## Histological details

Table 1: Detailed overview on the symptoms identified during the semi-quantitative histological evaluation. The experiment with larval and juvenile brown trout with the respective treatment are listed in rows and the assessed symptoms are listed in columns. Each symptom is divided into three severity categories (white: not detected, light grey: detected in moderate frequency/severity, dark grey: detected in high frequency/severity). Values depict the absolute number of samples showing the symptom. CIT=citalopram, VEN=venlafaxine.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | hyperplasia | | | inflammation | | | macrophage infiltration | | | intercellular spaces | | | vacuolisation | | | necrosis | | |
| Experiment | Treatment | no | medium | severe | no | medium | severe | no | medium | severe | no | medium | severe | no | medium | severe | no | medium | severe |
| larvae + CIT (7 °C) | control | 10 | 34 | 6 | 43 | 1 | 6 | 49 | 0 | 1 | 24 | 4 | 22 | 48 | 0 | 2 | 36 | 2 | 12 |
| larvae + CIT (7 °C) | 1 µg/L CIT | 17 | 32 | 2 | 43 | 4 | 4 | 46 | 1 | 4 | 35 | 2 | 14 | 50 | 0 | 1 | 34 | 10 | 7 |
| larvae + CIT (7 °C) | 10 µg/L CIT | 21 | 28 | 5 | 52 | 1 | 1 | 51 | 1 | 2 | 34 | 5 | 15 | 47 | 0 | 7 | 45 | 2 | 7 |
| larvae + CIT (7 °C) | 100 µg/L CIT | 4 | 24 | 23 | 47 | 1 | 3 | 45 | 1 | 5 | 17 | 1 | 33 | 50 | 0 | 1 | 34 | 5 | 12 |
| larvae + CIT (7 °C) | 1000 µg/L CIT | 7 | 23 | 12 | 31 | 1 | 10 | 26 | 0 | 16 | 19 | 1 | 22 | 41 | 0 | 1 | 27 | 4 | 11 |
| larvae + CIT (11 °C) | control | 14 | 17 | 4 | 17 | 5 | 13 | 33 | 0 | 2 | 32 | 0 | 3 | 35 | 0 | 0 | 24 | 4 | 7 |
| larvae + CIT (11 °C) | 1 µg/L CIT | 14 | 12 | 4 | 18 | 6 | 6 | 28 | 0 | 2 | 30 | 0 | 0 | 30 | 0 | 0 | 20 | 6 | 4 |
| larvae + CIT (11 °C) | 10 µg/L CIT | 17 | 16 | 0 | 16 | 6 | 11 | 31 | 0 | 2 | 31 | 0 | 2 | 33 | 0 | 0 | 23 | 3 | 7 |
| larvae + CIT (11 °C) | 100 µg/L CIT | 12 | 12 | 2 | 10 | 5 | 11 | 23 | 0 | 3 | 26 | 0 | 0 | 26 | 0 | 0 | 16 | 2 | 8 |
| larvae + CIT (11 °C) | 1000 µg/L CIT | 7 | 11 | 18 | 14 | 3 | 19 | 32 | 0 | 4 | 19 | 2 | 15 | 36 | 0 | 0 | 23 | 3 | 10 |
| juveniles + CIT | control | 0 | 30 | 0 | 12 | 5 | 13 | 28 | 2 | 0 | 12 | 4 | 14 | 13 | 4 | 13 | 14 | 7 | 9 |
