

1 **Supporting Information.** Pages S1 to S21. Supplementary Text. Supplementary Tables: Tables
2 S1 to S10. Supplementary Figures: Figures S1 to S8.

3 *Transcriptional changes in the ovaries of perch from*
4 *Chernobyl*

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21 **Supplementary Text**

22 **Methods**

23 **Assembly and Annotation of the European Perch.** The parameters used in Trim Galore were:
24 “--illumina -q 20 --stringency 5 -e 0.1 --length 20 --trim-n”. The parameters used in Trinity were:
25 “--seqType fq --max_memory 100G --CPU 24 --min_contig_length 200 --min_kmer_cov 1 --
26 SS_lib_type RF --verbose --full_cleanup”.

27

28 **Results**

29 **General health condition.** The whole-body weight and the total length were not statistically
30 significantly different between fish from different gonad phenotypes ($p = 0.468$ and 0.159
31 respectively) (Table S1). The Fulton condition index was significantly lower in fish with
32 developed/irradiated and underdeveloped gonads from the Cooling Pond (mean = 1 ± 0.7 , $p =$
33 0.005 and mean = 1 ± 0.2 , $p = 0.016$, respectively) and in fish with developed/irradiated gonads
34 from Yanovsky lake (mean = 1 ± 0.1 , $p = 0.021$) as compared to fish with developed gonads
35 (mean = 1.3 ± 0.1) (Table S1). The Fulton condition index of fish with underdeveloped gonads
36 from Yanovsky lake (mean = 1.1 ± 0.1 , $p = 0.052$) and developed/irradiated gonads from
37 Svyatoye (mean = 1.2 ± 0.1 , $p = 0.371$) were not statistically significantly different to fish with
38 developed gonads (Table S1). No difference was observed between the liver weight and the
39 hepatosomatic index of fish with different gonad phenotypes ($p = 0.372$ and $p = 0.08$
40 respectively) (Table S1).

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42 **De novo transcriptome assembly.** To assess completeness, the assembled transcriptome was
43 compared against a database of universal *vertebrata* single copy orthologs using BUSCO v2.0
44 (Simão et al, 2015). This showed that 91.1 % of the universal single copy orthologs were present

45 in a complete form in the transcriptome, the majority of which (84.1 %) were found duplicated
46 suggesting redundancy amongst the assembled transcripts. Of those that were not found in a
47 complete form, 4.8 % were present in a fragmented form, and only 4.1 % were missing entirely.
48 To assess the level of redundancy in the transcriptome, CD-HIT was used to cluster the
49 transcripts based on sequence similarity. Clustering of the transcriptome resulted in removal of
50 25,419 (4.8 %), 74,487 (13.9 %) and 99,724 (18.7%) transcripts when using sequence identity
51 thresholds of 100 %, 99 % and 98 % respectively, suggesting the presence of redundant
52 transcript sequences in the assembly. However, to avoid removing biologically distinct but
53 highly similar transcripts we proceeded with the unclustered transcripts, and instead controlled
54 for redundancy downstream. The majority (88.6 %) of the transcripts identified with an open
55 reading frame were annotated, whilst those with no open reading frame were generally not
56 annotated (Figure S3). In addition, the majority (73.8 %) of transcripts with no open reading
57 frame represented short fragments of less than 500 bases. Transcripts were filtered to remove
58 those annotated to bacterial, viral or archaeal species, those shorter than 500 bases in length,
59 those with a normalised abundance (FPKM value) lower than 1 in all samples, and those with no
60 open reading frame detected”.

61 **Functional classification of transcriptome.** In total, genes were found associated with 28, 39
62 and 53 distinct terms of the three main functional categories of “cellular component”, “molecular
63 function” and “biological process” respectively. The 60 GO classes with more than 50 genes are
64 shown in Supplementary Figure S4.

65

66 **Supplementary Tables**

67 **Supplementary Table S1:** Table showing the hydrological parameters of the lakes and the total
 68 dose rate to perch. The total dose rate is the sum of the external (^{137}Cs) and internal (^{137}Cs and
 69 ^{90}Sr) dose rate.

Lake	Maximum depth (m)	Surface (km ²)	Distance from CNPP (km)	^{137}Cs deposition (kBq/m ²) "corrected to 6/05/1986"	Total dose rate (μGy/h) to perch in 2015
Yanovsky (H)	11	0.8	2.3	18650	9.8
Cooling Pond (H)	18	22	1.5	19180	7.9
Svyatoye (M)	5.1	0.25	225	1778	1.7
Stoyacheye (R)	17	0.46	167	293	0.1
Dvoriche (R)	3.5	0.128	95	100	0.1
Gorova (R)	2	0.05	88	10	0

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 71
 72 **Supplementary Table S2. A)** Nutrient concentrations (NO_3^- , NO_2^- and PO_4^{3-} in μg/L, mean ±
 73 SD, n = 3 per lake). **B)** Physicochemical parameters (Dissolved Oxygen, DO, in %, pH,
 74 temperature, conductivity in μS/cm). **C)** Major alkali and alkali-earth element water
 75 concentrations (Na, Mg, S, K and Ca in mg/L, mean ± SD, n = 3 per lake). **D)** Trace elements
 76 concentrations (As, Sr, Cd, Cs, Pb and U in μg/L, mean ± SD, n = 3 per lake) determined by
 77 ICP-MS. All the measurements were performed in the surface water samples of the 6 lakes in
 78 September 2014 and 2015.

