	TACGACGGCTGAGCGTGAAA
DNA	Actin1_r10	TAGAGCCTCTGGACACCGGA
DNA	EIF4E_f1	CCTGAGGTGCAGGCTCCTTT
DNA	EIF4E_r1	AGAGCACGAGCATCAAGCCA
DNA	EIF4E_f4	CTGTTCGCCAACCAACGCAA
DNA	EIF4E_r4	AACGGGTTGAGTGTCGGGTC
DNA	EIF4E_f6	CCGACACTCAACCCGTTTGG
DNA	EIF4E_r6	ACGCGGACAGCGGTTCTAAA
DNA	EIF4E_f7	TCAGACCCGACACTCAACCC
DNA	EIF4E_r7	AACGCGGACAGCGGTTCTAA
DNA	EIF4E_f10	CCGCAAGTTCTGCCTTCTGTG
DNA	EIF4E_r10	TTGCGTTGGTTGGCGAACAG
DNA	TUBA_1f	GGCAATGATGCCCTGTTCGG
DNA	TUBA_1r	ACCAGCGGGAAGTGGATACG
DNA	TUBA_2f	TATCAACGGTCGTCCGCCAA
DNA	TUBA_2r	CTAGCATGCACACGCTTCGG
DNA	TUBA_6f	TCTACACCCCGAAGCGTGTG
DNA	TUBA_6r	ACCCTCTCGGGTGTACCAGT
DNA	TUBA_8f	CCGGGGTACGAACTCAGCTT
DNA	TUBA_8r	TTGGCCCACAGCCGATAACT
DNA	TUBA_10f	TGACTTGGTACCACTCGGCG
DNA	TUBA_10r	GCAACCGGCTTGACCAATGT
DNA	L32_f1	AAGGCCGAGATCGCTCACTC
DNA	L32_r1	CCTCCATTCTTAGGCGGGCA
DNA	L32_f4	TCAAGAGGGTTGGTCCGAGC
DNA	L32_r4	CCGATGTTTGGCATCGGTCG
DNA	L32_f5	GCGATTCAGAGGTTGCCGAC
DNA	L32_r5	AGTGAGCGATCTCGGCCTTC
DNA	L32_f8	ATCGCTCACTCCGTATCGGC
DNA	L32_r8	TCCTCCATTCTTAGGCGGGC
DNA	L32_f7	ACCGGGGAGGTTCACAACTC
DNA	L32_r7	CCGGCGGTTGTTCTTCAAGG

