***Supplementary material***

Analysis of hepatic deiodinase 2 mRNA levels in natural fish lake populations exposed to different levels of putative thyroid disrupters

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***Analytical procedures.***

***Hepatic mRNA analysis by RT-qPCR*.** Liver samples were homogenised in TRIzol Reagent (Gibco, Paisley, UK) using Eppendorf-fitting, RNase free pestles (Iberlabo, Madrid, Spain). RNA was extracted in TRIzol as specified by the supplier. Total RNA concentration was estimated by spectrophotometric absorption at 260 nm in a Nanodrop Spectrophotometer ND-1000 (NanoDrop Technologies; Delaware, USA) and treated with DNAse I (F. Hoffmann-La Roche Ltd, Basel, Switzerland) to remove contaminating genomic DNA. RNA integrity was assessed by electrophoresis in a Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara CA). RNA was treated with DNAseI to remove genomic DNA contamination. Ten µg of DNaseI-digested RNA were copied to cDNA by reverse transcriptase (Omniscript, Qiagen, Valencia, CA, USA) and stored at -20ºC. Aliquots corresponding to 40 ng of the original RNA preparation were used to quantify specific transcripts in a Abi Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) by the SYBR GREEN method (Applied Biosystems). Sequences for dio2, cyp1a and ß-Actin primers used in this work were the following:

ß-Actin Forward: 5'- CTGTCTTCCCCTCCATCGTC-3'

ß-Actin Reverse: 5'- TCTTGCTCTGAGCCTCGTCTC-3'

dio2 Forward: 5'- CGCTCCTTCGAGGTCAGG -3'

dio2 Reverse: 5'-ACCACCCTCTCCTCCAGTGAT-3'

CYP1A Forward: 5'-CACTGACTCCCTCATTGACCAC-3'

CYP1A Reverse: 5'-ACAGATCATTGACAATGCCCAC-3'

The relative amounts of cDNA present in the samples were calculated from the number of cycles required for amplification reaction to reach fluorescence above the threshold level in the RT-qPCR reaction (CT values), according to the equation [1](#_ENREF_1):



In which EAct and ETG correspond to Real-Time efficiency for ß-actin and the target gene, respectively. Efficiency values for dio2, cyp1a and ß-Actin primers on real samples were calculated using a series of standard dilutions as 102%, 101%, and 106%, respectively. These values were considered close enough one each other and to 100% to not to require any correction for efficiency on the calculations of relative mRNA abundance values. The suitability of ß-Actin as reference gene for these samples has been tested previously[2](#_ENREF_2). Results are expressed as copies of dio2 or cyp1a mRNA per 1000 copies of ß-actin mRNA. A typical experiment calculated CT values as averages of three replicates.

To confirm the sequence of the amplified PCR products, they were purified using GFX PCR Purification Kit (Amersham Biosciences, Backinghamshire, UK) and inserted into the pTZ57R/T plasmid (InsT/Aclone PCR Product Cloning Kit, Fermentas, Burlington, Canada). DNA sequencing was performed using Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). Amplified sequences were compared to previously reported sequences of homologous genes from different salmonids using ClustalW from Bioedit Sequence Alignment Editor (BioEdit v7.0.5, Ibis Therapeutics, Carlsbad, CA).