| juveniles + CIT | 1 µg/L CIT | 0 | 26 | 4 | 14 | 6 | 10 | 30 | 0 | 0 | 13 | 1 | 16 | 12 | 1 | 17 | 16 | 6 | 8 |
| juveniles + CIT | 10 µg/L CIT | 0 | 29 | 1 | 17 | 3 | 10 | 29 | 1 | 0 | 18 | 0 | 12 | 16 | 1 | 13 | 15 | 4 | 11 |
| juveniles + CIT | 100 µg/L CIT | 0 | 30 | 0 | 14 | 4 | 12 | 29 | 0 | 1 | 11 | 4 | 15 | 4 | 5 | 21 | 12 | 6 | 12 |
| juveniles + CIT | 1000 µg/L CIT | 1 | 17 | 10 | 8 | 5 | 15 | 27 | 0 | 1 | 5 | 1 | 22 | 6 | 0 | 22 | 17 | 1 | 10 |
| larvae + VEN (7 °C) | control | 20 | 0 | 10 | 24 | 0 | 6 | 24 | 4 | 2 | 25 | 1 | 4 | 27 | 0 | 3 | 18 | 6 | 6 |
| larvae + VEN (7 °C) | 1 µg/L VEN | 20 | 0 | 9 | 20 | 0 | 9 | 24 | 3 | 2 | 23 | 1 | 5 | 25 | 0 | 4 | 14 | 9 | 6 |
| larvae + VEN (7 °C) | 10 µg/L VEN | 19 | 0 | 11 | 25 | 0 | 5 | 25 | 4 | 1 | 22 | 0 | 8 | 26 | 0 | 4 | 16 | 8 | 6 |
| larvae + VEN (7 °C) | 100 µg/L VEN | 14 | 1 | 15 | 21 | 0 | 9 | 23 | 3 | 4 | 22 | 1 | 7 | 22 | 0 | 8 | 18 | 4 | 8 |
| larvae + VEN (7 °C) | 1000 µg/L VEN | 23 | 0 | 7 | 24 | 0 | 6 | 28 | 2 | 0 | 26 | 0 | 4 | 17 | 0 | 3 | 21 | 5 | 4 |
| larvae + VEN (11 °C) | control | 19 | 0 | 11 | 14 | 5 | 11 | 16 | 12 | 2 | 26 | 0 | 4 | 30 | 0 | 0 | 13 | 9 | 8 |
| larvae + VEN (11 °C) | 1 µg/L VEN | 13 | 0 | 12 | 12 | 2 | 11 | 14 | 6 | 5 | 20 | 0 | 5 | 22 | 0 | 3 | 13 | 3 | 9 |
| larvae + VEN (11 °C) | 10 µg/L VEN | 22 | 0 | 8 | 14 | 2 | 14 | 17 | 9 | 4 | 26 | 0 | 4 | 27 | 0 | 3 | 12 | 7 | 11 |
| larvae + VEN (11 °C) | 100 µg/L VEN | 19 | 0 | 10 | 12 | 5 | 12 | 17 | 9 | 3 | 24 | 0 | 5 | 25 | 0 | 4 | 12 | 6 | 11 |
| larvae + VEN (11 °C) | 1000 µg/L VEN | 18 | 0 | 11 | 18 | 1 | 10 | 18 | 6 | 5 | 24 | 0 | 5 | 23 | 0 | 6 | 11 | 7 | 11 |
| juveniles + VEN | control | 2 | 18 | 5 | 12 | 4 | 9 | 24 | 0 | 1 | 17 | 0 | 8 | 18 | 0 | 7 | 19 | 3 | 3 |
| juveniles + VEN | 1 µg/L VEN | 2 | 21 | 8 | 20 | 6 | 5 | 29 | 0 | 2 | 13 | 0 | 18 | 26 | 0 | 5 | 27 | 1 | 2 |
| juveniles + VEN | 10 µg/L VEN | 5 | 20 | 3 | 22 | 1 | 5 | 26 | 0 | 2 | 15 | 0 | 13 | 24 | 0 | 4 | 20 | 2 | 6 |
| juveniles + VEN | 100 µg/L VEN | 4 | 19 | 7 | 14 | 3 | 13 | 28 | 0 | 2 | 17 | 1 | 12 | 26 | 0 | 4 | 20 | 2 | 8 |
| juveniles + VEN | 1000 µg/L VEN | 4 | 20 | 4 | 15 | 2 | 11 | 26 | 0 | 2 | 13 | 0 | 15 | 21 | 0 | 7 | 23 | 3 | 2 |