Overexpressed Genes

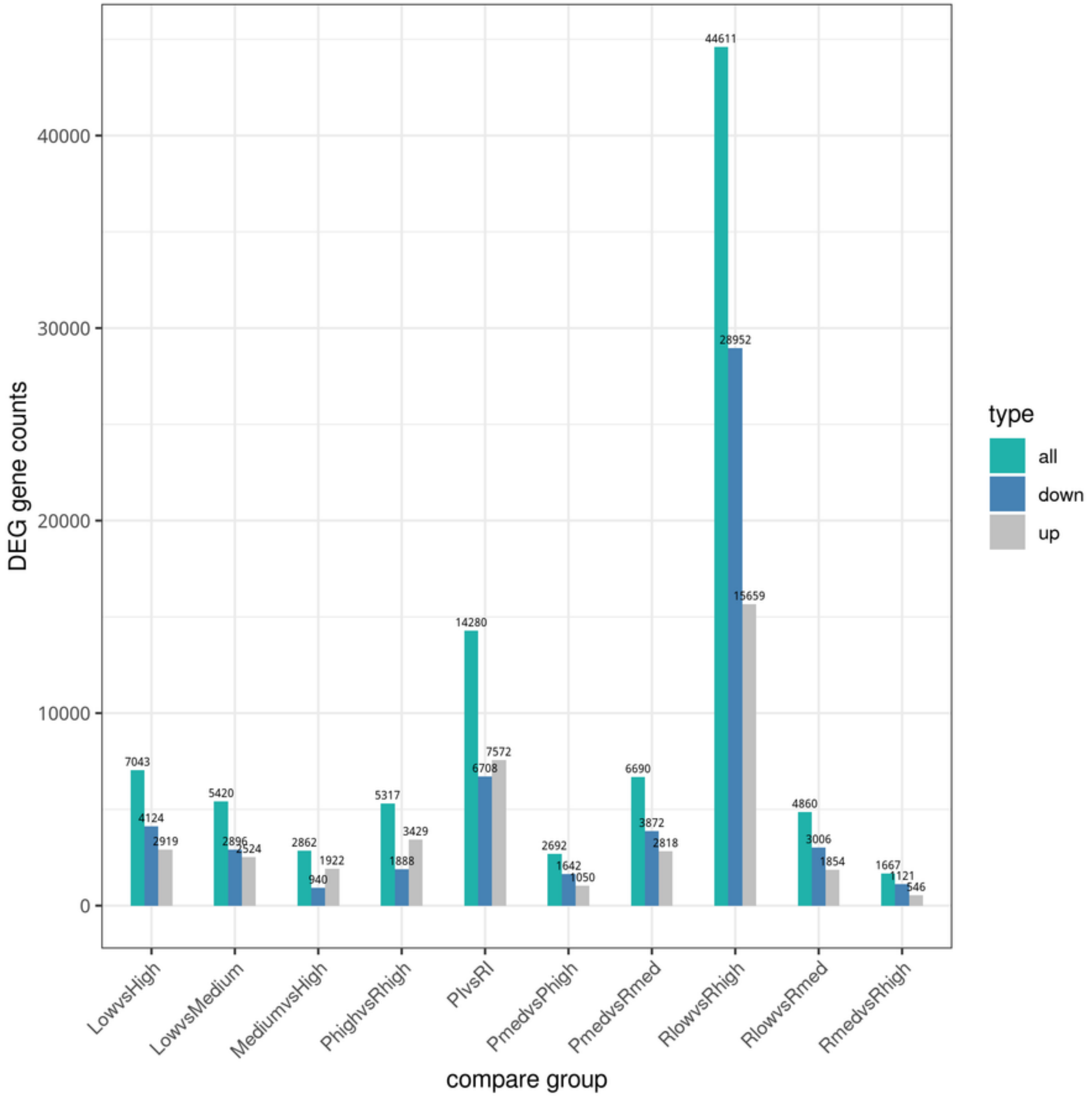
Type	Sequence name	Sequence
DNA	cox1_f1	AGATAGCTGCCGTGAGGGTG
DNA	cox1_r1	GCCCGTGCTCCTTTCTCTCT
DNA	cox1_f5	GGCACCTTGGTTTCACTCGT
DNA	cox1_r5	ATCACCCTCACGGCAGCTAT
DNA	cox1_f7	GGAGCCGTAAACTGAGTCTGCT
DNA	cox1_r7	CATGAGCCCATCACGCAACC
DNA	cox1_f10	AGCGGCGGCAGCTAAAATTG
DNA	cox1_r10	ATCCGGCTCTCTATAGCCCA
DNA	cox2_f1	AGTTGGTTTAGGCGGCCTGG
DNA	cox2_r1	CGTTTGGTCACAACAGGGGC
DNA	cox2_f3	ACCATTGATGACCCACGGCT
DNA	cox2_r3	ACTCCCTCACCGACCGATTT
DNA	cox2_f4	TCTGCCCCTGTTGTGACCAAA
DNA	cox2_r4	AGCCGTGGGTCATCAATGGT
DNA	cox2_f7	AAATCGGTCGGTGAGGGAGT
DNA	cox2_r7	CCAAGACAGAGCTTCCCCCT
DNA	cox1_f1	GGGCCCACACGATAAAGCCT
DNA	cox1_r1	AGGGGGTGGAGACCCAATCT
DNA	cox1_f2	GGGGGTATGGGTGTTGTCACT
DNA	cox1_r2	AAGCGGTATACCACGTCGCT
DNA	cox1_f7	GCTAGATCTACAGCGCCTCCT
DNA	cox1_r7	GGGTAGGCACAGGCTGAACT
DNA	cox1_f10	ACAAAAGCATGGGCTGTCACT