***PCBs and PBDE analysis.***

Muscle samples were ground with activated sodium sulphate until a fine powder was obtained. Cellulose cartridges were filled with this mixture and TBB and PCB209 were added as standards. A Soxhlet extraction was done with n-hexane:dichloromethane (4:1) for 18 h. The extract was cleaned up with sulfuric acid (5 times), concentrated by vacuum rotatory evaporation (20ºC, 20 Torr) and concentrated to near dryness under a gentle flow of nitrogen. Finally, the extract was redissolved in 50µl of isooctane. Before chromatographic analysis, an internal standard of PCB142 was added to correct for instrument variability. Samples were analyzed for PCBs by GC-ECD (Hewlett-Packard 5890 series II) with a 60 m x 0.25 mm i.d. DB-5 capillary column (J&W Scientific, Folsom, CA) coated with 5% phenyl/95% methylpolysiloxane (film thickness 0.25 m). The GC operated in splitless mode, and the oven temperature program started at 90ºC (held for 2 min), ramped to 150ºC at 15ºC·min-1 and then ramped to 290ºC at 4ºC·min-1 (holding time 20 min). Injector and detector temperatures were 280 and 310ºC, respectively. Helium and nitrogen were used as carrier (1.5 mL·min-1) and makeup (60 mL·min-1) gases, respectively. PBDE were analyzed by negative ion chemical ionization mass spectrometry coupled to gas chromatography (GC-MS-NICI). A GC system from Agilent Technologies 6890A (USA) was coupled to an MS detector 5973N. The system was equipped with a HP-5MS capillary column (60 m x 0.25 mm x 0.25 m film thickness). The oven temperature program was from 110ºC (held for 1 min) to 180ºC at 8ºC·min-1 (held for 1 min), then to 240ºC at 2ºC·min-1 (held for 5 min) and finally to 310ºC at 2ºC·min-1 (held for 15 min). Helium was used as a carrier gas (10 psi) and ammonia as ionization gas (1.6·10 Pa). Transfer line and quadrupole temperatures were 280 and 150ºC, respectively. Quantification was performed at a m/z value of 79 [Br]- which is the base peak of all PBDEs monitored. Confirmation was done at m/z values of 81 [Br]-, 161 [HBr2]-, 327, 405, 483, 563 and 643, corresponding to [M]- or [M-HBr 2]-. Retention time shifts could not be higher than 1 sec.

1 Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29** (2001).

2 Quirós, L. *et al.* Physiological response to persistent organic pollutants in fish from mountain lakes: Analysis of Cyp1A gene expression in natural populations of Salmo trutta. *Environmental Science & Technology* **41**, 5154-5160, doi:10.1021/es070455p (2007).

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| Table ST1. Physical characeristics of lakes sampled in this study | | | | | |  |
| Region | Lake name | Latitude N | Longitude E | Altitude ASL (m) | Average Temperature (ºC) |  |
| Pyrenees | Vidal d'Amunt | 42.53281 | 0.99351 | 2688 | 1.0 |  |
| Pyrenees | Xic de Colomina | 42.52149 | 0.99564 | 2425 | 2.7 |  |
| Pyrenees | Cavallers | 42.59257 | 0.85779 | 1800 | 5.5 |  |
| Pyrenees | Llebreta | 42.55083 | 0.89031 | 1620 | 6.2 |  |
| Tatra Mountains | Veľké Hinçovo | 49.17970 | 20.06060 | 1946 | -0.7 |  |
| Tatra Mountains | Morskie Oko | 49.19780 | 20.07220 | 1395 | 2.4 |  |