## Number of fish

Table 2: Number of individual fish assessed per parameter in the experiment with brown trout larvae exposed to citalopram.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | brown trout larvae 7°C | | | | | brown trout larvae 11°C | | | | |
| treatment | control | 1 µg/L | 10 µg/L | 100 µg/L | 1000 µg/L | control | 1 µg/L | 10 µg/L | 100 µg/L | 1000 µg/L |
| histology class catergorisation | 50 | 51 | 54 | 51 | 42 | 35 | 30 | 33 | 26 | 35 |
| histology glycogen categorisation | 51 | 51 | 54 | 50 | 42 | 35 | 30 | 33 | 26 | 36 |
| AChE activity | 45 | 46 | 40 | 17 | 46 | 20 | 19 | 18 | 28 | 23 |
| CbE activity (NPA Substrate) | 45 | 46 | 40 | 17 | 46 | 20 | 19 | 18 | 28 | 23 |
| CbE activity (NPV Substrate) | 45 | 46 | 40 | 17 | 46 | 20 | 19 | 18 | 28 | 23 |

Table 3: Number of individual fish assessed per parameter in the experiment with brown trout larvae exposed to venlafaxine.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | brown trout larvae 7°C | | | | | brown trout larvae 11°C | | | | |
| treatment | control | 1 µg/L | 10 µg/L | 100 µg/L | 1000 µg/L | control | 1 µg/L | 10 µg/L | 100 µg/L | 1000 µg/L |
| histology class catergorisation | 30 | 29 | 30 | 30 | 30 | 30 | 25 | 30 | 30 | 29 |
| histology glycogen categorisation | 30 | 29 | 30 | 29 | 30 | 30 | 25 | 30 | 30 | 29 |
| AChE activity | 30 | 30 | 30 | 30 | 30 | 30 | 29 | 29 | 30 | 28 |
| CbE activity (NPA Substrate) | 30 | 30 | 30 | 30 | 30 | 30 | 29 | 29 | 30 | 28 |
| CbE activity (NPV Substrate) | 30 | 30 | 30 | 30 | 30 | 30 | 29 | 29 | 30 | 28 |
| SOD activity | 30 | 30 | 30 | 30 | 30 | 29 | 29 | 30 | 30 | 30 |

Table 4: Number of individual fish assessed per parameter in the experiment with juvenile brown trout exposed to either citalopram or venlafaxine; CIT=citalopram, VEN=venlafaxine, n.a.= not assessed.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | juvenile brown trout + CIT | | | | | juvenile brown trout + VEN | | | | |
| treatment | control | 1 µg/L CIT | 10 µg/L CIT | 100 µg/L CIT | 1000 µg/L CIT | control | 1 µg/L VEN | 10 µg/L VEN | 100 µg/L VEN | 1000 µg/L VEN |
| histology class catergorisation | 30 | 30 | 30 | 30 | 28 | 25 | 30 | 28 | 30 | 28 |
| histology glycogen categorisation | 30 | 30 | 30 | 29 | 27 | 25 | 30 | 28 | 30 | 28 |
| AChE activity | 29 | 26 | 26 | 27 | 26 | 23 | 27 | 26 | 27 | 25 |
| CbE activity (NPA Substrate) | 29 | 26 | 26 | 27 | 26 | 23 | 27 | 26 | 27 | 25 |
| CbE activity (NPV Substrate) | 29 | 26 | 26 | 27 | 26 | 23 | 27 | 26 | 27 | 25 |
| SOD activity | n.a. | n.a. | n.a. | n.a. | n.a. | 26 | 30 | 29 | 30 | 28 |
| Hsp 70 level | 30 | 30 | 30 | 27 | 30 | 26 | 30 | 29 | 29 | 28 |

## Statistical details

Table 5: Detailed statistical information about the respective parameters.