DNA	cox1_r10	GTGGTCGGCACCTCCCTTAG
DNA	ND4_f1	AAGGCTCACGTAGAAGCCCC
DNA	ND4_r1	TCTAGCTGCCGCAAACACCT
DNA	ND4_3f	GGGCGTACAGGCCTATGCTA
DNA	ND4_r3	ATCTAGCTGCCGCAAACACC
DNA	ND4_f7	GGGCCGCGTACAGGCTTTAT
DNA	ND4_r7	CCTACACACAGTGCTACCGC
DNA	ND4_f9	CCGTTTTGGGCGTACAGGC
DNA	ND4_r9	TGCTACGCCTCCTCACAAACT
DNA	ND5_3f	AGGGTGTCAAGATAGGCGGG
DNA	ND5_r3	GCATGCTCTAACACTGAGTCCT
DNA	ND5_f5	TTACCCAGAAGACTAGGGGCAT
DNA	ND5_r5	CCAACTACGCCCTCCTTGCC
DNA	ND5_f7	GGGTGTCAAGATAGGCGGGTT
DNA	ND5_r7	AGCATGCTCTAACACTGAGTCC
DNA	ND5_f9	AAGGGTGTCAAGATAGGCGGG
DNA	ND5_r9	AGCATGCTCTAACACTGAGTCCTT
DNA	16S_f2	CCAAGAGTTGCTAGGGGCGA
DNA	16S_r2	CACTGACCCCACGCCAGTAA
DNA	16S_f3	GGTCGCAACCCTGTCGTTTG
DNA	16S_r3	ACCCGGTACGCCGATCAAAA
DNA	16S_f4	TTGATACAGCCCCCACCACG
DNA	16S_r4	TCGCCCCTAGCAACTCTTGG
DNA	16S_f5	TTTTGGTAAACAGGCGGGCG
DNA	16S_r5	GGAGCAGCCATCCTGAGTGT
DNA	16S_f9	TGGGCGCGGATAGAAACCAA
DNA	16S_r9	GACAGGGTTGCGACCTCGAT