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| Table ST2. Chemical and gene expression data for the surveyed fish populations | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  | | |  | | | | |  | | |  | | | |  | | |  | | |  | | |  | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  |  | | | | | | |  |  | | | | |  |  | | | | | | |  |  | | | | | | |  |  | | | | |  |  | | | | | | |
|  | | Unit |  | |  | |  | | Llebreta | | | |  | |  | | | |  | |  | | | |  | | Cavallers | | | |  | | | | | | |  |  | | | | | | |  | Xic de Colomina | | | | | | | | | | | | |  |  | | | | | | |  | Vidal d'Amunt | | | | | | | | | | | | |  |  | | | | | | |  | Morskie Oko | | | | | | | | | | | | |  |  | | | | | | |  | Veľké Hinçovo | | | | | | | | | | | | |
|  | |  | | |  | | Median | |  | | Min. | | | | - | | Max. | | | |  | | Median | | | |  | | Min. | | | | - | | | Max. | | | | |  | | | Median | | | | | |  | | | Min. | | | - | | | Max. | | | | |  | | | Median | | | | |  | | | Min. | | | - | | | Max. | | | | | |  | | Median | | | | | |  | | Min. | | | | - | | Max. | | | | | |  | | Median | | | | | |  | | Min. | | | | - | | Max. | | |
| *n* | | number of fish | | | | | 14 | |  | |  | | | |  | |  | | | |  | | 14 | | | |  | |  | | | |  | | |  | | | | |  | | | 12 | | | | | |  | | |  | | |  | | |  | | | | |  | | | 12 | | | | |  | | |  | | |  | | |  | | | | | |  | | 13 | | | | | |  | |  | | | |  | |  | | | | | |  | | 14 | | | | | |  | |  | | | |  | |  | | |
| Sexa | |  | | |  | | 1.93 | |  | |  | | | |  | |  | | | |  | | 1.71 | | | |  | |  | | | |  | | |  | | | | |  | | | 2.00 | | | | | |  | | |  | | |  | | |  | | | | |  | | | 1.75 | | | | |  | | |  | | |  | | |  | | | | | |  | | 1.25 | | | | | |  | |  | | | |  | |  | | | | | |  | | 1.11 | | | | | |  | |  | | | |  | |  | | |
| Age | | years | | |  | | 6 | |  | | 4 | | | | - | | 9 | | | |  | | 4 | | | |  | | 3 | | | | - | | | 7 | | | | |  | | | 6 | | | | | |  | | | 5 | | | - | | | 8 | | | | |  | | | 5 | | | | |  | | | 5 | | | - | | | 9 | | | | | |  | | 6 | | | | | |  | | 3 | | | | - | | 17 | | | | | |  | | 6 | | | | | |  | | 5 | | | | - | | 7 | | |
| Lenght | | cm | | |  | | 33 | |  | | 26 | | | | - | | 36 | | | |  | | 28 | | | |  | | 15 | | | | - | | | 32 | | | | |  | | | 24 | | | | | |  | | | 21 | | | - | | | 29 | | | | |  | | | 30 | | | | |  | | | 24 | | | - | | | 33 | | | | | |  | | 18 | | | | | |  | | 13 | | | | - | | 54 | | | | | |  | | 16 | | | | | |  | | 14 | | | | - | | 18 | | |
| Weight | | g | | |  | | 358 | |  | | 190 | | | | - | | 560 | | | |  | | 273 | | | |  | | 30 | | | | - | | | 430 | | | | |  | | | 150 | | | | | |  | | | 105 | | | - | | | 215 | | | | |  | | | 280 | | | | |  | | | 185 | | | - | | | 355 | | | | | |  | | 57 | | | | | |  | | 18 | | | | - | | 2400 | | | | | |  | | 45 | | | | | |  | | 23 | | | | - | | 56 | | |