79 **A**

Lakes	NO_3^-	NO_2^-	PO_4^{3-}
Yanovsky (H)	76.1 ± 44.7	9.8 ± 14.7	6.0 ± 1.1
Cooling Pond (H)	57.9 ± 21.9	11.9 ± 3.9	15.0 ± 15.5
Svyatoye (M)	159.3 ± 81.8	2.0 ± 1.0	1.7 ± 0.5
Stoyacheye (R)	259.2 ± 182.7	1.5 ± 0.5	1.4 ± 0.9
Dvoriche (R)	201.0 ± 39.7	2.1 ± 0.2	1.4 ± 0.4
Gorova (R)	79.6 ± 68.7	2.5 ± 0.1	5.6 ± 1.0

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81 **B**

Lake	DO (%)	pH	T (°C)	Conductivity (μS/cm)
Yanovsky (H)	108	8	17.7	277
Cooling Pond (H)	48	8.6	15.6	318
Svyatoye (M)	110	6.3	18.9	120
Stoyacheye (R)	125	7.1	19.3	226
Dvoriche (R)	95	6.3	20.1	186
Gorova (R)	55	8.3	17.2	255

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85 **C**

Lake	Na	Mg	S	K	Ca
Yanovsky (H)	16.1 ± 1.3	4.9 ± 0.2	13.8 ± 1.8	4.8 ± 0.7	25.4 ± 1.4
Cooling Pond (H)	11.1 ± 1.4	7.9 ± 0.2	8.1 ± 1.1	3.2 ± 0.6	37.0 ± 1.3
Svyatoye (M)	2.02 ± 0.2	2.7 ± 0.1	0.3 ± 0.1	4.0 ± 0.2	18.0 ± 0.3
Stoyacheye (R)	2.7 ± 0.1	5.6 ± 0.3	0.8 ± 0.2	6.0 ± 0.3	33.2 ± 1.7
Dvoriche (R)	3.6 ± 0.1	5.7 ± 0.1	1.0 ± 0.1	2.4 ± 0.03	28.2 ± 0.5
Gorova (R)	6.2 ± 1.3	7.0 ± 0.3	2.4 ± 1.2	3.6 ± 0.7	37.8 ± 6.4

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87 **D**

Lake	As	Sr	Cd	Cs	Pb	U
Yanovsky (H)	0.7 ± 0.2	117.5 ± 6.8	0.008 ± 0.009	0.005 ± 0.0007	0.02 ± 0.03	0.2 ± 0.1
Cooling P. (H)	0.8 ± 0.1	188.4 ± 14.2	0.002 ± 0.001	0.004 ± 0.0006	0.1 ± 0.3	0.6 ± 0.06
Svyatoye (M)	0.3 ± 0.001	64.4 ± 1.1	nd	0.01 ± 0.0009	nd	0.0006 ± 0.00005

Stoyacheye (R)	0.7 ± 0.05	61.5 ± 2.9	0.001 ± 0.001	0.006 ± 0.0002	nd	0.03 ± 0.003
Dvoriche (R)	0.9 ± 0.05	71.4 ± 1.8	0.0004 ± 0.002	0.02 ± 0.0007	0.01 ± 0.028	0.003 ± 0.0006
Gorova (R)	0.9 ± 0.2	159.8 ± 5.7	0.002 ± 0.001	0.002 ± 0.0007	0.006 ± 0.014	0.2 ± 0.03

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89 **Supplementary Table S3:** Table showing the composition of the 6 different pools used for next
90 generation sequencing analyses. Sampling sites show either reference (R), medium (M) or high
91 (H) levels of radiation.

Pool Id.	Lakes	Gonad Phenotype	Number of fish
1	Yanovsky (H)	Developed/irradiated	3
2		Undeveloped	3
3	Cooling Pond (H)	Developed/irradiated	3
4		Undeveloped	3
5	Svyatoye (M)	Developed/irradiated	3
6	Gorova (R)		3
	Dvoriche (R)	Developed	3
	Stoyacheye (R)		3

92 **Supplementary Table S4:** List of the target genes and the associated primers and probe used to
93 perform the qPCR validation.

Gene name	Primers	Probe
<i>skal</i>	F: TCCAGCACATTAAGGACAACGT R: GCTTCCATTTGCTGGCTCTT	CCTGCACACATGCCCCGGAA
<i>cp2g1</i>	F: GGTGGACCAGGCTGATGACT R: ACCGTAGCCCTTGGTAACTTTG	CACAGGCAGAGCGCCAATGCC
<i>hus1</i>	F: TCTCTCATGGACGATATTGCACAT R: GGCCAGGGTGGACATCAG	ACCTGCTGACCCATGAGGAACTGCTG
<i>gthb1</i>	F: ACCGAATGTAACCCCGAAAAC R: TTAAAATGAGACACAGCTGGATAACG	CGTACTGCGGGCACTTTCACGGAC
<i>star</i>	F: CGTCAATGTTAAGAAGCCATGTG R: ACCAACCTGCATCATCATTACG	AGCGGCTTTTTCTGTATCCTCGTCCA
<i>actb</i>	F: CGTGACCTCACAGACTACCTCATG R: CCTTGATGTCACGCACGATT	ATCCTGACAGAGCGTGGTTACTCATTACCC

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95 **Supplementary Table S5:** Number of reads from *Perca fluviatilis* sequencing. Counts are given
 96 for the raw data, the quality trimmed data and the data mapped back to the de novo assembled
 97 transcriptome. For each library, the percent of G and C nucleotides, and the percentage of bases
 98 with a quality score greater than 20 (Q20) or 30 (Q30) are shown.

Phenotype (Exposure Level)	Total Raw PE Reads	%GC	%Q20	%Q30	Total Trimmed PE Reads	Total Mapped Reads	Percent Mapped Reads
Developed/irradiated (High Level 1)	24,529,588	48	97.70%	95.60%	24,499,661	19,390,487	79.00%
Undeveloped (High Level 1)	24,350,401	49	97.80%	95.90%	24,320,952	20,127,478	82.70%
Developed/irradiated (High Level 2)	20,898,790	47	98.10%	96.50%	20,872,080	15,395,334	73.70%
Undeveloped (High Level 2)	29,294,698	49	97.80%	96.00%	29,257,591	24,930,050	85.10%
Developed/irradiated (Medium Level)	28,909,427	48	98.00%	96.30%	28,873,161	22,765,363	78.70%
Developed	23,802,568	47	97.80%	96.00%	23,766,562	18,650,271	78.40%

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101 **Supplementary Table S6:** Transcriptome assembly statistics from Trinity.