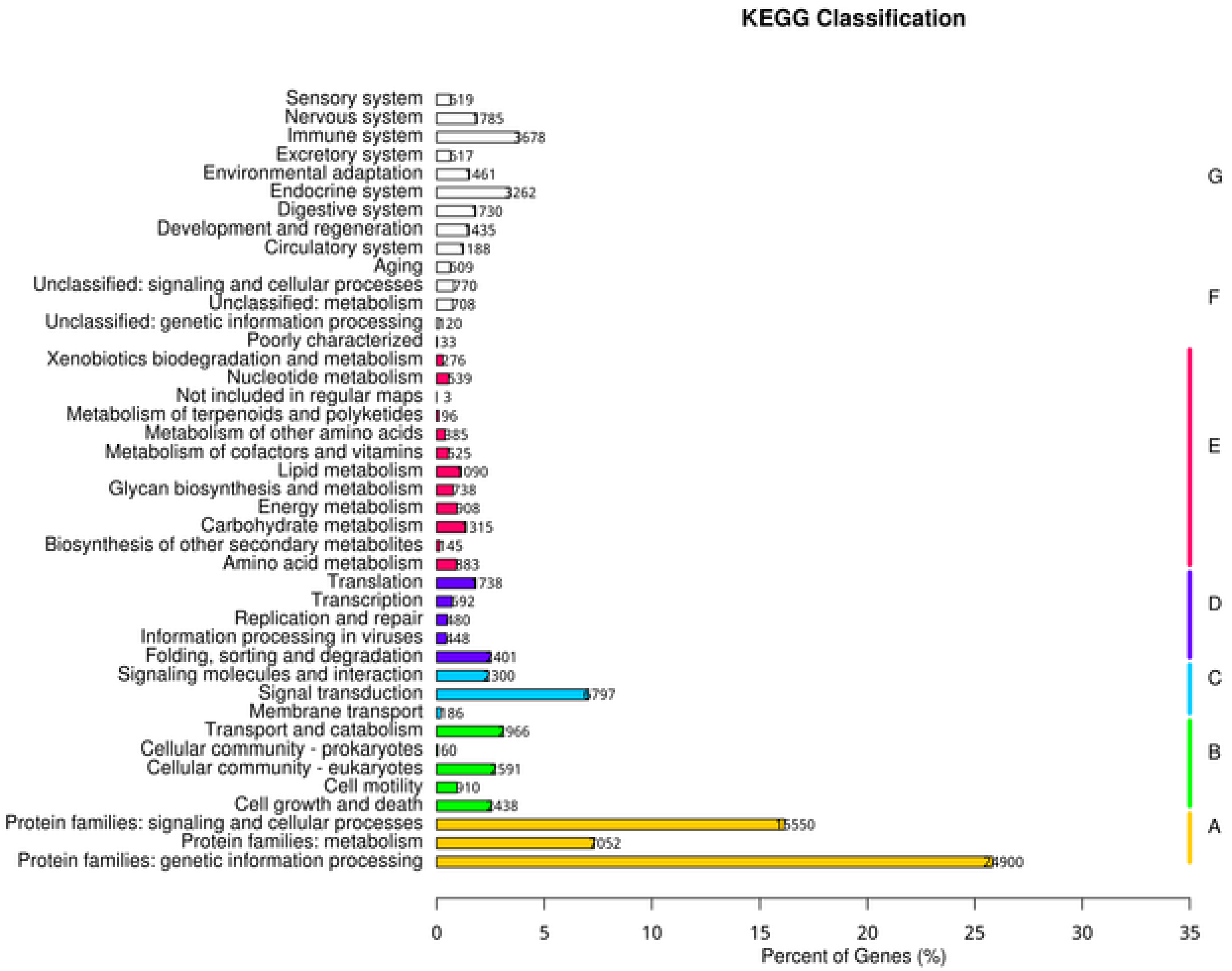
Transcriptome of fish intestine

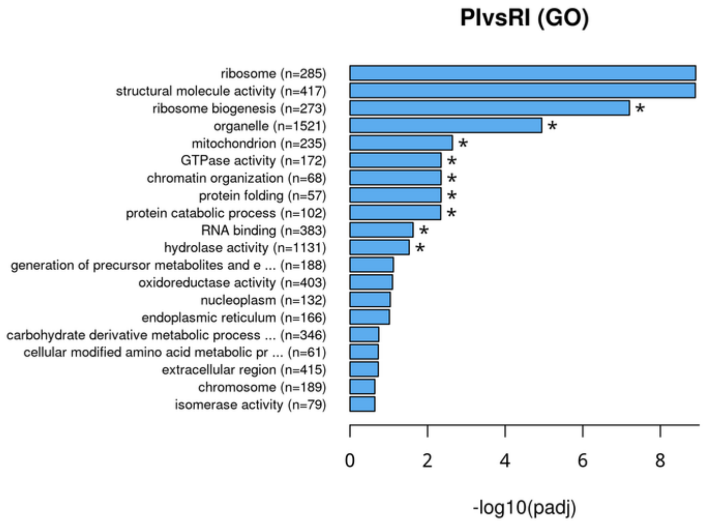


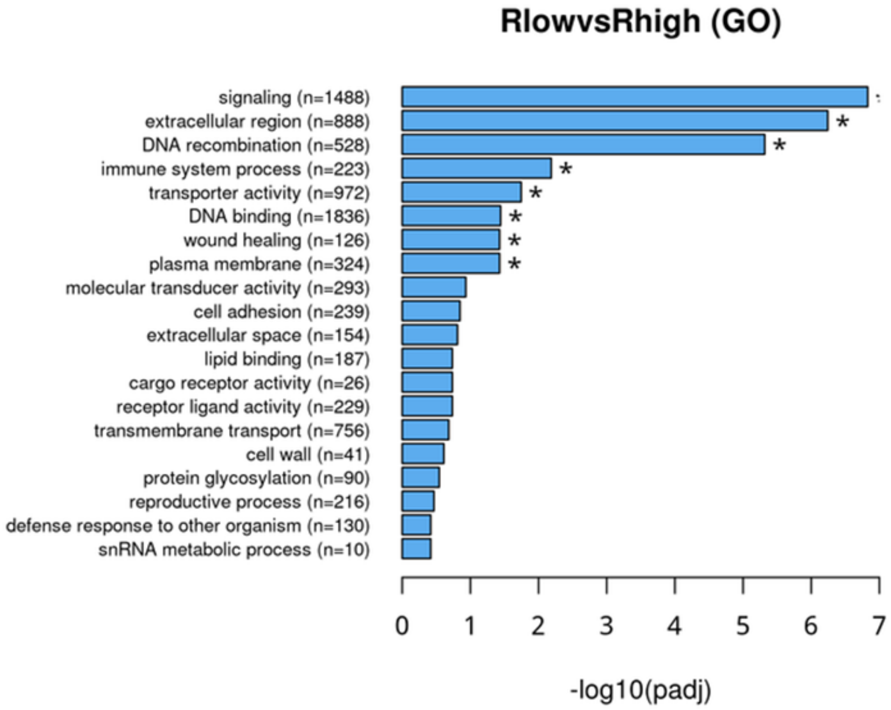
Transcriptome of fish intestine