| dio2 | | mRNA copiesb | | | | | 0.63 | |  | | 0.03 | | | | - | | 7.62 | | | |  | | 3.03 | | | |  | | 0.45 | | | | - | | | 40.39 | | | | |  | | | 14.63 | | | | | |  | | | 3.25 | | | - | | | 104.19 | | | | |  | | | 6.34 | | | | |  | | | 0.95 | | | - | | | 40.67 | | | | | |  | | 1.48 | | | | | |  | | 0.29 | | | | - | | 12.45 | | | | | |  | | 0.87 | | | | | |  | | 0.04 | | | | - | | 2.82 | | |
| cyp1a | | mRNA copiesb | | | | | 271.69 | |  | | 38.21 | | | | - | | 486.33 | | | |  | | 383.72 | | | |  | | 48.70 | | | | - | | | 2907.95 | | | | |  | | | 1084.79 | | | | | |  | | | 632.88 | | | - | | | 2789.49 | | | | |  | | | 871.07 | | | | |  | | | 309.93 | | | - | | | 1414.21 | | | | | |  | | 717.25 | | | | | |  | | 57.51 | | | | - | | 2907.95 | | | | | |  | | 1328.69 | | | | | |  | | 1013.96 | | | | - | | 2203.81 | | |
| aHCH | | ng/gc | | |  | | 0.40 | |  | | 0.14 | | | | - | | 0.55 | | | |  | | 0.32 | | | |  | | 0.25 | | | | - | | | 1.18 | | | | |  | | | 0.10 | | | | | |  | | | 0.05 | | | - | | | 1.65 | | | | |  | | | 0.21 | | | | |  | | | 0.11 | | | - | | | 0.92 | | | | | |  | | 0.14 | | | | | |  | | 0.06 | | | | - | | 0.48 | | | | | |  | | 0.27 | | | | | |  | | 0.15 | | | | - | | 0.38 | | |
| HeCB | | ng/g | | |  | | 0.40 | |  | | 0.11 | | | | - | | 0.59 | | | |  | | 0.48 | | | |  | | 0.33 | | | | - | | | 0.79 | | | | |  | | | 0.39 | | | | | |  | | | 0.17 | | | - | | | 0.73 | | | | |  | | | 0.58 | | | | |  | | | 0.36 | | | - | | | 1.45 | | | | | |  | | 0.12 | | | | | |  | | 0.01 | | | | - | | 0.65 | | | | | |  | | 0.36 | | | | | |  | | 0.29 | | | | - | | 0.48 | | |
| gHCH | | ng/g | | |  | | 0.69 | |  | | 0.43 | | | | - | | 4.83 | | | |  | | 1.02 | | | |  | | 0.19 | | | | - | | | 2.30 | | | | |  | | | 1.48 | | | | | |  | | | 0.91 | | | - | | | 3.67 | | | | |  | | | 2.04 | | | | |  | | | 1.64 | | | - | | | 3.45 | | | | | |  | | 1.16 | | | | | |  | | 0.13 | | | | - | | 2.48 | | | | | |  | | 2.54 | | | | | |  | | 0.32 | | | | - | | 3.80 | | |
| PCB28 | | ng/g | | |  | | 0.24 | |  | | 0.03 | | | | - | | 0.49 | | | |  | | 0.18 | | | |  | | 0.09 | | | | - | | | 0.45 | | | | |  | | | 0.27 | | | | | |  | | | 0.15 | | | - | | | 0.42 | | | | |  | | | 0.24 | | | | |  | | | 0.04 | | | - | | | 1.03 | | | | | |  | | 0.24 | | | | | |  | | 0.07 | | | | - | | 1.17 | | | | | |  | | 0.45 | | | | | |  | | 0.11 | | | | - | | 1.34 | | |
| PCB52 | | ng/g | | |  | | 0.31 | |  | | 0.04 | | | | - | | 0.61 | | | |  | | 0.08 | | | |  | | 0.02 | | | | - | | | 0.33 | | | | |  | | | 1.89 | | | | | |  | | | 0.54 | | | - | | | 3.50 | | | | |  | | | 0.31 | | | | |  | | | 0.01 | | | - | | | 0.42 | | | | | |  | | 0.27 | | | | | |  | | 0.12 | | | | - | | 0.63 | | | | | |  | | 0.18 | | | | | |  | | 0.02 | | | | - | | 0.85 | | |