|  |  |  |
| --- | --- | --- |
| parameter | experiment | detailed information |
| histology class categorisation | brown trout larvae + citalopram 7°C | likelihood-ratio-χ²-test: *df*=16, *χ²*=40.467, *p*=0.0007, pairwise comparison [control|1000 µg/L] *p*=0.0047 |
| histology class categorisation | brown trout larvae + citalopram 11°C | likelihood-ratio-χ²-test: *df*=16, *χ²*=30.986, *p*=0.0135, pairwise comparison [control|1000 µg/L] *p*=0.0036 |
| histology class categorisation | brown trout larvae + venlafaxine 7°C | likelihood-ratio-χ²-test: *df*=16, *χ²*=15.494, *p*=0.4888 |
| histology class categorisation | brown trout larvae + venlafaxine 11°C | likelihood-ratio-χ²-test: *df*=16, *χ²*=10.701, *p*=0.8275 |
| histology class categorisation | juvenile brown trout + citalopram | likelihood-ratio-χ²-test: *df*=16, *χ²*=30.706, *p*=0.0147, pairwise comparison [control|1000 µg/L] *p*=0.0095 |
| histology class categorisation | juvenile brown trout + venlafaxine | likelihood-ratio-χ²-test: *df*=16, *χ²*=19.575, *p*=0.24 |
| histology glycogen categorisation | brown trout larvae + citalopram 7°C | likelihood-ratio-χ²-test: *df*=8, *χ²*=19.807, *p*=0.0111, pairwise comparison revealed no difference to control |
| histology glycogen categorisation | brown trout larvae + citalopram 11°C | likelihood-ratio-χ²-test: *df*=8, *χ²*=48.951, p<0.0001, pairwise comparison [control|1000 µg/L] *p*=0.0008 |
| histology glycogen categorisation | brown trout larvae + venlafaxine 7°C | likelihood-ratio-χ²-test: *df*=8, *χ²*=6.98, *p*=0.5388 |
| histology glycogen categorisation | brown trout larvae + venlafaxine 11°C | likelihood-ratio-χ²-test: *df*=8, *χ²*=9.941, *p*=0.2692 |
| histology glycogen categorisation | juvenile brown trout + citalopram | likelihood-ratio-χ²-test: *df*=8, *χ²*=8.804, *p*=0.3591 |
| histology glycogen categorisation | juvenile brown trout + venlafaxine | likelihood-ratio-χ²-test: *df*=8, *χ²*=9.911, *p*=0.2713 |
| AChE activity | brown trout larvae + citalopram 7°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=1.0282, *p*=0.3941 |
| AChE activity | brown trout larvae + citalopram 11°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=0.9551, *p*=0.436 |
| AChE activity | brown trout larvae + venlafaxine 7°C | nested ANOVA: *df*=4,10, *F*=1.404, *p*=0.2362 |
| AChE activity | brown trout larvae + venlafaxine 11°C | welch ANOVA: *df*=4,70.415, *F*=2.0841, *p*=0.092 |
| AChE activity | juvenile brown trout + citalopram | nested ANOVA: *df*=4,10, *F*=1.8406, *p*=0.1257 |
| AChE activity | juvenile brown trout + venlafaxine | nested ANOVA: *df*=4,10, *F*=0.5178, *p*=0.7228 |
| CbE activity (NPA substrate) | brown trout larvae + citalopram 7°C | fourth root transformation, nested ANOVA: *df*=4,10, *F*=0.9768, *p*=0.4216 |
| CbE activity (NPA substrate) | brown trout larvae + citalopram 11°C | nested ANOVA: *df*=4,10, *F*=0.6241, *p*=0.6465 |
| CbE activity (NPA substrate) | brown trout larvae + venlafaxine 7°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=1.1056, *p*=0.3567 |
| CbE activity (NPA substrate) | brown trout larvae + venlafaxine 11°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=3.0054, *p*=0.0206, post-hoc Dunnett’s test revealed no difference to control |
| CbE activity (NPA substrate) | juvenile brown trout + citalopram | logarithm transformation, nested ANOVA: *df*=4,10, *F*=0.3276, *p*=0.859 |
| CbE activity (NPA substrate) | juvenile brown trout + venlafaxine | nested ANOVA: *df*=4,10, *F*=0.8819, *p*=0.4772 |
| CbE activity (NPV substrate) | brown trout larvae + citalopram 7°C | nested ANOVA: *df*=4,10, *F*=0.8061, *p*=0.5227 |
| CbE activity (NPV substrate) | brown trout larvae + citalopram 11°C | nested ANVOA: *df*=4,10, *F*=1.1402, *p*=0.3425 |
| CbE activity (NPV substrate) | brown trout larvae + venlafaxine 7°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=1.0453, *p*=0.3865 |
| CbE activity (NPV substrate) | brown trout larvae + venlafaxine 11°C | logarithm transformation, nested ANOVA: *df*=4,10, *F*=1.9203, *p*=0.1106 |
| CbE activity (NPV substrate) | juvenile brown trout + citalopram | logarithm transformation, nested ANOVA: *df*=4,10, *F*=1.1392, *p*=0.3415 |
| CbE activity (NPV substrate) | juvenile brown trout + venlafaxine | nested ANOVA: *df*=4,10, *F*=0.8819, *p*=0.4772 |
| SOD activity | brown trout larvae + venlafaxine 7°C | brown trout larvae 7°C: nested ANOVA: *df*=4,10, *F*=3.7377, *p*=0.0064, post-hoc Dunnett’s Test [control 10 µg/L] *p*=0.0442, [control|100 µg/L] *p*=0.0048, [control|1000 µg/L] *p*=0.082 |
| SOD activity | brown trout larvae + venlafaxine 11°C | nested ANOVA: *df*=4,10, *F*=1.5387, *p*=1946 |
| SOD activity | juvenile brown trout + venlafaxine | nested ANOVA: *df*=4,10, *F*=1.5804, *p*=0.1834 |
| Hsp70 level | juvenile brown trout + citalopram | logarithm transformation, nested ANOVA: *df*=4,10, *F*= 1.7073, *p*=0.1522 |
| Hsp70 level | juvenile brown trout + venlafaxine | logarithm transformation, nested ANOVA: *df*=4,10, *F*=3.2671, *p*=0.0138, post-hoc Dunnett’s test revealed no difference to control |