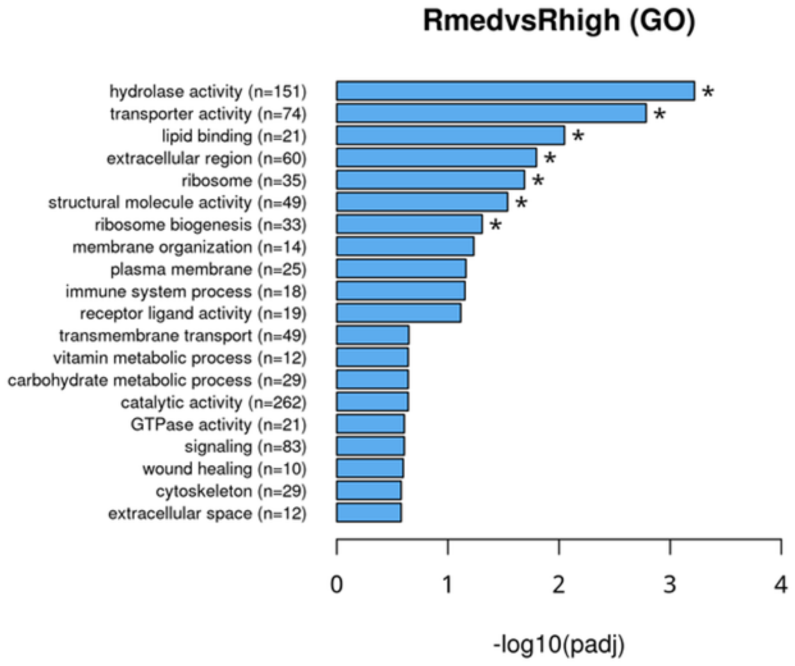
R- reference
P- polluted
Low – low number of parasites
Medium – medium number of parasites
High- high number of parasites