| PCB101 | | ng/g | | |  | | 0.11 | |  | | 0.03 | | | | - | | 0.16 | | | |  | | 0.39 | | | |  | | 0.12 | | | | - | | | 0.67 | | | | |  | | | 0.24 | | | | | |  | | | 0.01 | | | - | | | 0.67 | | | | |  | | | 0.29 | | | | |  | | | 0.06 | | | - | | | 0.64 | | | | | |  | | 0.19 | | | | | |  | | 0.07 | | | | - | | 12.27 | | | | | |  | | 1.11 | | | | | |  | | 0.22 | | | | - | | 1.77 | | |
| PCB118 | | ng/g | | |  | | 0.47 | |  | | 0.21 | | | | - | | 0.78 | | | |  | | 0.64 | | | |  | | 0.40 | | | | - | | | 0.91 | | | | |  | | | 0.77 | | | | | |  | | | 0.10 | | | - | | | 1.67 | | | | |  | | | 0.62 | | | | |  | | | 0.23 | | | - | | | 1.51 | | | | | |  | | 0.22 | | | | | |  | | 0.09 | | | | - | | 19.99 | | | | | |  | | 0.31 | | | | | |  | | 0.16 | | | | - | | 36.41 | | |
| PCB153 | | ng/g | | |  | | 1.21 | |  | | 0.62 | | | | - | | 2.02 | | | |  | | 1.44 | | | |  | | 0.56 | | | | - | | | 2.39 | | | | |  | | | 2.52 | | | | | |  | | | 1.00 | | | - | | | 6.31 | | | | |  | | | 2.67 | | | | |  | | | 1.27 | | | - | | | 12.97 | | | | | |  | | 1.75 | | | | | |  | | 0.36 | | | | - | | 208.66 | | | | | |  | | 5.74 | | | | | |  | | 3.58 | | | | - | | 8.23 | | |
| PCB138 | | ng/g | | |  | | 1.05 | |  | | 0.63 | | | | - | | 1.63 | | | |  | | 1.48 | | | |  | | 0.23 | | | | - | | | 1.89 | | | | |  | | | 2.25 | | | | | |  | | | 0.89 | | | - | | | 5.91 | | | | |  | | | 2.49 | | | | |  | | | 1.35 | | | - | | | 11.96 | | | | | |  | | 1.20 | | | | | |  | | 0.32 | | | | - | | 147.27 | | | | | |  | | 6.97 | | | | | |  | | 0.68 | | | | - | | 11.26 | | |
| PCB180 | | ng/g | | |  | | 0.84 | |  | | 0.47 | | | | - | | 1.32 | | | |  | | 0.85 | | | |  | | 0.51 | | | | - | | | 1.47 | | | | |  | | | 1.59 | | | | | |  | | | 0.54 | | | - | | | 4.08 | | | | |  | | | 1.13 | | | | |  | | | 0.59 | | | - | | | 6.59 | | | | | |  | | 1.29 | | | | | |  | | 0.28 | | | | - | | 231.17 | | | | | |  | | 4.74 | | | | | |  | | 2.52 | | | | - | | 6.30 | | |
| ppDDE | | ng/g | | |  | | 4.38 | |  | | 2.00 | | | | - | | 6.50 | | | |  | | 3.37 | | | |  | | 2.05 | | | | - | | | 5.38 | | | | |  | | | 7.70 | | | | | |  | | | 4.00 | | | - | | | 19.30 | | | | |  | | | 15.62 | | | | |  | | | 9.79 | | | - | | | 35.28 | | | | | |  | | 9.61 | | | | | |  | | 2.51 | | | | - | | 1273.72 | | | | | |  | | 48.04 | | | | | |  | | 29.56 | | | | - | | 69.11 | | |
| p,p´DDT | | ng/g | | |  | | 0.60 | |  | | 0.46 | | | | - | | 0.82 | | | |  | | 0.71 | | | |  | | 0.50 | | | | - | | | 1.26 | | | | |  | | | 1.08 | | | | | |  | | | 0.76 | | | - | | | 2.15 | | | | |  | | | 1.19 | | | | |  | | | 0.66 | | | - | | | 2.48 | | | | | |  | | 0.56 | | | | | |  | | 0.22 | | | | - | | 30.25 | | | | | |  | | 1.59 | | | | | |  | | 0.32 | | | | - | | 2.50 | | |
| PBDE 28+33 | | pg/g | | |  | | 78.54 | |  | | 41.33 | | | | - | | 104.23 | | | |  | | 100.14 | | | |  | | 99.00 | | | | - | | | 172.67 | | | | |  | | | 286.88 | | | | | |  | | | 158.64 | | | - | | | 515.15 | | | | |  | | | 248.19 | | | | |  | | | 190.12 | | | - | | | 535.22 | | | | | |  | | 76.60 | | | | | |  | | 36.47 | | | | - | | 172.74 | | | | | |  | | 87.81 | | | | | |  | | 3.17 | | | | - | | 144.99 | | |