General Assembly Statistics	
Number of assembled transcripts	534,099
Number of assembled “genes”	309,937
Assembly GC content	46.31 %
Total number of raw PE reads	151,785,472
Total number of filtered PE reads	151,590,007
Total number of mapped PE reads	121,258,983
Transcript Statistics	
Median Contig Length	374 bp
Mean Contig Length	871.54 bp
Contig N10	5,937 bp
Contig N20	4,261 bp

Contig N30	3,238 bp
Contig N40	2,483 bp
Contig N50	1,879 bp
Gene Statistics	
Median Contig Length	321 bp
Mean Contig Length	607.55 bp
Contig N10	5,038 bp
Contig N20	3,334 bp
Contig N30	2,283 bp
Contig N40	1,486 bp
Contig N50	909 bp

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103 **Supplementary Table S7:** A) Differential expression of some genes between the
 104 Undeveloped/Irradiated, Developed/Irradiated and Developed/Reference phenotypes assessed by
 105 the NGS and qPCR techniques. ↑: up-regulated; ↓: down-regulated; =: non significant ($-1 <$
 106 $\log_2(\text{FC}) < 1$). Results show \log_2 Fold Change ($\log_2(\text{FC})$).

	Undeveloped/Irradiated compared to Developed/Reference		Undeveloped/Irradiated compared to Developed/Irradiated		Developed/Irradiated compared to Developed/Reference	
	NGS	qPCR	NGS	qPCR	NGS	qPCR
<i>ska1</i>	=	1.8	=	1.9	=	=
<i>gthb1</i>	↓7.6	↓4.8	↓7.1	↓4.3	=	=
<i>cp2g1</i>	=	1.4	=	1.3	=	=
<i>star</i>	↓9	↓3.8	↓8.5	↓3.6	=	=
<i>hus1</i>	↑10.3	↑3.7	=	=	↑10.3	↑3

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108 B) Gene expression results measured in individual gonad samples from the different lakes that
 109 were used to form the pools for NGS analyses. The *bactin* gene is used as the reference. The data
 110 are expressed as mean ($2^{-\Delta\text{Ct}}$) \pm SD, $n = 3$.

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Phenotype	Lake	Gene name				
		<i>ska1</i>	<i>gthb1</i>	<i>cp2g1</i>	<i>star</i>	<i>hus1</i>
Dev_Ref	Gorova	0.14 ± 0.02	0.011 ± 0.003	0.004 ± 0.002	0.009 ± 0.003	0.02 ± 0.008
	Dvoriche	0.11 ± 0.04	0.011 ± 0.006	0.001 ± 0.002	0.005 ± 0.002	0.02 ± 0.01
	Stoyacheye	0.07 ± 0.03	0.007 ± 0.003	0.004 ± 0.003	0.004 ± 0.0005	0.01 ± 0.005
Dev_Irr	Yanovsky	0.08 ± 0.06	0.005 ± 0.003	0.00002 ± 0.00002	0.005 ± 0.003	0.3 ± 0.6
	Cooling Pond	0.08 ± 0.02	0.009 ± 0.003	0.001 ± 0.001	0.005 ± 0.001	0.03 ± 0.003
	Svyatoye	0.13 ± 0.07	0.006 ± 0.003	0.004 ± 0.004	0.005 ± 0.001	0.01 ± 0.003
Undev_Irr	Yanovsky	0.26 ± 0.04	0.0004 ± 0.0004	0.005 ± 0.007	0.0004 ± 0.0004	0.07 ± 0.02
	Cooling Pond	0.45 ± 0.18	0.0003 ± 0.0002	0.004 ± 0.006	0.0004 ± 0.0002	0.4 ± 0.5

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118 **Supplementary Table S8:** Excel sheet giving the differential expression analysis results from
119 DESeq2 for comparisons between the phenotypes a) undeveloped gonads vs developed gonads,
120 b) undeveloped gonads vs developed/irradiated gonads, c) developed/irradiated gonads vs
121 developed gonads. Samples from different sampling sites were combined as replicates.
122 BaseMean, log2FoldChange, lfcSE, stat, pvalue and padj represent standard output from the
123 DESeq2 package. FPKM values represent the mean over all sampling sites. Transcripts were
124 classified as being significantly differentially expressed if they showed a fold change greater
125 than 2-fold (upregulated or downregulated), an adjusted p value less than 0.05, and a mean

126 normalized FPKM expression value greater than 1 for at least one of the two conditions. The raw
127 read counts and normalized FPKM values for all 534,099 transcripts are provided.

128 **Supplementary Table S9:** Excel sheet giving the gene ontology results for the comparisons
129 between the phenotypes for a) undeveloped gonads vs developed gonads, b) undeveloped gonads
130 vs developed/irradiated gonads, c) developed/irradiated gonads vs developed gonads. Gene
131 ontology terms are collected into three classes; biological process, molecular function and
132 cellular component.

133 **Supplementary Table S10.** Genes related to gonad development and pathway previously found responding to radiation exposure (*).