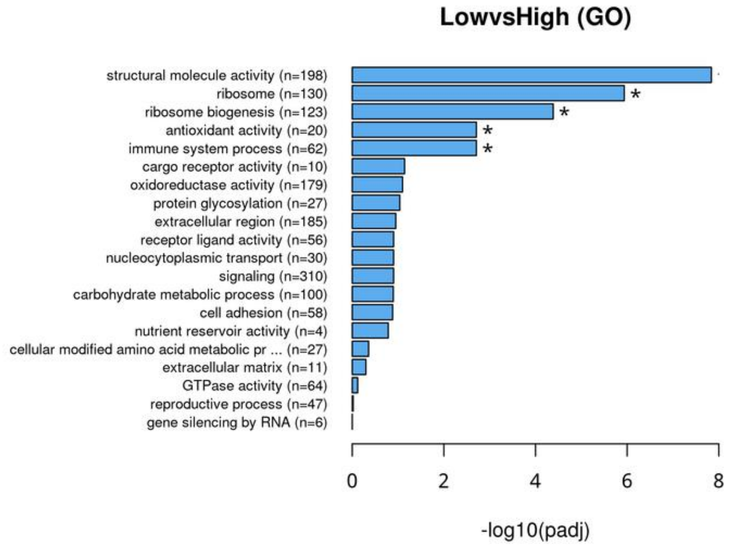


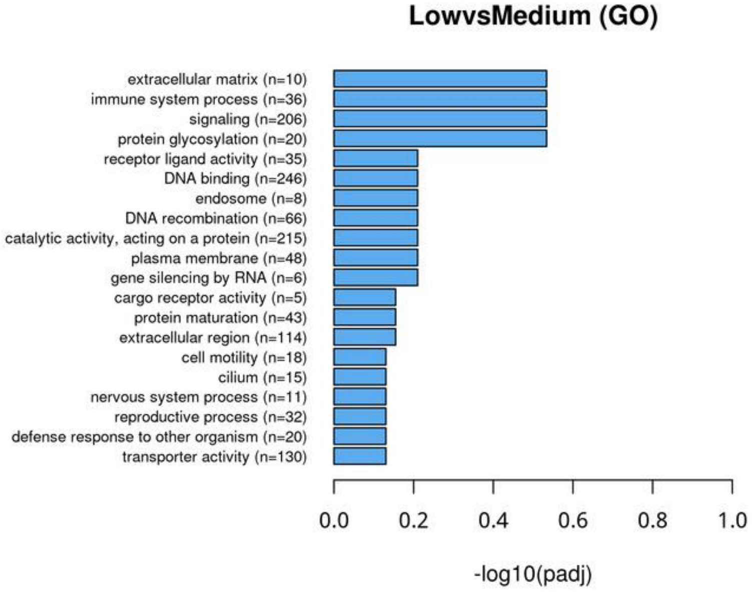

KEGG Classification
Sensory system
519
Nervous system
1785
Immune system
3678
Excretory system
517
Environmental adaptation
1461
Endocrine system
3262
Digestive system
1730
Development and regeneration
1435
Circulatory system
1188
Aging
509
Unclassified: signaling and cellular processes
770
Unclassified: metabolism
708
Unclassified: genetic information processing
120
Poorly characterized
33
Xenobiotics biodegradation and metabolism
276
Nucleotide metabolism
539
Not included in regular maps
3
Metabolism of terpenoids and polyketides
96
Metabolism of other amino acids
385
Metabolism of cofactors and vitamins
525
Lipid metabolism
1090
Glycan biosynthesis and metabolism
738
Energy metabolism
908
Carbohydrate metabolism
1315
Biosynthesis of other secondary metabolites
145
Amino acid metabolism
883
Translation
1738
Transcription
692
Replication and repair
480
Information processing in viruses
448
Folding, sorting and degradation
2401
Signaling molecules and interaction
2300
Signal transduction
6797
Membrane transport
186
Transport and catabolism
2966
Cellular community - prokaryotes
60
Cellular community - eukaryotes
2591
Cell motility
910
Cell growth and death
2438
Protein families: signaling and cellular processes
15550
Protein families: metabolism
7052
Protein families: genetic information processing
24900
G
F
E
D
C
B
A
0
5
10
15
20
25
30
35
Percent of Genes (%)

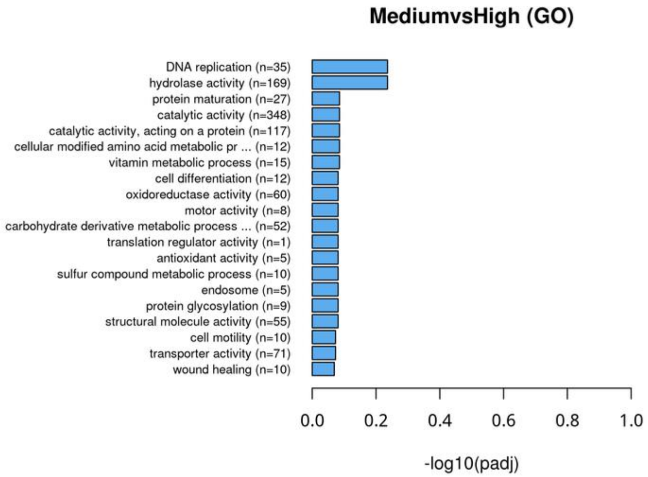

PlvsRl (GO)
ribosome (n=285)
structural molecule activity (n=417)
ribosome biogenesis (n=273) *
organelle (n=1521) *
mitochondrion (n=235) *
GTPase activity (n=172) *
chromatin organization (n=68) *
protein folding (n=57) *
protein catabolic process (n=102) *
RNA binding (n=383) *
hydrolase activity (n=1131) *
generation of precursor metabolites and e ... (n=188)
oxidoreductase activity (n=403)
nucleoplasm (n=132)
endoplasmic reticulum (n=166)
carbohydrate derivative metabolic process ... (n=346)
cellular modified amino acid metabolic pr ... (n=61)
extracellular region (n=415)
chromosome (n=189)
isomerase activity (n=79)
0
2
4
6
8
-log10(padj)