| PBDE 47 | | pg/g | | |  | | 339.85 | |  | | 135.59 | | | | - | | 610.72 | | | |  | | 333.83 | | | |  | | 188.68 | | | | - | | | 438.69 | | | | |  | | | 425.87 | | | | | |  | | | 369.02 | | | - | | | 531.13 | | | | |  | | | 545.57 | | | | |  | | | 356.42 | | | - | | | 948.59 | | | | | |  | | 122.02 | | | | | |  | | 55.59 | | | | - | | 589.35 | | | | | |  | | 108.82 | | | | | |  | | 71.79 | | | | - | | 253.82 | | |
| PBDE 100 | | pg/g | | |  | | 168.45 | |  | | 101.72 | | | | - | | 308.49 | | | |  | | 134.84 | | | |  | | 112.83 | | | | - | | | 500.76 | | | | |  | | | 286.93 | | | | | |  | | | 137.18 | | | - | | | 332.88 | | | | |  | | | 256.60 | | | | |  | | | 138.75 | | | - | | | 796.55 | | | | | |  | | 42.58 | | | | | |  | | 16.07 | | | | - | | 76.97 | | | | | |  | | 31.42 | | | | | |  | | 3.55 | | | | - | | 37.38 | | |
| PBDE 99 | | pg/g | | |  | | 237.82 | |  | | 133.33 | | | | - | | 392.60 | | | |  | | 328.97 | | | |  | | 167.99 | | | | - | | | 432.98 | | | | |  | | | 292.35 | | | | | |  | | | 224.14 | | | - | | | 394.90 | | | | |  | | | 304.97 | | | | |  | | | 232.22 | | | - | | | 636.08 | | | | | |  | | 136.28 | | | | | |  | | 61.16 | | | | - | | 764.32 | | | | | |  | | 120.40 | | | | | |  | | 76.92 | | | | - | | 153.78 | | |
| PBDE 154 | | pg/g | | |  | | 80.16 | |  | | 62.39 | | | | - | | 233.75 | | | |  | | 201.59 | | | |  | | 65.21 | | | | - | | | 226.32 | | | | |  | | | 292.25 | | | | | |  | | | 248.79 | | | - | | | 334.06 | | | | |  | | | 257.11 | | | | |  | | | 181.45 | | | - | | | 730.97 | | | | | |  | | 35.42 | | | | | |  | | 11.92 | | | | - | | 51.62 | | | | | |  | | 33.55 | | | | | |  | | 21.01 | | | | - | | 39.46 | | |
| PBDE153 | | pg/g | | |  | | 93.66 | |  | | 77.42 | | | | - | | 149.79 | | | |  | | 95.45 | | | |  | | 62.84 | | | | - | | | 107.07 | | | | |  | | | 246.69 | | | | | |  | | | 212.41 | | | - | | | 362.58 | | | | |  | | | 243.58 | | | | |  | | | 123.54 | | | - | | | 512.42 | | | | | |  | | 30.06 | | | | | |  | | 12.36 | | | | - | | 39.92 | | | | | |  | | 24.00 | | | | | |  | | 13.16 | | | | - | | 27.59 | | |
| a) 1=male, 2= female, averages | | | | | | | | | | | |  |  | | | | |  |  | | | | |  |  | | | | |  |  | | | |  | | |  | | | | |  | | |  | | | | |  | | |  | | |  | | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | |
| b) Referred to 1000 copies of ß-actin mRNA | | | | | | | | | | | |  |  | | | | |  |  | | | | |  |  | | | | |  |  | | | |  | | |  | | | | |  | | |  | | | | |  | | |  | | |  | | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | |
| c) Weigh per gram of net tissue (muscle) | | | | | | | | | | | |  |  | | | | |  |  | | | | |  |  | | | | |  |  | | | |  | | |  | | | | |  | | |  | | | | |  | | |  | | |  | | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | | | | | |  | |  | | | | | |  | |  | | | |  | |  | |