Gene	Comparison	Bibliographical Notes
<i>Nltp</i>	Dev/irr vs Dev/ref (+21)	Encodes the non-specific lipid transfer protein. Involved in intracellular transport and signalling of lipids (Peretti <i>et al.</i> , 2020).
<i>Ldah</i>	Dev/irr vs Dev/ref (+3) Undev/irr vs Dev/ref (+3)	Encodes the lipid droplet associated hydrolase.
<i>At11b</i>	Dev/irr vs Dev/ref (-10)	Encodes the phospholipid transporting ATPase IF
<i>Odo1</i>	Dev/irr vs Dev/ref (+9) Undev/irr vs Dev/ref (+9)	Catalyzes the conversion of 2-oxoglutarate, a substrate increased during the eggs development of the parasite worm in response to a higher energetic need (Beis and Barrett, 1975).
<i>Cyb5</i>	Dev/irr vs Dev/ref (-2) Undev/irr vs Dev/ref (+11)	Encodes an hemoprotein that modulates the synthesis of androgens by regulating the activity of CYP17A (Dharia <i>et al.</i> , 2004 ; Nakamura <i>et al.</i> , 2010,
<i>Cyp17a</i>	Undev/irr vs Dev/ref (-7)	
<i>Ovos</i>	Undev/irr vs Dev/ref (-7)	Identified in the egg white of birds (Stevens 1991; Saxena and Tayyab, 1997), in cancerous ovaries of hens (Lim <i>et al.</i> , 2011)
<i>Vegfc</i>	Undev/irr vs Dev/ref (-23)	Encodes a protein which is produced in granulosa and theca cells in humans (Agrawal <i>et al.</i> , 2002) Regulates oocyte quality (Bokal <i>et al.</i> , 20005) Regulates oocyte development (Devjak <i>et al.</i> , 2012)
<i>Prgr</i>	Undev/irr vs Dev/ref (-9)	Interact plays a major role in regulating female reproductive system (Gadkan-Sable <i>et al.</i> , 2005) Involved in oocyte maturation in the African clawed frog (<i>Xenopus laevis</i>) (Bayaa <i>et al.</i> , 2000; Tian <i>et al.</i> , 2000)
<i>K1C18</i>	Dev/irr vs Dev/ref (+5)	

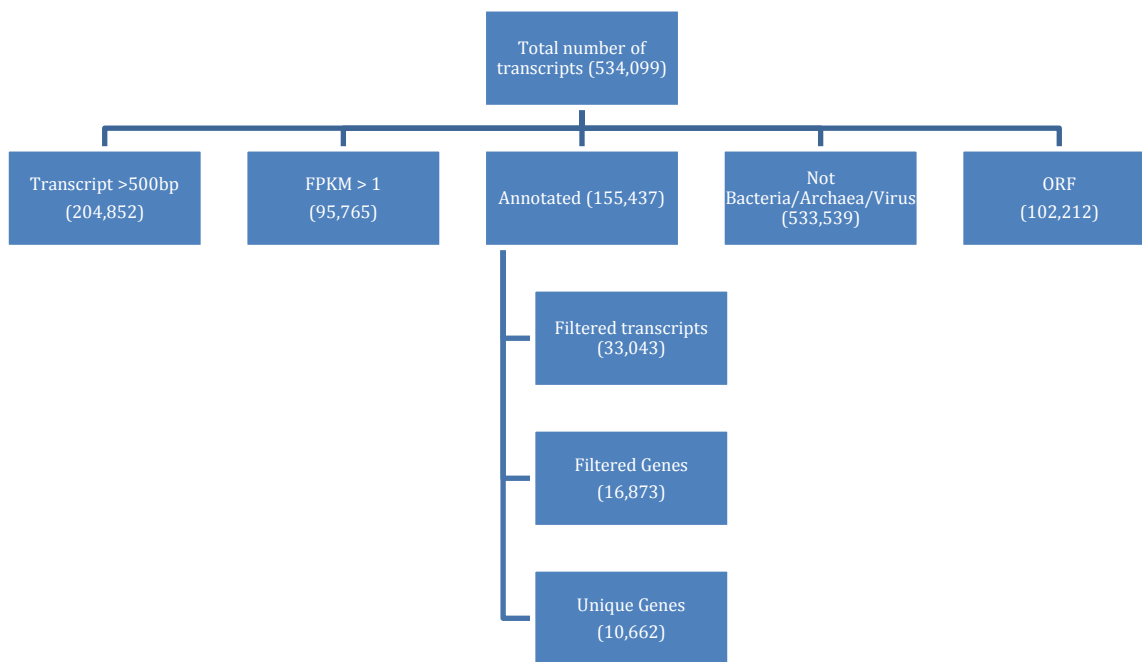
	Undev/irr vs Dev/ref (-6)	
<i>Nell2</i>	Undev/irr vs Dev/ref (-5)	Involved in the female reproductive cycle in mammals (Ryu <i>et al.</i> , 2011). Regulate the GnRH involved in timing of female puberty and gonad development (Ha <i>et al.</i> , 2008).
<i>Tcp4</i>	Undev/irr vs Dev/ref (-10)	Encodes a transcription factor involved in cell development and timing of reproductive maturation in plants (Sarvepalli and Nath, 2011).
<i>Mod5</i>	Dev/irr vs Dev/ref (+10) Undev/irr vs Dev/ref (+10)	Tumour suppressor gene
<i>Gps2</i>	Dev/irr vs Dev/ref (+10) Undev/irr vs Dev/ref (+9)	Tumour suppressor gene
<i>Rergl</i>	Dev/irr vs Dev/ref (+6) Undev/irr vs Dev/ref (+6)	Encodes the Ras-related and estrogen-regulated growth inhibitor protein. Found downregulated in human high grade ovarian serous cancer tissue samples (Lou <i>et al.</i> , 2018)
<i>Eif3</i>	Undev/irr vs Dev/ref (-10)	EIF3I initiates cell proliferation is involved in embryonic development (Sadato <i>et al.</i> 2018) <i>eif3</i> gene expression has been associated to gonadal development in freshwater crustaceans (Jiang <i>et al.</i> , 2014)
<i>Ggnb2</i>	Undev/irr vs Dev/ref (+1)	Involved in differentiation and in placental development (Li <i>et al.</i> 2016)
<i>Rad51*</i>	Dev/irr vs Dev/ref (+3) Undev/irr vs Dev/ref (+4)	Upregulated in marine mussels environmentally exposed to radionuclide discharges from the Sellafield nuclear facility, Ravenglass estuary, UK (AlAmri <i>et al.</i> , 2012).
<i>Hus1*</i>	Dev/irr vs Dev/ref (+10) Undev/irr vs Dev/ref (+10)	
<i>Wdhd1*</i>	Dev/irr vs Dev/ref (+10) Undev/irr vs Dev/ref (+11)	Initiates DNA replication (Chen <i>et al.</i> , 2017)

<i>Dmat1*</i>	Dev/irr vs Dev/ref (+10) Undev/irr vs Dev/ref (+11)	

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135 **Supplementary Figures**

136 **Supplementary Figure S1:** Filtering approach for assembled transcripts from Trinity *de novo*
137 transcriptome assembly. Transcripts were filtered to keep only those greater than or equal to
138 500bp in length with a minimum normalised abundance (FPKM) greater than 1 across all
139 samples and an identified open reading frame. Transcripts that showed a match to a non-
140 eukaryotic gene from the UniProt/SwissProt database were also removed.