RlowvsRhigh (GO)
signaling (n=1488)
extracellular region (n=888) *
DNA recombination (n=528) *
immune system process (n=223) *
transporter activity (n=972) *
DNA binding (n=1836) *
wound healing (n=126) *
plasma membrane (n=324) *
molecular transducer activity (n=293)
cell adhesion (n=239)
extracellular space (n=154)
lipid binding (n=187)
cargo receptor activity (n=26)
receptor ligand activity (n=229)
transmembrane transport (n=756)
cell wall (n=41)
protein glycosylation (n=90)
reproductive process (n=216)
defense response to other organism (n=130)
snRNA metabolic process (n=10)
0
1
2
3
4
5
6
7
-log10(padj)


RmedvsRhigh (GO)
hydrolase activity (n=151) *
transporter activity (n=74) *
lipid binding (n=21) *
extracellular region (n=60) *
ribosome (n=35) *
structural molecule activity (n=49) *
ribosome biogenesis (n=33) *
membrane organization (n=14)
plasma membrane (n=25)
immune system process (n=18)
receptor ligand activity (n=19)
transmembrane transport (n=49)
vitamin metabolic process (n=12)
carbohydrate metabolic process (n=29)
catalytic activity (n=262)
GTPase activity (n=21)
signaling (n=83)
wound healing (n=10)
cytoskeleton (n=29)
extracellular space (n=12)
0
1
2
3
4
-log10(padj)


LowvsHigh (GO)
structural molecule activity (n=198)
ribosome (n=130)
ribosome biogenesis (n=123)
antioxidant activity (n=20)
immune system process (n=62)
cargo receptor activity (n=10)
oxidoreductase activity (n=179)
protein glycosylation (n=27)
extracellular region (n=185)
receptor ligand activity (n=56)
nucleocytoplasmic transport (n=30)
signaling (n=310)
carbohydrate metabolic process (n=100)
cell adhesion (n=58)
nutrient reservoir activity (n=4)
cellular modified amino acid metabolic pr ... (n=27)
extracellular matrix (n=11)
GTPase activity (n=64)
reproductive process (n=47)
gene silencing by RNA (n=6)
*
*
*
*
0
2
4
6
8
-log10(padj)


LowvsMedium (GO)
extracellular matrix (n=10)
immune system process (n=36)
signaling (n=206)
protein glycosylation (n=20)
receptor ligand activity (n=35)
DNA binding (n=246)
endosome (n=8)
DNA recombination (n=66)
catalytic activity, acting on a protein (n=215)
plasma membrane (n=48)
gene silencing by RNA (n=6)
cargo receptor activity (n=5)
protein maturation (n=43)
extracellular region (n=114)
cell motility (n=18)
cilium (n=15)
nervous system process (n=11)
reproductive process (n=32)
defense response to other organism (n=20)
transporter activity (n=130)
0.0
0.2
0.4
0.6
0.8
1.0
-log10(padj)


MediumvsHigh (GO)
DNA replication (n=35)
hydrolase activity (n=169)
protein maturation (n=27)
catalytic activity (n=348)
catalytic activity, acting on a protein (n=117)
cellular modified amino acid metabolic pr ... (n=12)
vitamin metabolic process (n=15)
cell differentiation (n=12)
oxidoreductase activity (n=60)
motor activity (n=8)
carbohydrate derivative metabolic process ... (n=52)
translation regulator activity (n=1)
antioxidant activity (n=5)
sulfur compound metabolic process (n=10)
endosome (n=5)
protein glycosylation (n=9)
structural molecule activity (n=55)
cell motility (n=10)
transporter activity (n=71)
wound healing (n=10)
0.0
0.2
0.4
0.6
0.8
1.0
-log10(padj)

Thank you for your attention!