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| Supplementary Table ST3. Analysis of the distribution of age and reproductive status' values among the sampled fish populations | | | | | | |
| **Descriptives** |  |  |  |  |  |  |
|  |  | *n* | Mean | Std. Deviation |  |  |
| **Age** | Llebreta | 14 | 6.214285714 | 1.42389344 |  |  |
|  | Cavallers | 14 | 4.5 | 1.224744871 |  |  |
|  | Xic de Colomina | 12 | 6.25 | 0.753778361 |  |  |
|  | Vidal d'Amunt | 12 | 5.416666667 | 1.164500153 |  |  |
|  | Morskie Oko | 13 | 6.384615385 | 2.218800785 |  |  |
|  | Veľké Hinçovo | 14 | 5.857142857 | 0.949262293 |  |  |
|  | Total | 79 | 5.759493671 | 1.486824097 |  |  |
| **Reproductive status** | Llebreta | 14 | 2.642857143 | 1.823232246 |  |  |
|  | Cavallers | 14 | 2.714285714 | 1.437335753 |  |  |
|  | Xic de Colomina | 12 | 1.833333333 | 0.937436867 |  |  |
|  | Vidal d'Amunt | 12 | 2.833333333 | 1.403458931 |  |  |
|  | Morskie Oko | 12 | 2.866666667 | 1.952775802 |  |  |
|  | Veľké Hinçovo | 14 | 2.714285714 | 1.38278267 |  |  |
|  | Total | 78 | 2.607692308 | 1.51681221 |  |  |
|  |  |  |  |  |  |  |
| **ANOVA** |  |  |  |  |  |  |
|  |  | Sum of Squares | df | Mean Square | F | Sig. |
| **Age** | Between Groups | 34.61536143 | 5 | 6.923072286 | 3.667120486 | 0.005137304 |
|  | Within Groups | 137.8150183 | 73 | 1.887876963 |  |  |
|  | Total | 172.4303797 | 78 |  |  |  |
| **Reproductive status** | Between Groups | 8.946813187 | 5 | 1.789362637 | 0.765918816 | 0.577376731 |
|  | Within Groups | 168.2085714 | 72 | 2.336230159 |  |  |
|  | Total | 177.1553846 | 77 |  |  |  |
|  |  |  |  |  |  |  |
| **Post Hoc Tests (Tukey's B)** | |  |  |  |  |  |
| **Age** |  |  |  |  |  |  |
| **Lake** | *n* | Means for groups in homogeneous subsets (alpha = 0.05) | |  |  |  |
|  |  | 1 | 2 |  |  |  |
| Cavallers | 14 | 4.5 |  |  |  |  |
| Vidal d'Amunt | 12 | 5.416666667 | 5.416666667 |  |  |  |
| Veľké Hinçovo | 14 | 5.857142857 | 5.857142857 |  |  |  |
| Llebreta | 14 |  | 6.214285714 |  |  |  |
| Xic de Colomina | 12 |  | 6.25 |  |  |  |
| Morskie Oko | 13 |  | 6.384615385 |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| **Reproductive status** | |  |  |  |  |  |
| **Lake** | *n* | Means for groups in homogeneous subsets (alpha = 0.05) | |  |  |  |
|  |  | 1 |  |  |  |  |
| Xic de Colomina | 12 | 1.833333333 |  |  |  |  |
| Llebreta | 14 | 2.642857143 |  |  |  |  |
| Cavallers | 14 | 2.714285714 |  |  |  |  |
| Veľké Hinçovo | 14 | 2.714285714 |  |  |  |  |
| Vidal d'Amunt | 12 | 2.833333333 |  |  |  |  |
| Morskie Oko | 12 | 2.866666667 |  |  |  |  |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Supplementary Table ST4. Analysis of the distribution of dio2 and cyp1a mRNA levels in male and female fish according their reproductive statusa,b | | | | | | | |  |