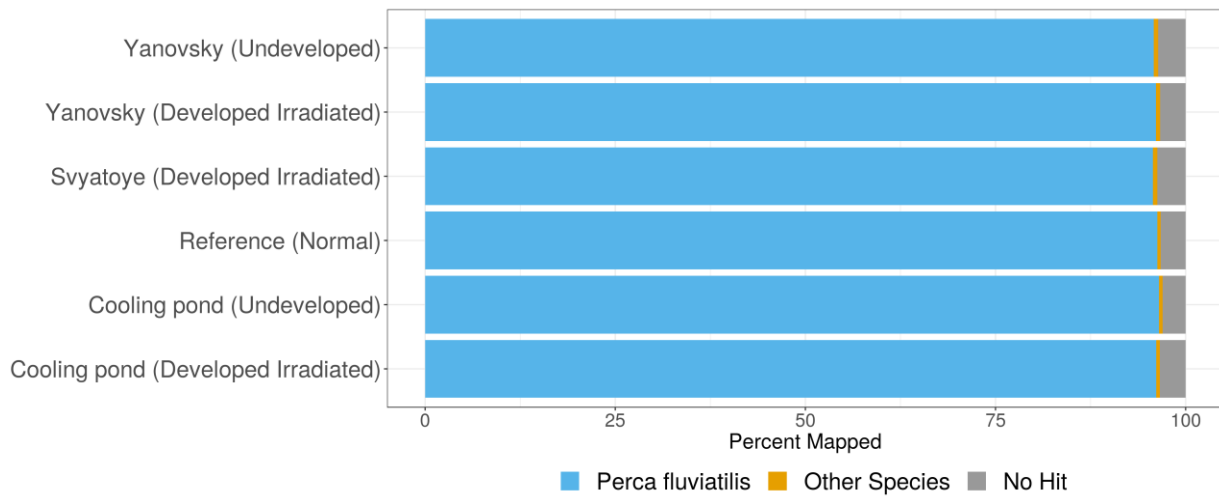
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152 **Supplementary Figure S2:** Proportion of reads for each sample that map to the European perch

153 (*Perca fluviatilis*) genome (blue), other related species predominantly stickleback (*Gasterosteus*

154 *aculeatus*), and reads that have no hit against any of the ~50 species analysed.



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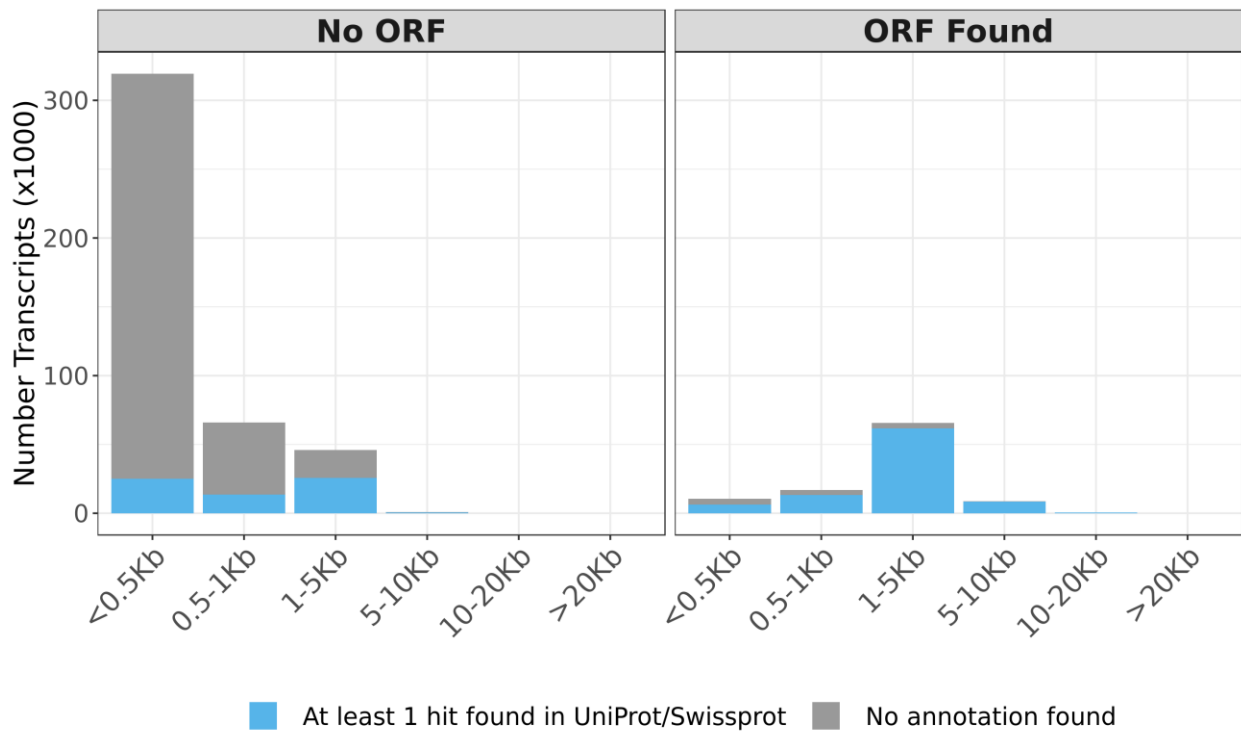
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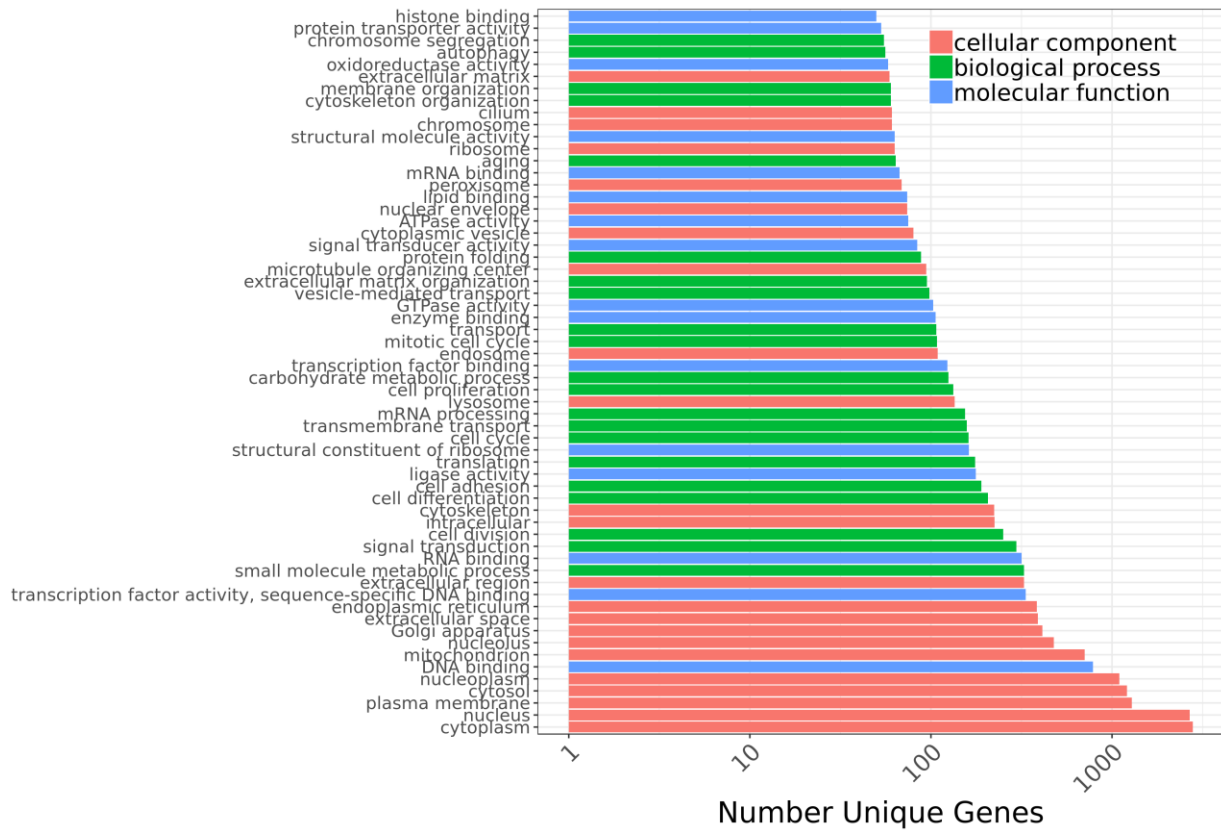
Supplementary Figure S3: Proportion of annotated transcripts split based on whether or not an open reading frame was identified for the transcript, and based on the transcript size. Those transcripts with a significant hit against genes in the Uniprot/Swissprot database are coloured blue.



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181 **Supplementary Figure S4:** Number of unique genes present in each of the top GO classes for
 182 cellular component, biological process and molecular function. GO classes with more than 50
 183 genes are shown.

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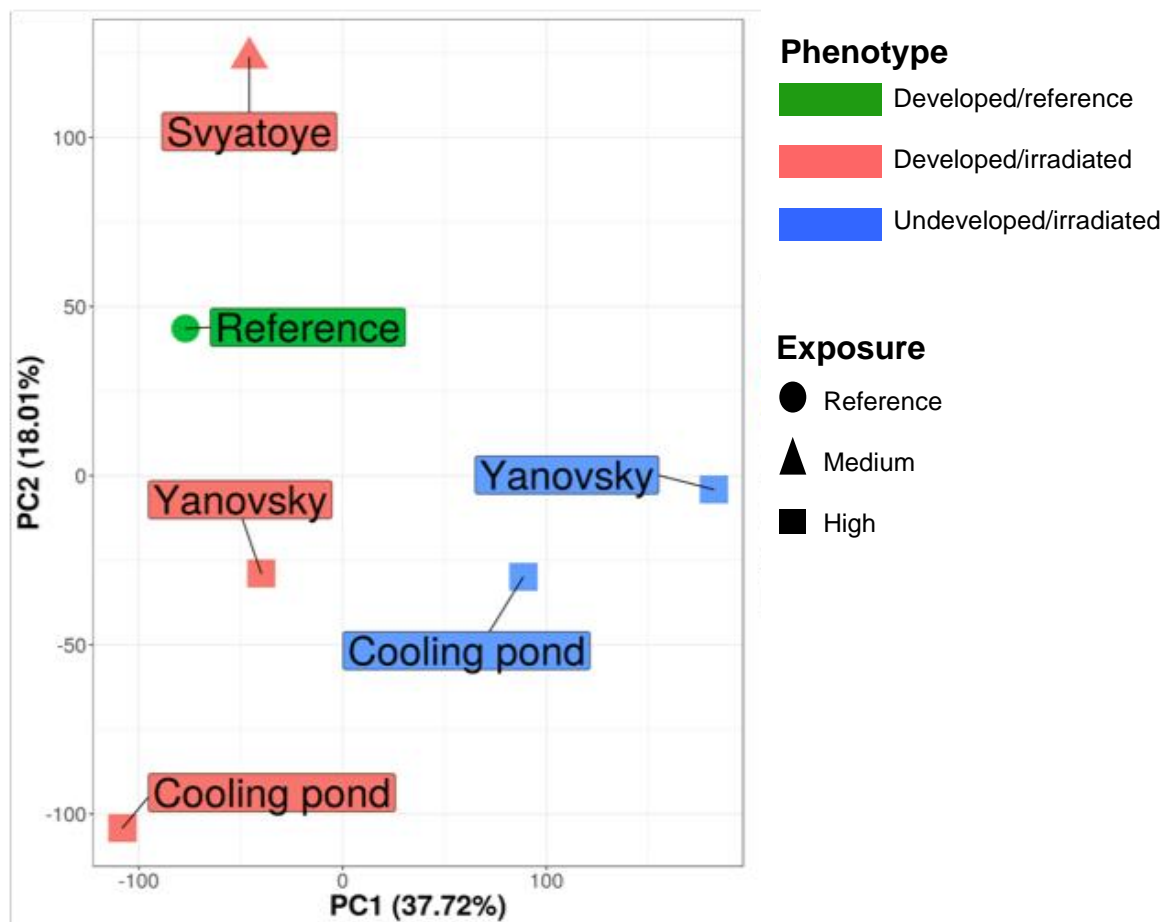
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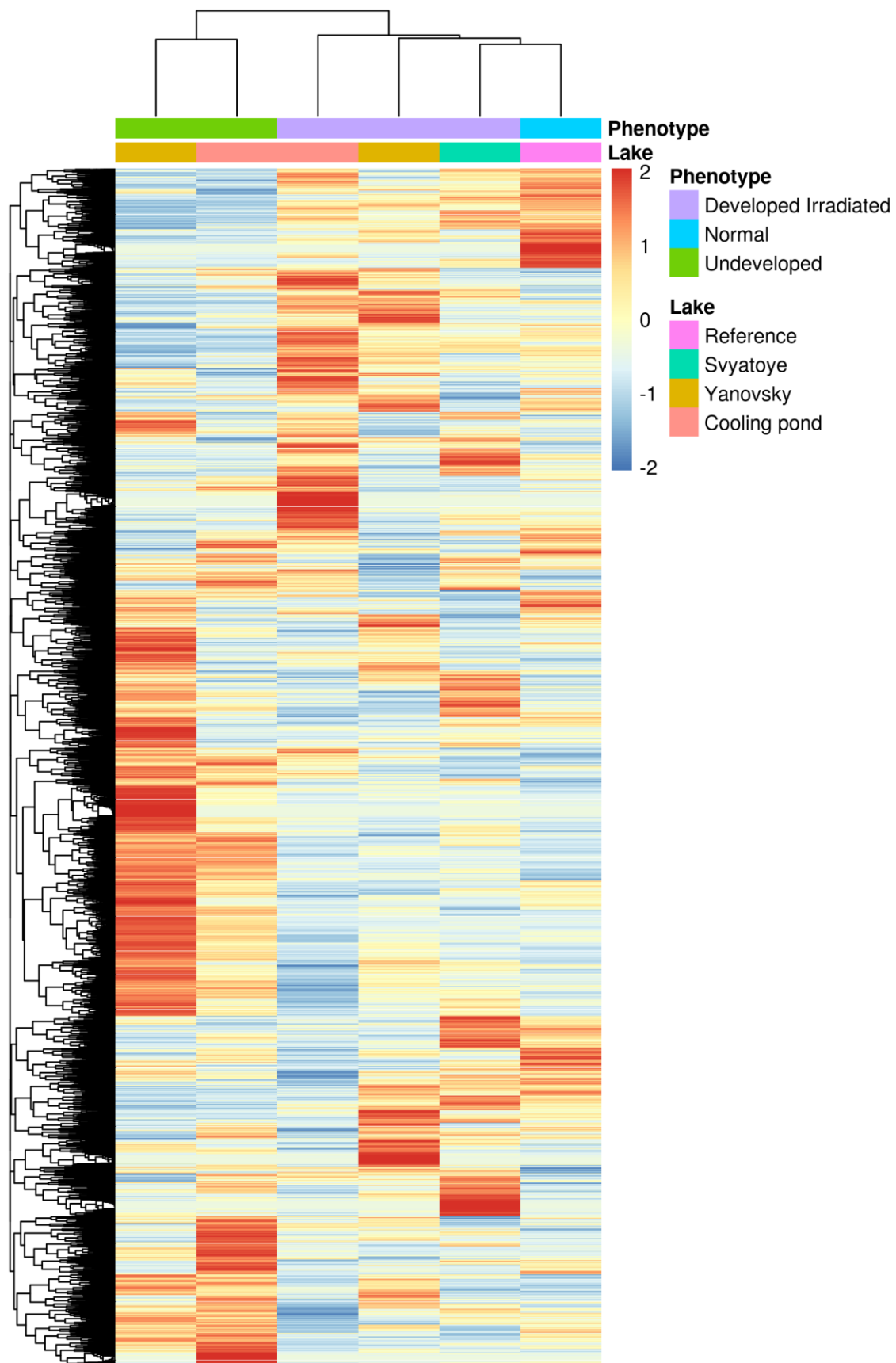
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191 **Supplementary Figure S5:** Principal component analysis showing the distribution of the
192 different gonad phenotypes (developed/reference, developed/irradiated and
193 undeveloped/irradiated) and associated exposure levels (Reference, Medium, High) based on
194 their normalised gene expression profiles. The largest source of variation in the gene expression
195 data was found between the undeveloped phenotypes (blue) and both reference and irradiated
196 developed phenotypes (green and red), which accounts for 37.7% of the variance in the data.

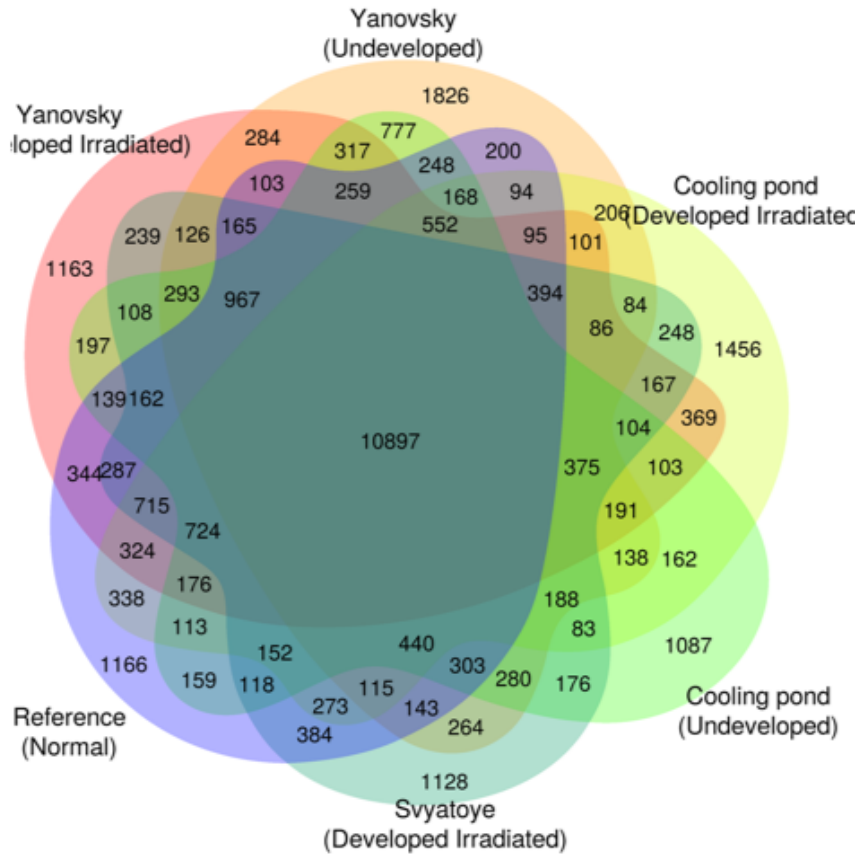


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198 **Supplementary Figure S6:** Heatmap showing the normalized abundance levels (FPKM) across
199 all 33,047 filtered transcripts. Abundance levels are normalized on a row-by-row basis such that
200 each row has mean 0 and standard deviation 1, so that transcripts with expression above the

201 mean across all samples appear red whilst transcripts with expression below the mean appear
202 blue.



204 **Supplementary Figure S7.** Venn diagram showing the overlap between the expressed
 205 transcripts (FPKM > 1) for each sample.



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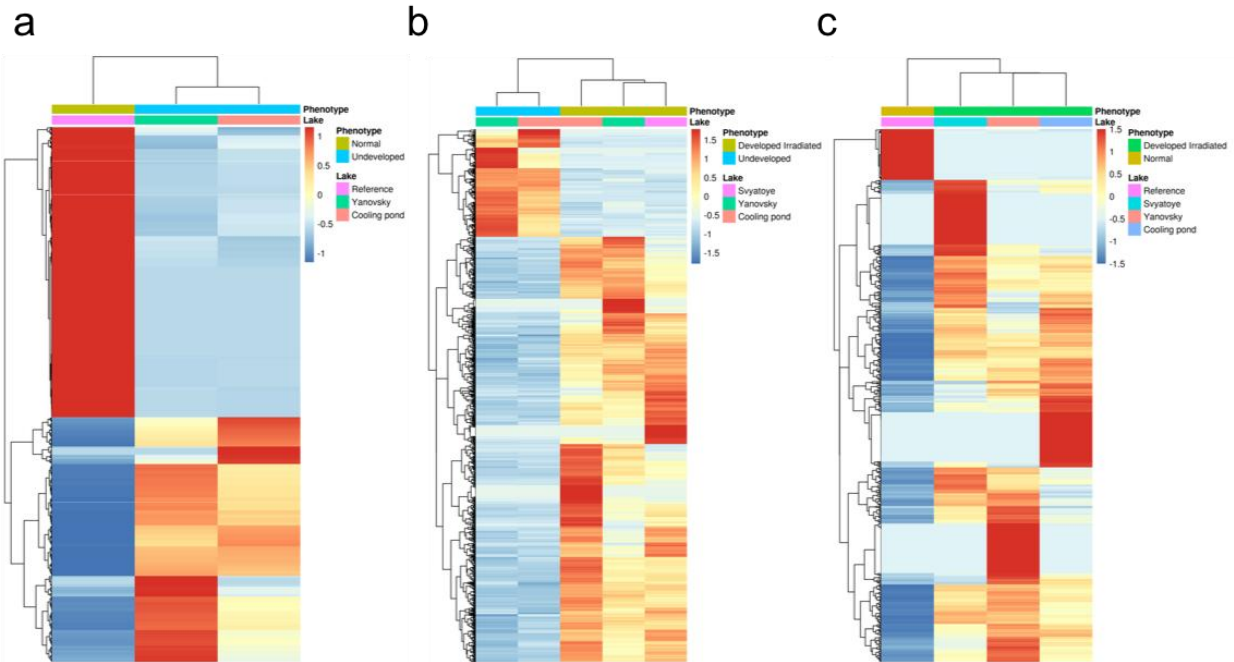
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213 **Supplementary Figure S8:** Results of differential expression analysis between different
214 conditions for a) undeveloped gonads vs developed/reference gonads (1,825 transcripts), b)
215 undeveloped gonads vs developed/irradiated gonads (1,994 transcripts), c) developed/irradiated
216 gonads vs developed/reference gonads (610 transcripts). Heatmaps show the normalized
217 abundance levels (FPKM) across all differentially regulated genes identified when using all
218 exposure levels as pseudo-replicates. Abundance levels were further normalized such that each
219 row has mean 0 and standard deviation 1, so that transcripts with expression above the mean
220 appear red whilst transcripts with expression below the mean appear blue.



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