| **Descriptives** |  |  |  |  |  |  |  |  |
|  | Sex | Reproductive Status | *n* | Mean | Std. Deviation |  |  |  |
| **dio2 (log values)** | Male | 1-2 | 17 | 0.291158539 | 0.715531518 |  |  |  |
|  |  | 3-5 | 6 | 0.133592914 | 0.367284384 |  |  |  |
|  | Female | 1-2 | 21 | 0.498250701 | 0.957867865 |  |  |  |
|  |  | 3-4 | 20 | 0.609404285 | 0.595431177 |  |  |  |
|  |  | 5-7 | 6 | -0.16890681 | 0.65332076 |  |  |  |
|  | Total |  | 70 | 0.391273746 | 0.758816634 |  |  |  |
| **cyp1a (log values)** | Male | 1-2 | 17 | 2.935898307 | 0.34170676 |  |  |  |
|  |  | 3-5 | 7 | 3.082568227 | 0.482477799 |  |  |  |
|  | Female | 1-2 | 21 | 2.76290303 | 0.380755823 |  |  |  |
|  |  | 3-4 | 20 | 2.558539516 | 0.472628977 |  |  |  |
|  |  | 5-7 | 6 | 2.476709523 | 0.519663266 |  |  |  |
|  | Total |  | 71 | 2.754088168 | 0.452045162 |  |  |  |
|  |  |  |  |  |  |  |  |  |
| **ANOVA** |  |  |  |  |  |  |  |  |
|  |  |  | Sum of Squares | df | Mean Square |  | Sig. |  |
| **dio2 (log values)** |  | Between Groups | 3.643546012 | 4 | 0.910886503 |  | 0.174695473 |  |
|  |  | Within Groups | 36.0868392 | 65 | 0.555182142 |  |  |  |
|  |  | Total | 39.73038521 | 69 |  |  |  |  |
| **cyp1a (log values)** |  | Between Groups | 2.54527852 | 4 | 0.63631963 |  | 0.010688093 |  |
|  |  | Within Groups | 11.75885945 | 66 | 0.178164537 |  |  |  |
|  |  | Total | 14.30413797 | 70 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| **Post Hoc Tests (Tukey's B)** | |  |  |  |  |  |  |  |
| **dio2 (log values)** |  |  |  |  |  |  |  |  |
| **Sex (Reproductive status)** |  | *n* | Means for groups in homogeneous subsets (alpha = 0.05) | |  |  |  |  |
|  |  |  | 1 |  |  |  |  |  |
| Female (5-7) |  | 6 | -0.16890681 |  |  |  |  |  |
| Male (3-5) |  | 6 | 0.133592914 |  |  |  |  |  |
| Male (1-2) |  | 17 | 0.291158539 |  |  |  |  |  |
| Female (1-2) |  | 21 | 0.498250701 |  |  |  |  |  |
| Female (3-4) |  | 20 | 0.609404285 |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
| **cyp1a (log values)** |  |  |  |  |  |  |  |  |
| **Sex (Reproductive status)** |  | *n* | Means for groups in homogeneous subsets (alpha = 0.05) | |  |  |  |  |
|  |  |  | 1 | 2 |  |  |  |  |
| Female (5-7) |  | 6 | 2.476709523 |  |  |  |  |  |
| Female (3-4) |  | 20 | 2.558539516 |  |  |  |  |  |
| Female (1-2) |  | 21 | 2.76290303 | 2.76290303 |  |  |  |  |
| Male (1-2) |  | 17 | 2.935898307 | 2.935898307 |  |  |  |  |
| Male (3-5) |  | 7 |  | 3.082568227 |  |  |  |  |
| a) Defined as in supplementary Table S2 | | | |  |  |  |  |  |
| b) Reproductive status were aggregated to ensure sufficient *n* values for all groups | | | | |  |  |  |  |
|  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |