Anguillicola crassus infection affects mRNA expression levels in gas gland tissue of European yellow and silver eel

Gabriel Schneebauer1,2, Ron P. Dirks3, Bernd Pelster1,2*

1 Institute of Zoology, University of Innsbruck, Innsbruck, Austria, 2 Center for Molecular Biosciences, University Innsbruck, Innsbruck, Austria, 3 ZF-screens, Leiden, The Netherlands

* bernd.pelster@uibk.ac.at

Abstract

Using Illumina sequencing, we investigated transcriptional changes caused by the nematode Anguillicola crassus within yellow and silver eels by comparing swimbladder samples of uninfected yellow with infected yellow eels, and uninfected silver with infected silver eels, respectively. In yellow eel gas gland, the infection caused a modification of steady state mRNA levels of 1675 genes, most of them being upregulated. Functional annotation analysis based on GO terms was used to categorize identified genes with regard to swimbladder metabolism or response to the infection. In yellow eels, the most prominent category was 'immune response', including various inflammatory components, complement proteins, and immunoglobulins. The elevated expression of several glucose and monocarboxylate transporters indicated an attempt to maintain the level of glucose metabolism, even in due to the infection thickened swimbladder tissue. In silver eel swimbladder tissue, on the contrary, the mRNA levels of only 291 genes were affected. Genes in the categories 'glucose metabolism' and 'ROS metabolism' barely responded to the infection and even the reaction of the immune system was much less pronounced compared to infected yellow eels. However, in the category 'extracellular matrix', the mRNA levels of several mucin genes were strongly elevated, suggesting increased mucus production as a defense reaction against the parasite. The present study revealed a strong reaction to an Anguillicola crassus infection on mRNA expression levels in swimbladder tissue of yellow eels, whereas in silver eels the changes were almost negligible. A possible explanation for this difference is that the silvering process requires so much energy that there is not much scope to cope with the additional challenge of a nematode infection. Another possible explanation could be that gas-secreting activity of the silver eel swimbladder was largely reduced, which could coincide with a reduced responsiveness to other challenges, like a nematode infection.

Introduction

As catadromous fish, European eels Anguilla anguilla spend most of their lifetime in European fresh- and coastal water systems as so called yellow eels. After a transformation named
silvering, which prepares eels for their long-distance migration and represents the beginning of sexual maturation [1], they return to the species’ expected spawning grounds in the Sargasso Sea for reproduction [2,3]. Because of this complex lifecycle, eels are particularly vulnerable to potential stressors such as overfishing [4], habitat loss [5], pollution [6], changing ocean currents [7], decline of primary production due to increasing sea surface temperature [8], or parasites [9,10]. Almost certain, these stressors somehow act synergistically and have caused a recruitment decline of about 95% since the 1980s [11], resulting in *A. anguilla* being listed as critically endangered species by the International Union for the Conservation of Nature and Natural Resources™ since 2010 [12].

After eels have passed the continental shelf on their spawning migration, they start performing diel vertical migrations, swimming at depths of 600–1000 m during daytime and 100–300 m during nighttime [13–15]. These daily changes in hydrostatic pressure significantly affect pressure and volume of the swimbladder, functioning as a buoyancy organ [16–19].

During the silvering process, eels not only change body color, their eyes enlarge, neuromasts appear along the lateral line, and body fat content increases [20–22], but also the swimbladder undergoes changes. These changes are thought to improve its gas secreting capacity in order to cope with the significant changes in hydrostatic pressure, encountered during the vertical migrations. Slightly increased wall thickness and vascularization, guanine deposition into the wall to dampen diffusional gas loss and enlargement of the *retia mirabilia* to enhance countercurrent concentration performance [23–25], for example, resulted in a fivefold increase in gas deposition in the American eel *Anguilla rostrata* [23]. The underlying molecular processes of these silvering related improvements and the effects of silvering on various metabolic pathways relevant for swimbladder metabolism, on mRNA level, have been addressed in a recent study [26].

In 1980, the parasitic nematode *Anguillicola crassus* was introduced to Europe by importing infected Japanese eels *Anguilla japonica* from Taiwan to Germany and spread almost throughout the entire eel population within only 10 years [27,28]. Larval stages of the parasite are taken up by the eels via food consumption, invade the swimbladder and, as adults, feed on blood and tissue [27]. This feeding activity and an increasing number of nematodes in the swimbladder lumen, for example, reduce the gas secreting capability of the gas gland cells and swimbladder wall elasticity, and cause various severe pathological changes that can eventually result in loss of swimbladder function [29–31]. The infection with *Anguillicola crassus* has also been shown to impair silvering related improvements in swimbladder function like the ROS defense capacity [32]. In addition, mRNA levels of certain genes, relevant for swimbladder metabolism [26], or the silvering process in general [33] appear to be affected by the nematode infection. However, a comprehensive study on the transcriptional changes in gas gland tissue provoked by the nematode in yellow or in silver eels is missing.

In this study, we therefore investigated the effects of an *Anguillicola crassus* infection on swimbladder tissue at the mRNA level by comparing the swimbladder transcriptome of uninfected yellow eels with infected yellow eels, and of uninfected silver eels with infected silver eels. For comparative reasons, we particularly focused on expression changes related to (1) glucose metabolism and (2) ion exchange, which are required for acid production and release in order to switch on the Root effect for gas secretion [17,18]; (3) angiogenesis, required for appropriate blood supply to the swimbladder [23]; (4) ROS defense, required to avoid oxidative stress related to hyperbaric oxygen tensions [32,34–36]; (5) extracellular matrix, involved in reducing diffusional gas loss from the swimbladder [23–25]; (6) immune response, required to defeat the nematode infection [28,37]; and (7) maturation, which occurs in silver eels during spawning migration [38], because these aspects have been addressed in a previous study, analyzing the transcriptional changes related to silvering [26].
Materials and methods

Animals

All experiments were performed with European eels (*Anguilla anguilla*). Uninfected yellow eels were caught by local fishermen in Lake Constance, Bregenz, Austria (N 47° 30’ 54”, E 9° 44’ 35”), and kept in an outdoor freshwater basin at the Institute of Zoology at the University of Innsbruck, until sampling. Infected yellow eels were caught by local fishermen in the River Elbe, close to Winsen (Luhe), Germany (N 53° 24’ 7.7”, E 10° 9’ 27.9”), and kept in an outdoor freshwater basin at the Thu¨nen Institute of Fisheries Ecology, Ahrensburg, Germany, until sampling. All silver eels were caught by local fishermen in the IJsselmeer, The Netherlands (N 52° 49’ 50”, E 5° 25’ 47”), and kept in large tanks at Leiden University until sampling. Recent studies have shown that the European eel is a panmictic species [39,40] and therefore we assumed that the different sampling points should not bias the results of this study. Table 1 shows the morphometrics of the animals, chosen for the experiments, with the silvering index calculated according to Durif et al. [41], and the ocular index calculated according to Pankhurst [42].

Only swimbladders showing no sign of infection (0 or 1 parasite inside the bladder) or heavily infected swimbladders were selected for the analysis (Table 1). The swimbladder of all infected eels had a similar appearance: thickened, multilayered swimbladder epithelium, exudate inside the bladder, almost no gas filling. We did not include tissue of swimbladders in a transitional state, i.e. with only few nematodes or one or more of the criteria mentioned before (thickened, multilayered swimbladder epithelium; exudate inside the bladder; almost no gas filling) not fulfilled.

Tissue preparation

Eels were either killed with an overdose of neutralized tricaine methanesulfonate (MS-222; Sigma-Aldrich, St. Louis, MO, USA), or anesthetized with MS-222 and subsequently decerebrated and spinally pithed. The swimbladder was dissected, freed from connective tissue to reveal the actual gas gland tissue, cleaned from *Anguillicola crassus* specimen if necessary, immediately shock frozen in liquid nitrogen, and stored at -80°C until further use. Infected swimbladders contained between 5 and 30 parasites, and the swimbladder wall was markedly thickened and nontransparent as stated previously [30]. Tissue sampling was performed in compliance with the Austrian law, the guidelines of the Austrian Federal Minister for Education, Arts, and Culture, and also the Dutch and German law. The tissue sampling procedure was approved by the Tierversuchskommission of the University of Innsbruck.

### Table 1. Morphometrics, silvering index according to Durif et al. [41], and ocular index according to Pankhurst [42].

|                      | Uninfected yellow | Infected yellow | Uninfected silver | Infected silver |
|----------------------|-------------------|-----------------|-------------------|-----------------|
| Body mass (g)        | 339.33 ± 7.89     | 235.60 ± 30.77  | 1437.36 ± 472.69  | 830.77 ± 56.21  |
| Body length (cm)     | 59.33 ± 1.36      | 51.80 ± 2.18    | 82.72 ± 6.08      | 73.20 ± 2.20    |
| Pectoral fin length (mm) | 23.30 ± 0.46   | 22.92 ± 1.40    | 38.08 ± 2.28      | 36.13 ± 1.11    |
| Horizontal eye diameter (mm) | 5.73 ± 0.35 | 5.94 ± 0.38     | 10.42 ± 0.74      | 10.02 ± 0.34    |
| Vertical eye diameter (mm) | 5.43 ± 0.21 | 5.80 ± 0.33     | 10.44 ± 0.70      | 9.83 ± 0.14     |
| Silvering index      | 2.00 ± 0.00       | 2.40 ± 0.22     | 4.00 ± 0.32       | 4.17 ± 0.28     |
| Ocular index         | 4.16 ± 0.41       | 5.24 ± 0.42     | 10.35 ± 0.72      | 10.67 ± 0.62    |
| Number of parasites  | 0                 | 16.7 ± 3.4      | 0.4 ± 0.2         | 11.8 ± 2.5      |

Uninfected yellow eels (N = 7), infected yellow eels (N = 5), uninfected silver eels (N = 5), and infected silver eels (N = 6). Overall mean values ± S.E.M.

https://doi.org/10.1371/journal.pone.0183128.t001
RNA isolation and Illumina RNASeq analysis

Total RNA was isolated from gas gland tissue using the Qiagen miRNeasy kit (Qiagen, Venlo, Netherlands) as established and described in detail in a previous study [26]. Briefly, quality and integrity of the isolated RNA were checked on an Agilent Bioanalyzer 2100 total RNA Nano series II chip (Agilent, Amstelveen, Netherlands). Illumina RNAseq libraries were prepared from 2 μg total RNA using the Illumina TruSeq™ RNA Sample Prep Kit v2 according to the manufacturer’s instructions (Illumina Inc. San Diego, CA, USA). All RNAseq libraries (150–750 bp inserts) were sequenced on an Illumina HiSeq2500 sequencer as 2 × 50 nucleotides paired-end reads according to the manufacturer’s protocol. Image analysis and base calling were done using the Illumina pipeline [43,44]. The data discussed in this publication have been deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE102221 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102221).

Illumina data processing

Data processing was performed as described previously [26,43,44]. Briefly, reads (10–20 million per sample) were aligned to the draft genome sequence of European eel [45], using TopHat (version 2.0.5) [46]. Secondary alignments of reads were excluded by filtering the files using SAMtools (version 0.1.18) [47]. Aligned fragments per predicted gene were counted from SAM alignment files using the Python package HTSeq (version 0.5.3p9) [48]. In order to make comparisons across samples possible, these fragment counts were corrected for the total amount of sequencing performed for each sample. As a correction scaling factor, library size estimates determined using the R/Bioconductor (release 2.11) package DESeq [49] were employed. Read counts were normalized by dividing the raw counts obtained from HTSeq by its scale factor. Detailed read coverage for individual genes was extracted from the TopHat alignments using SAMtools. Differentially expressed genes between uninfected yellow and infected yellow eels and also between uninfected silver and infected silver eels were identified using DESeq, the cut-off for significance was set to P < 0.01. Gene ontology annotations were used for a detailed pathway and biological process analysis of differentially expressed genes.

Results

General observations

Comparing uninfected and infected yellow and silvers eels, even at a significance level of p < 0.01 a large number of genes showed different expression levels, especially in yellow eels. In yellow eel gas gland tissue, an Anguillicola crassus infection resulted in 1675 differentially transcribed genes of which 1138 were upregulated and 537 were downregulated. In silver eels, the infection resulted in only 291 differentially transcribed genes of which 169 were upregulated and 122 were downregulated (Fig 1). Ninety-nine genes were transcribed differentially in infected yellow eels as well as in infected silver eels. Twenty-two genes were differently affected in infected yellow and silver eels (Fig 2; Table 2). Thirteen of these genes were upregulated in yellow eels but downregulated in silver eels, and 9 genes were downregulated in yellow eels but upregulated in silver eels.

Elevated in infected silver eels, but expressed at a lower level in infected yellow eels were NADPH oxidase oxygenizer 1 (noxox1) and two Ca^{2+} binding proteins, C2 calcium-dependent domain containing protein 4c (c2c4c) and the ef-hand calcium-binding protein 1 (neca1).
Elevated in infected yellow eels but reduced in infected silver eel gas gland tissue due to the infection with *Anguillicola crassus*, and the number of genes affected in both groups (green). The lower part shows the numbers of genes either up- or downregulated. Diagrams were generated with Venn Diagram Plotter (https://omics.pnl.gov/software/venn-diagram-plotter).

Fig 3 shows the results of a GO enrichment analysis for GO biological processes and GO molecular function, focusing on the 10 categories with the largest number of hits, and combining the remaining genes as ‘others’. With respect to biological processes, a very large number of diverse processes showed a number of genes with modified expression levels, so that in infected yellow and silver eels 92.5% and 91.2% of the modified genes, respectively, were combined as ‘others’. Processes affected in both, infected yellow and silver eels, included ‘signal transduction’, ‘multicellular organismal development’, ‘immune response’, ‘cell adhesion’, ‘transport’, ‘cell differentiation’, and ‘nervous system development’. Processes included in the 10 categories with a larger number of hits in infected yellow eels, but not in silver eels, were ‘apoptosis’, ‘regulation of transcription’, and ‘response to drug’. In infected silver eels, in turn, ‘proteolysis’, ‘inflammatory response’, and ‘G-protein coupled receptor protein signaling pathway’ were among the 10 categories with a larger number of hits. The same analysis for GO molecular function revealed less diversity, and 75.2% and 76.2% of the genes were listed as ‘others’ in infected yellow and infected silver eels, respectively. The molecular function with the largest number of hits was ‘protein binding’, contributing 9.5% and 8.8% to the total number of modified genes in infected yellow and infected silver eels, respectively. ‘DNA binding’ was among the 10 categories with the largest number of hits in infected yellow eels, but not in silver eels, and ‘transferase activity’ was among the 10 top categories in infected silver eels, but not in yellow eels.
Transcriptional changes in yellow eel gas gland tissue related to the nematode infection

As the next step, we performed the GO enrichment analysis focusing on genes of specific functional categories expected to be important for swimbladder function, i.e. glucose and lactate metabolism, ROS defense, ion transport, extracellular matrix, and vasculogenesis and angiogenesis. We also included immune defense and maturation, which have been reported to be important categories in a previous study [26]. Especially in yellow eels, a large number of genes were affected in the expression level. We therefore restricted our analysis to genes showing at least a 3-fold difference in the mRNA expression level.

**Glucose and lactate metabolism.** In gas gland tissue of infected yellow eels, 4 genes involved in monocarboxylate transport and glucose transport showed a significantly higher mRNA expression level than in uninfected yellow eels (Table 3). In addition, the mRNA level of fructose-bisphosphate aldolase A increased 5.66-fold, while the glucokinase mRNA level decreased 12.5-fold.

**ROS defense.** Also important for swimbladder function is ROS defense to avoid tissue damage due to high oxygen partial pressures, and 40 genes related to ROS were affected in their mRNA expression level in infected yellow eels (Table 4). The expression level of several transcription factors was significantly increased (fosb; fos; junb), and at least two copies of each of these transcription factors were affected. The expression level of one copy of fos and
one of fosb was elevated more than 20-fold. Cytochrome b-245 heavy chain (cy24b) and cytochrome p450 1b1 (cp1b1) were found with elevated expression levels.

Ion transport. With respect to ion transport, 56 genes showed modified expression levels in infected yellow eels, and only 18 of these were reduced (S1 Table). In addition to monocarboxylate transporter 1, which was present at very high levels in infected yellow eel gas gland tissue, two amino acid transporters were elevated almost 4-fold (y+1 amino acid transporter 2, ylat2; and sodium-dependent neutral amino acid transporter b at1; s6a19). In infected yellow eel gas gland tissue, a large number of Na\(^+\), K\(^+\), or Cl\(^-\)-transporting proteins were expressed with significantly modified mRNA levels: orphan sodium and chloride-dependent neurotransmitter transporter ntt73, s6a15; voltage-dependent anion-selective channel protein 2, vdac2; transient receptor potential cation channel subfamily a member 1, trpa1; solute carrier family 12 member 2, s12a2; electrogenic sodium bicarbonate cotransporter 1, s4a4; potassium voltage-gated channel subfamily c member 1, kcnc1; chloride channel protein 2, clcn2; calcium-activated potassium channel subunit alpha-1, kcnal1; solute carrier family 12 member 5, s12a5; cystic fibrosis transmembrane conductance regulator, cfr; sodium channel protein type 5 subunit alpha, scn5a; amiloride-sensitive cation channel neuronal, accn1; solute carrier family 13 member 3, s13a3. Seven of these genes showed an increased expression level, while 6 of these transporters, like cfr, clcn2, and s12a5, showed a reduced expression level. Interestingly, sodium potassium-transporting ATPase subunit beta-2 (at1b2) also showed a more than 8-fold reduction in the expression level.

### Table 2. Differentially transcribed and contradictorily regulated genes in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.

| Gene   | Name                   | Description                          | Yellow | Silver |
|--------|------------------------|--------------------------------------|--------|--------|
| g26738 | hfe                    | hereditary hemochromatosis protein    | 0.41   | 24.64  |
| g24844 | rergl                  | ras-related and estrogen-regulated growth inhibitor-like protein | 0.41   | 4.38   |
| g11737 | noxo1                  | nadph oxidase organizer 1            | 0.38   | 15.96  |
| g2232  | unt13c                 | protein unc-13 homolog c             | 0.35   | 7.78   |
| g27646 | c2c4c                  | c2 calcium-dependent domain-containing protein 4c | 0.26   | 40.43  |
| g28445 | st17a                  | serine threonine-protein kinase 17a  | 0.22   | 9.56   |
| g16142 | licn1                  | testican-1                           | 0.21   | 16.89  |
| g11645 | neca1                  | n-terminal el-hand calcium-binding protein 1 | 0.15   | 13.17  |
| g17980 | irg1                   | immune-responsive gene 1 protein     | 0.04   | 3.66   |
| g14663 | cp1b1                  | cytochrome p450 1b1                   | 11.08  | 0.22   |
| g8112  | rimb2                  | rims-binding protein 2               | 9.59   | 0.25   |
| g5564  | s39ac                  | zinc transporter zip12               | 7.17   | 0.22   |
| g28431 | mpl3c                  | microtubule-associated proteins 1a 1b light chain 3c | 5.25   | 0.03   |
| g12939 | cdkn3                  | cyclin-dependent kinase inhibitor 3  | 4.49   | 0.09   |
| g26398 | nptx1                  | neuronal pentraxin-1                 | 3.88   | 0.09   |
| g850   | degs2                  | sphingolipid delta-desaturase c4-hydroxylase des2 | 3.42   | 0.15   |
| g14690 | cpas1                  | circularly permuted ras protein 1     | 2.84   | 0.24   |
| g26753 | cxl11                  | c-x-c motif chemokine 11             | 2.71   | 0.17   |
| g9811  | ctl2b                  | protein cta-2-beta                   | 2.55   | 0.13   |
| g18466 | akr                    | homeobox protein akr                  | 2.49   | 0.11   |
| g9993  | a33                    | zinc-binding protein a33             | 2.44   | 0.26   |
| g20093 | pclo                   | protein piccolo                      | 2.13   | 0.23   |

Fold cha. = Fold change; pval = 0.000 indicates P values < 0.0005

https://doi.org/10.1371/journal.pone.0183128.t002
Fig 3. Most important targets of an *Anguillicola crassus* infection. The ten most prominent biological processes, affected by the infection with *Anguillicola crassus* in yellow (A) and silver eel (B) gas gland tissue, respectively. The ten most prominent molecular functions, affected by the infection with *Anguillicola crassus* in yellow (C) and silver eel (D) gas gland tissue, respectively.

https://doi.org/10.1371/journal.pone.0183128.g003

Table 3. Differentially transcribed genes (fold change >3) based on GO terms “glucose metabolism” or “lactate metabolism” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.

| Gene     | Name          | Description                              | Yellow Fold cha. | pval | Silver Fold cha. | pval |
|----------|---------------|------------------------------------------|------------------|------|------------------|------|
| g42062   | sc5a8         | sodium-coupled monocarboxylate transporter 1 | Inf              | 0.000|                  |      |
| g14954   | sc5a8         | sodium-coupled monocarboxylate transporter 1 | 18.00            | 0.002|                  |      |
| g21440   | aldoa         | fructose-bisphosphate aldolase a          | 5.66             | 0.000|                  |      |
| g13449   | gtr5          | solute carrier family facilitated glucose transporter member 5 | 5.12             | 0.000|                  |      |
| g42042   | tec           | tyrosine-protein kinase tec               | 4.44             | 0.006|                  |      |
| g27889   | fyn           | tyrosine-protein kinase fyn               | 3.46             | 0.000|                  |      |
| g23774   | ppara         | peroxisome proliferator-activated receptor alpha | 0.31             | 0.005|                  |      |
| g21936   | npas4         | neuronal pas domain-containing protein 4  | 0.29             | 0.003|                  |      |
| g3113    | hlx4          | glucokinase                              | 0.08             | 0.009|                  |      |
| g12848   | gtr3          | solute carrier family facilitated glucose transporter member 3 | 3.18             | 0.000| 6.18             | 0.003|
| g7770    | mt12b         | monocarboxylate transporter 12-b         | 4.91             | 0.006|                  |      |
| g22031   | acs2l         | acetyl-coenzyme a synthetase 2- mitochondrial | 4.37             | 0.007|                  |      |
| g21839   | k6pf          | 6- muscle type                           | 0.14             | 0.008|                  |      |

Fold cha. = Fold change; pval = 0.000 indicates P values < 0.0005

https://doi.org/10.1371/journal.pone.0183128.t003
Table 4. Differentially transcribed genes (fold change >3) based on GO terms related to “ROS defense” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.

| Gene | Name | Description | Yellow | Silver |
|------|------|-------------|--------|--------|
| g38341 | angi7 | angiopoietin-related protein 7 | Inf | 0.004 |
| g11898 | fosb | protein fosb | 21.55 | 0.000 |
| g12410 | fos | proto-oncogene c-fos | 20.06 | 0.000 |
| g24314 | dscam | down syndrome cell adhesion molecule homolog flags: precursor | 11.14 | 0.005 |
| g5407 | hspbb | heat shock protein beta-11 | 10.00 | 0.000 |
| g5637 | cy24b | cytochrome b-245 heavy chain | 9.01 | 0.001 |
| g10399 | plcz1 | 1-phosphatidylinositol- bisphosphate phosphodiesterase zeta-1 | 8.85 | 0.003 |
| g16623 | tutlb | protein turtle homolog b | 5.62 | 0.004 |
| g5642 | dus2 | dual specificity protein phosphatase 2 | 5.15 | 0.000 |
| g12409 | fos | proto-oncogene c-fos | 4.96 | 0.000 |
| g5410 | hspbb | heat shock protein beta-11 | 4.84 | 0.000 |
| g12220 | rir2 | ribonucleoside-diphosphate reductase subunit m2 | 4.78 | 0.000 |
| g11400 | junb | transcription factor jun-b | 4.20 | 0.000 |
| g6522 | trpa1 | transient receptor potential cation channel subfamily a member 1 | 4.13 | 0.005 |
| g3425 | plcb2 | 1-phosphatidylinositol- bisphosphate phosphodiesterase beta-2 | 4.08 | 0.002 |
| g12361 | junb | transcription factor jun-b | 4.06 | 0.000 |
| g7816 | hmox | heme oxygenase | 4.05 | 0.000 |
| g17581 | lox5 | arachidonate 5-lipoxygenase | 3.86 | 0.001 |
| g3322 | fos | proto-oncogene c-fos | 3.70 | 0.000 |
| g15583 | nud17 | nucleoside diphosphate-linked moiety x motif 17 | 3.38 | 0.000 |
| g17446 | paxi | Paxilin | 3.35 | 0.000 |
| g12497 | kpcb | protein kinase c beta type | 3.34 | 0.004 |
| g10644 | arrd4 | arrestin domain-containing protein 4 | 3.26 | 0.000 |
| g5757 | plcg2 | 1-phosphatidylinositol- bisphosphate phosphodiesterase gamma-2 | 3.23 | 0.000 |
| g4039 | cdk1 | cell division protein kinase 1 | 3.19 | 0.000 |
| g24609 | kcc1d | calcium calmodulin-dependent protein kinase type 1d | 3.19 | 0.001 |
| g14921 | ets1a | protein c-ets-1-a | 3.11 | 0.003 |
| g7735 | cy24b | cytochrome b-245 heavy chain | 3.07 | 0.000 |
| g28565 | rir2 | ribonucleoside-diphosphate reductase subunit m2 | 3.03 | 0.005 |
| g7976 | adam9 | disintegrin and metalloproteinase domain-containing protein 9 | 3.03 | 0.001 |
| g26034 | cp27a | sterol 26- mitochondrial | 3.02 | 0.000 |
| g21936 | npas4 | neuronal pas domain-containing protein | 0.99 | 0.003 |
| g17084 | achb2 | neuronal acetylcholine receptor subunit beta-2 | 0.24 | 0.005 |
| g14480 | myh7 | myosin-7 | 0.16 | 0.000 |
| g2246 | mk10 | mitogen-activated protein kinase 10 | 0.14 | 0.000 |
| g2030 | pa24c | cytosolic phospholipase a2 gamma | 0.12 | 0.000 |
| g21973 | nptx1 | neuronal pentraxin-1 | 0.07 | 0.000 |
| g14663 | cp1b1 | cytochrome p450 1b1 | 11.08 | 0.000 |
| g26398 | nptx1 | neuronal pentraxin-1 | 3.88 | 0.004 |
| g12711 | mmp9 | matrix metalloproteinase-9 | 3.64 | 0.000 |
| g26738 | hfe | hereditary hemochromatosis protein | 24.64 | 0.000 |
| g19822 | co5a1 | collagen alpha-1 chain flags: precursor | 0.33 | 0.005 |
| g24694 | mmp17 | matrix metalloproteinase-17 | 0.08 | 0.000 |

Fold cha. = Fold change; pval = 0.000 indicates P values < 0.0005

https://doi.org/10.1371/journal.pone.0183128.t004
Table 5. Differentially transcribed genes (fold change >3) based on GO terms related to “extracellular matrix” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.

| Gene   | Name            | Description                                      | Yellow |         | Silver |         |
|--------|-----------------|--------------------------------------------------|--------|---------|--------|---------|
| g16623 | tutlb protein   | protein turtle homolog b                         | 5.62   | 0.004   | 16.49  | 0.000   |
| g22618 | chia acidic mammalian chitinase                | 5.05    | 0.000   |         |         |
| g7750  | tsp4b thrombospondin-4-b                       | 4.97    | 0.000   |         |         |
| g1692  | tsp1 thrombospondin-1 flags: precursor         | 4.84    | 0.000   |         |         |
| g14358 | co6a6 collagen alpha-6 chain flags: precursor | 3.40    | 0.000   |         |         |
| g5094  | scub3 signal cub and egf-like domain-containing protein 3 flags: precursor | 3.38    | 0.010   |         |         |
| g12721 | smc2 structural maintenance of chromosomes protein 2 | 3.32    | 0.000   |         |         |
| g12271 | cspg2 versican core protein                    | 3.12    | 0.000   |         |         |
| g12322 | impg2 interphotoreceptor matrix proteoglycan 2 | 0.20    | 0.001   |         |         |
| g23617 | chia acidic mammalian chitinase                | 6.21    | 0.000   |         |         |
| g34568 | muc5a mucin-5ac                                  | 3.02    | 0.002   | 24.74  | 0.000   |
| g24192 | muc5b mucin-5b                                   | 41.31   | 0.000   |         |         |
| g35363 | muc5a mucin-5ac                                  | 21.34   | 0.001   |         |         |
| g28800 | muc5b mucin-5b                                   | 19.61   | 0.000   |         |         |
| g18964 | muc5a mucin-5ac c                                | 13.31   | 0.000   |         |         |
| g19822 | co5a1 collagen alpha-1 chain flags: precursor  | 0.33    | 0.005   |         |         |
| g17364 | tecta alpha-tectorin flags: precursor           | 0.04    | 0.004   |         |         |

Fold cha. = Fold change; pval = 0.000 indicates P values < 0.0005

https://doi.org/10.1371/journal.pone.0183128.t005

**Extracellular matrix.** The mRNA expression level of 11 genes was modified in infected yellow eel gas gland tissue, and all but one were elevated (Table 5). Connective tissue growth factor (ctgf) was more than 5-fold elevated, and the level of collagen alpha 6 (co6a6) and versican core protein (cspg2) was increased. Acidic mammalian chitinase (chia) was 5-6-fold elevated. Similarly, thrombospondin-1 (tsp1) and thrombospondin-4b (tsp4b) were almost 5-fold elevated. Of the various mucin genes only mucin 5ac (muc5a) was 3-fold elevated.

**Angiogenesis or vasculogenesis.** In infected yellow eels, 51 genes related to angiogenesis or vasculogenesis were modified, and only 9 of these genes were reduced in their expression level (Table 6). Expression of angiopoietin-related protein 7 (angl7), signal cub and egf-like domain-containing protein 3 (scub3), bone morphogenetic protein 1 (bmp1), and several copies of thrombospondin (tsp1; tsp4b) were expressed at a significantly higher level.

**Immune defense.** The largest number of genes affected by the infection of the swimbladder with the nematode was related to immune defense. In infected yellow eels, 167 genes were modified in their expression level, and only 24 of these genes were reduced in their expression level (Table 7). Many-fold elevated in their expression level were genes coding for immunoglobulin light chain, immunoglobulin heavy chain variable region, complement proteins (co3; cfah; fhr2; c1r; co4a; co7), several interleukins (interleukin 12subunit beta, il12b; interleukin-18 receptor 1, il18r; interleukin-6 receptor subunit beta, il6rb; interleukin-17 receptor b, il17rb), and interferon regulatory factor (irf4). In addition, several heat shock proteins showed increased mRNA expression levels (heat shock 70 kda, hsp70; heat shock protein beta, hspbb; heat shock protein 105 kda, hs105).

**Maturation.** Of the genes related to maturation, 67 were modified in their expression level, and 15 of these genes showed reduced expression levels (S2 Table). Two copies of fer-1-like protein 4 (frl4) were 16- and 25-fold elevated in the mRNA expression level. A number...
Table 6. Differentially transcribed genes (fold change >3) based on GO terms “angiogenesis” or “vasculogenesis” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.

| Gene   | Name   | Description                                                                 | Yellow |          | Silver |          |
|--------|--------|------------------------------------------------------------------------------|--------|----------|--------|----------|
|        |        |                                                                               | Fold cha. | pval    | Fold cha. | pval    |
| angi7  | g38341 | angiopoietin-related protein 7                                              | Inf    | 0.004    |         |         |
| cy24b  | g6637  | cytochrome b-245 heavy chain                                                | 9.01   | 0.001    |         |         |
| plcz1  | g10399 | 1-phosphatidylinositol- bisphosphate phosphodiesterase zeta-1               | 8.85   | 0.003    |         |         |
| hnr2   | g17733 | complement factor h-related protein 2                                        | 7.64   | 0.000    |         |         |
| tsp1   | g1691  | thrombospondin-1 flags; precursor                                           | 6.46   | 0.000    |         |         |
| agr2   | g8980  | type-2 angiotensin ii receptor                                               | 5.26   | 0.001    |         |         |
| ctgf   | g3764  | connective tissue growth factor                                              | 5.14   | 0.000    |         |         |
| tsp4b  | g7750  | thrombospondin-4-b                                                          | 4.97   | 0.000    |         |         |
| tsp1   | g1692  | thrombospondin-1 flags; precursor                                           | 4.84   | 0.000    |         |         |
| cxc4r4 | g17339 | c-x-c chemokine receptor type 4                                              | 4.66   | 0.000    |         |         |
| tec    | g42042 | tyrosine-protein kinase tec                                                  | 4.44   | 0.006    |         |         |
| frem2  | g23394 | fras1-related extracellular matrix protein 2                                | 4.21   | 0.000    |         |         |
| junb   | g11400 | transcription factor jun-b                                                   | 4.20   | 0.000    |         |         |
| co7    | g20805 | complement component c7 flags; precursor                                    | 4.11   | 0.000    |         |         |
| plcb2  | g3425  | 1-phosphatidylinositol- bisphosphate phosphodiesterase beta-2               | 4.08   | 0.002    |         |         |
| junb   | g12361 | transcription factor jun-b                                                   | 4.06   | 0.000    |         |         |
| hmox   | g7816  | heme oxygenase                                                              | 4.05   | 0.000    |         |         |
| cy61   | g4725  | protein cyr61                                                                | 4.00   | 0.000    |         |         |
| cyt1f  | g16847 | cystatin-f                                                                   | 3.98   | 0.000    |         |         |
| lyve1  | g11223 | lymphatic vessel endothelial hyaluronic acid receptor 1                      | 3.97   | 0.000    |         |         |
| myo1f  | g21342 | myosin-if                                                                    | 3.96   | 0.000    |         |         |
| sem3c  | g513   | semaphorin-3c                                                                | 3.96   | 0.001    |         |         |
| sem4b  | g21035 | semaphorin-4b flags; precursor                                              | 3.81   | 0.000    |         |         |
| c3p1   | g27449 | protein c3p1                                                                 | 3.80   | 0.000    |         |         |
| myo1f  | g20848 | myosin-if                                                                    | 3.73   | 0.000    |         |         |
| myo1f  | g29877 | myosin-if                                                                    | 3.68   | 0.004    |         |         |
| angi7  | g8377  | angiopoietin-related protein 7                                              | 3.65   | 0.001    |         |         |
| itb2   | g35688 | integrin beta-2                                                              | 3.63   | 0.000    |         |         |
| ptprh  | g12898 | receptor-type tyrosine-protein phosphatase h                                 | 3.47   | 0.000    |         |         |
| cno73  | g5296  | sec6-like protein c14orf73                                                  | 3.44   | 0.000    |         |         |
| scub3  | g5094  | signal cub and egf-like domain-containing protein 3 flags; precursor         | 3.38   | 0.010    |         |         |
| par11  | g18110 | poly polymerase 11                                                           | 3.36   | 0.002    |         |         |
| bmp1   | g9669  | bone morphogenetic protein 1                                                | 3.34   | 0.000    |         |         |
| myo1e  | g21341 | myosin-ie                                                                    | 3.14   | 0.000    |         |         |
| myo1f  | g20847 | myosin-if                                                                    | 3.12   | 0.000    |         |         |
| ets1a  | g14921 | protein c-ets-1-a                                                            | 3.11   | 0.003    |         |         |
| cy24b  | g7735  | cytochrome b-245 heavy chain                                                | 3.07   | 0.000    |         |         |
| co4    | g26070 | complement c4 contains:                                                     | 3.06   | 0.000    |         |         |
| sh2d7  | g1347  | sh2 domain-containing protein 7                                              | 3.05   | 0.010    |         |         |
| ccl20  | g11499 | c-c motif chemokine 20                                                       | 3.05   | 0.000    |         |         |
| f13a   | g22834 | coagulation factor xiii a chain                                              | 0.33   | 0.000    |         |         |
| npas4  | g21936 | neuronal pas domain-containing protein 4                                     | 0.29   | 0.003    |         |         |
| tnni2  | g3471  | troponin fast skeletal muscle                                                | 0.13   | 0.000    |         |         |
| cramp  | g13604 | cathelin-related antimicrobial peptide                                       | 0.13   | 0.000    |         |         |
| dlld   | g6339  | delta-like protein d                                                         | 0.12   | 0.000    |         |         |

(Continued)
of genes listed under the GO term maturation has also been listed under different GO terms, like, for example angi7; protein fsb, fos, hspbb, tsp1, tsp4b, gtr5, cfrtr.

### Transcriptional changes in silver eel gas gland tissue related to the nematode infection

**Glucose metabolism.** Overall, 10 genes of glucose and lactate metabolism were affected by the infection in yellow eels, while only 4 genes were affected in silver eels (Table 3). None of the genes involved in glycolysis was affected in infected silver eels, and only one glucose transport and one monocarboxylate transporter showed a higher mRNA expression level.

**ROS defense.** While 40 genes related to ROS were affected in the mRNA expression level in infected yellow eels, only 6 genes were affected in infected silver eels (Table 4). Among these 6 genes matrix metalloproteinase-9 (mmp9) and hereditary hemochromatosis protein (hfe) showed a more than 20-fold increased expression level in infected silver eels, while the other 4 genes showed largely reduced expression levels. Cytochrome p450 1b1 (cp1b1), which was significantly elevated in infected yellow eels, was about 5-fold downregulated in infected silver eels.

**Ion transport.** In infected silver eels gas gland tissue, 19 genes showed modified expression levels, with 5 downregulated and 14 upregulated genes (S1 Table). Only 2 ion transport
| Gene | Name | Description | Yellow | Silver |
|------|------|-------------|--------|--------|
|      |      |             | Fold cha. | pval   |
|      |      |             | pval   | Fold cha. | pval   |
| g38341 | angi7 | angiopoietin-related protein 7 | Inf     | 0.004  |
| g13085 | mlrv  | myosin regulatory light chain ventricular cardiac muscle isoform | 26.82   | 0.005  |
| g11898 | fosb  | protein fosb | 21.55   | 0.000  |
| g16766 | i23o1 | indoleamine -dioxygenase 1 | 18.77   | 0.001  |
| g43990 | ns1ba | influenza virus ns1a-binding protein homolog a | 16.15   | 0.002  |
| g14949 | co3   | complement c3 contains: | 12.91   | 0.000  |
| g38820 | gima1 | gtpase imap family member 1 | 12.32   | 0.008  |
| g41857 | immunoglobulin heavy chain variable region | 12.27   | 0.000  |
| g31601 | immunoglobulin light chain | 12.00   | 0.008  |
| g34285 | lv302 | ig lambda chain v-iii region loi | 10.25   | 0.000  |
| g37595 | hsp70 | heat shock 70 kda protein | 10.04   | 0.000  |
| g5407  | hspbb | heat shock protein beta-11 | 10.00   | 0.001  |
| g6637  | cy24b | cytochrome b-245 heavy chain | 9.01    | 0.000  |
| g34987 | fucl4 | fucolectin-4 flags: precursor | 8.42    | 0.000  |
| g30502 | gima4 | gtpase imap family member 4 | 8.37    | 0.000  |
| g2443  | rg54  | regulator of g-protein signaling 4 | 8.15    | 0.000  |
| g34201 | mhc class i antigen | 7.87    | 0.000  |
| g22923 | cfah  | complement factor h | 7.80    | 0.000  |
| g17733 | fhr2  | complement factor h-related protein 2 | 7.64    | 0.000  |
| g9234  | c1tqf | c1tq-related factor | 7.61    | 0.010  |
| g10835 | hs105 | heat shock protein 105 kda | 7.57    | 0.000  |
| g42422 | igkc  | ig kappa chain c region | 7.56    | 0.000  |
| g24709 | vsig1 | v-set and immunoglobulin domain-containing protein 1 | 7.55    | 0.005  |
| g20323 | il12b | interleukin-12 subunit beta | 7.21    | 0.000  |
| g35498 | immunoglobulin light chain | 6.86    | 0.000  |
| g9306  | dnjb4 | homolog subfamily b member 4 | 6.64    | 0.000  |
| g1691  | tsp1  | thrombospondin-1 flags: precursor | 6.46    | 0.000  |
| g16469 | tri69 | tripartite motif-containing protein 69 | 6.12    | 0.000  |
| g26875 | lysc  | lysozyme c | 5.86    | 0.000  |
| g10077 | c1r   | complement c1r subcomponent | 5.62    | 0.000  |
| g16623 | tuttb | protein turtle homolog b | 5.62    | 0.004  |
| g20076 | cd3g  | t-cell surface glycoprotein cd3 gamma chain | 5.58    | 0.000  |
| g6900  | nfac2 | nuclear factor of activated t- cytoplasmic 2 | 5.46    | 0.000  |
| g39678 | irf4  | interferon regulatory factor 4 | 5.38    | 0.000  |
| g24053 | gima4 | gtpase imap family member 4 | 5.21    | 0.000  |
| g36156 | ha1d  | h-2 class i histocompatibility k-d alpha chain | 5.16    | 0.000  |
| g11916 | tcc4  | t-cell receptor gamma chain c region 5 10–13 | 5.09    | 0.000  |
| g12086 | l3bp  | galectin-3-binding protein b | 5.06    | 0.000  |
| g22618 | chia  | acidic mammalian chitinase | 5.05    | 0.000  |
| g24469 | elf3  | ets-related transcription factor elf-3 | 5.02    | 0.001  |
| g42742 | mhc class i antigen | 5.00    | 0.000  |
| g1342  | dnja4 | homolog subfamily a member 4 | 4.99    | 0.000  |
| g7750  | tsp4b | thrombospondin-4-b | 4.97    | 0.000  |
| g23321 | ccr9  | c-c chemokine receptor type 9 | 4.97    | 0.000  |
| g12409 | fos   | proto-oncogene c-fos | 4.96    | 0.000  |

(Continued)
Table 7. (Continued)

| Gene   | Name               | Description                          | Yellow     | Silver    |
|--------|--------------------|--------------------------------------|------------|-----------|
|        |                    |                                      | Fold cha.  | pval      | Fold cha. | pval      |
| g26051 | zap70              | tyrosine-protein kinase zap-70       | 4.91       | 0.000     |           |           |
| g5410  | hspbβ              | heat shock protein beta-11           | 4.84       | 0.000     |           |           |
| g1493  | rgsβ               | regulator of g-protein signaling 8   | 4.83       | 0.000     |           |           |
| g23965 | lac6               | Ig lambda-6 chain c region           | 4.72       | 0.000     |           |           |
| g10121 | ick                | tyrosine-protein kinase ick          | 4.67       | 0.000     |           |           |
| g1848  | ccl25              | c-c motif chemokine 25               | 4.66       | 0.000     |           |           |
| g17339 | cxcr4              | c-x-c chemokine receptor type 4      | 4.66       | 0.000     |           |           |
| g40079 | immunoglobulin light chain |                         | 4.66       | 0.000     |           |           |
| g278   | tnfa               | tumor necrosis factor               | 4.64       | 0.004     |           |           |
| g32441 | smp                | schwann cell myelin protein          | 4.62       | 0.000     |           |           |
| g10172 | slap2              | src-like-adapter 2                   | 4.59       | 0.000     |           |           |
| g19466 | irf4               | interferon regulatory factor 4       | 4.54       | 0.000     |           |           |
| g23648 | sepr               | seprase                             | 4.52       | 0.000     |           |           |
| g45177 | l3bpβ              | galectin-3-binding protein b         | 4.52       | 0.000     |           |           |
| g21248 | ccl4               | c-c motif chemokine 4                | 4.44       | 0.000     |           |           |
| g14635 | gima7              | gtpase imap family member 7          | 4.44       | 0.000     |           |           |
| g42042 | tec                | tyrosine-protein kinase tec          | 4.44       | 0.000     |           |           |
| g12085 | l3bpβ              | galectin-3-binding protein b         | 4.43       | 0.000     |           |           |
| g34980 | lysc3              | lysozyme c-3                        | 4.37       | 0.000     |           |           |
| g8265  | frim               | middle subunit short = ferritin m    | 4.29       | 0.000     |           |           |
| g41703 | zap70              | tyrosine-protein kinase zap-70       | 4.23       | 0.001     |           |           |
| g23394 | frem2              | fras1-related extracellular matrix protein 2 | 4.21       | 0.000     |           |           |
| g1769  | il2rg              | cytokine receptor common subunit gamma | 4.20       | 0.000     |           |           |
| g40915 | gima4              | gtpase imap family member 4          | 4.12       | 0.000     |           |           |
| g20805 | co7                | complement component c7 flags: precursor | 4.11       | 0.000     |           |           |
| g22235 | ibp3               | insulin-like growth factor-binding protein 3 | 4.06       | 0.000     |           |           |
| g7816  | hmox               | heme oxygenase                       | 4.05       | 0.000     |           |           |
| g1450  | cd22               | b-cell receptor cd22                 | 4.04       | 0.000     |           |           |
| g2603  | ten4               | teneurin-4                          | 3.99       | 0.009     |           |           |
| g9327  | il18r              | interleukin-18 receptor 1            | 3.99       | 0.006     |           |           |
| g16847 | cytlf              | cystatin-f                          | 3.98       | 0.000     |           |           |
| g11223 | lyve1              | lymphatic vessel endothelial hyaluronic acid receptor 1 | 3.97 | 0.000 |           |           |
| g21342 | myo1f              | myosin-if                           | 3.96       | 0.000     |           |           |
| g18814 | ylat2              | y+1 amino acid transporter 2         | 3.91       | 0.000     |           |           |
| g25574 | trn9               | tumor necrosis factor receptor superfamily member 9 | 3.87 | 0.000 |           |           |
| g2215  | chst1              | carbohydrate sulfotransferase 1      | 3.87       | 0.000     |           |           |
| g17581 | lox5               | arachidonate 5-lipoxygenase          | 3.86       | 0.001     |           |           |
| g2197  | bc11b              | b-cell lymphoma leukemia 11b         | 3.86       | 0.000     |           |           |
| g3335  | p2y14              | p2y purinoceptor 14                  | 3.81       | 0.003     |           |           |
| g21035 | sem4b              | semaphorin-4b flags: precursor       | 3.81       | 0.000     |           |           |
| g27449 | c3p1               | protein c3p1                         | 3.80       | 0.000     |           |           |
| g3210  | syub               | beta-synuclein                      | 3.77       | 0.005     |           |           |
| g16262 | ciks               | adapter protein ciks                 | 3.76       | 0.000     |           |           |
| g44240 | hvm45              | ig heavy chain v region mc101 flags: precursor | 3.75 | 0.000 |           |           |
| g20848 | myo1f              | myosin-if                           | 3.73       | 0.000     |           |           |
| g23368 | il6rb              | interleukin-6 receptor subunit beta  | 3.71       | 0.000     |           |           |

(Continued)
| Gene   | Name            | Description                                   | Yellow Fold cha. | Yellow pval | Silver Fold cha. | Silver pval |
|--------|-----------------|-----------------------------------------------|------------------|-------------|------------------|-------------|
| g39609 | igkc            | Ig kappa chain c region                       | 3.70             | 0.000       |                  |             |
| g3322  | fos             | Proto-oncogene c-fos                          | 3.70             | 0.000       |                  |             |
| g29877 | myo1f           | Myosin-if                                     | 3.68             | 0.004       |                  |             |
| g14600 | gpr4            | G-protein coupled receptor 4                  | 3.65             | 0.000       |                  |             |
| g36379 | co4a            | Complement c4-a                               | 3.64             | 0.000       |                  |             |
| g35688 | itb2            | Integrin beta-2                               | 3.63             | 0.000       |                  |             |
| g30636 | ajl1            | Galactose-binding lectin l-1                  | 3.62             | 0.000       |                  |             |
| g11126 | lr16b           | Leucine-rich repeat-containing protein 16b    | 3.61             | 0.000       |                  |             |
| g38019 | ksyk            | Tyrosine-protein kinase syk                    | 3.59             | 0.000       |                  |             |
| g4585  | a3lt2           | Alpha-1-galactosyltransferase 2               | 3.57             | 0.002       |                  |             |
| g26488 | nckpl           | Nck-associated protein 1-like                 | 3.49             | 0.000       |                  |             |
| g12898 | pfrh            | Receptor-type tyrosine-protein phosphatase h  | 3.47             | 0.000       |                  |             |
| g27889 | fyn             | Tyrosine-protein kinase fyn                   | 3.46             | 0.000       |                  |             |
| g35688 | itb2            | Integrin beta-2                               | 3.45             | 0.000       |                  |             |
| g8551  | nfkdb1          | Nuclear factor nf-kappa-b p105 subunit        | 3.44             | 0.001       |                  |             |
| g36600 | cmn1            | Chemokine-like receptor 1                     | 3.43             | 0.000       |                  |             |
| g41695 | hrmr1           | Major histocompatibility complex class i-related gene protein | 3.43 | 0.000 |          |             |
| g12291 | rac2            | Ras-related c3 botulinum toxin substrate 2    | 3.42             | 0.000       |                  |             |
| g28849 | igg2b           | Ig gamma-2b chain c region                    | 3.41             | 0.000       |                  |             |
| g5094  | scub3           | Signal cub and egf-like domain-containing protein 3 flags: precursor | 3.38 | 0.010 |          |             |
| g39226 | gma4            | Gtpase imap family member 4                   | 3.38             | 0.000       |                  |             |
| g18110 | par11           | Poly polymerase 11                            | 3.36             | 0.002       |                  |             |
| g9299  | gp183           | G-protein coupled receptor 183                | 3.36             | 0.000       |                  |             |
| g21657 | per1            | Perforin-1                                    | 3.34             | 0.000       |                  |             |
| g12497 | kpcb            | Protein kinase c beta type                    | 3.34             | 0.004       |                  |             |
| g16269 | svey1           | Von willebrand factor type egf and pentraxin domain-containing protein 1 | 3.33 | 0.006 |          |             |
| g1218  | dock2           | Dedicator of cytokinesis protein 2            | 3.30             | 0.000       |                  |             |
| g264   | aif1l           | Allograft inflammatory factor 1-like          | 3.29             | 0.000       |                  |             |
| g35485 | gma7            | Gtpase imap family member 7                   | 3.29             | 0.000       |                  |             |
| g23133 | pi2r            | Prostacyclin receptor                         | 3.28             | 0.000       |                  |             |
| g13206 | fbx40           | F-box only protein 40                         | 3.26             | 0.000       |                  |             |
| g25649 | tutla           | Protein turtle homolog a                      | 3.25             | 0.002       |                  |             |
| g5757  | plcg2           | 1-Phosphatidylinositol--bisphosphate phosphodiesterase gamma-2  | 3.23 | 0.000 |          |             |
| g15532 | cmn1            | Chemokine-like receptor 1                     | 3.22             | 0.000       |                  |             |
| g11604 | cci19           | C-c motif chemokine 19                        | 3.21             | 0.000       |                  |             |
| g4039  | cdk1            | Cell division protein kinase 1                | 3.19             | 0.000       |                  |             |
| g43465 | co7             | Complement component c7 flags: precursor      | 3.17             | 0.000       |                  |             |
| g21341 | myo1e           | Myosin-ie                                     | 3.14             | 0.000       |                  |             |
| g41479 | nap1            | Irl and pyd domains-containing protein 1       | 3.14             | 0.000       |                  |             |
| g20847 | myo1f           | Myosin-if                                     | 3.12             | 0.000       |                  |             |
| g14921 | ets1a           | Protein c-ets-1-a                             | 3.11             | 0.003       |                  |             |
| g4833  | il6ra           | Interleukin-6 receptor subunit alpha          | 3.10             | 0.000       |                  |             |
| g7735  | cy24b           | Cytochrome b-245 heavy chain                  | 3.07             | 0.000       |                  |             |
| g26070 | co4             | Complement c4 contains:                      | 3.06             | 0.000       |                  |             |
| g11499 | ccl20           | C-c motif chemokine 20                        | 3.05             | 0.000       |                  |             |
| g12107 | grn             | Granulin 1                                   | 3.05             | 0.000       |                  |             |

(Continued)
| Gene   | Name               | Description                                                                 | Yellow | Silver |
|--------|--------------------|------------------------------------------------------------------------------|--------|--------|
|        |                    |                                                                              | Fold cha. | pval   | Fold cha. | pval   |
| g9518  | tec                | tyrosine-protein kinase tec                                                  | 3.04   | 0.000  |           |        |
| g2101  | thms1              | protein themis                                                              | 3.01   | 0.000  |           |        |
| g22834 | f13a               | coagulation factor xiii a chain short = coagulation factor xiii              | 0.33   | 0.000  |           |        |
| g16693 | pamr1              | inactive serine protease pamr1                                              | 0.31   | 0.000  |           |        |
| g23774 | ppara              | peroxisome proliferator-activated receptor alpha short = ppar-alpha         | 0.31   | 0.005  |           |        |
| g3029  | tri25              | e3 ubiquitin isg15 ligase trim25                                             | 0.29   | 0.000  |           |        |
| g21936 | npas4              | neuronal pas domain-containing protein 4                                    | 0.29   | 0.003  |           |        |
| g13774 | ap1s2              | ap-1 complex subunit sigma-2                                                | 0.28   | 0.002  |           |        |
| g28238 | pvr3               | poliovirus receptor-related protein 3-like flags: precursor                | 0.26   | 0.000  |           |        |
| g15087 | actc               | alpha cardiac muscle 1                                                      | 0.25   | 0.005  |           |        |
| g10993 | s100p              | protein s100-p                                                               | 0.23   | 0.000  |           |        |
| g19703 | cadm3              | cell adhesion molecule 3                                                    | 0.19   | 0.005  |           |        |
| g6116  | acts               | alpha skeletal muscle                                                        | 0.18   | 0.000  |           |        |
| g30946 | gima7              | gtpase imap family member 7                                                  | 0.17   | 0.000  |           |        |
| g21654 | h1                 | histone h1 contains:                                                        | 0.17   | 0.000  |           |        |
| g16176 | pdyn               | proenkephalin-b                                                             | 0.16   | 0.000  |           |        |
| g28779 | cadm3              | cell adhesion molecule 3                                                    | 0.16   | 0.002  |           |        |
| g31407 | gima4              | gtpase imap family member 4                                                  | 0.16   | 0.000  |           |        |
| g2246  | mk10               | mitogen-activated protein kinase 10                                          | 0.14   | 0.000  |           |        |
| g21392 | nalp1              | lrr and pyd domains-containing protein 1                                     | 0.13   | 0.000  |           |        |
| g13604 | cramp              | cathelin-related antimicrobial peptide                                       | 0.13   | 0.000  |           |        |
| g2030  | pa24c              | cytosolic phospholipase a2 gamma                                             | 0.12   | 0.000  |           |        |
| g3113  | hxk4               | glucokinase                                                                 | 0.08   | 0.009  |           |        |
| g11642 | col2               | cofflin-2                                                                   | 0.06   | 0.000  |           |        |
| g25966 | gima5              | gtpase imap family member 5                                                  | 0.05   | 0.003  |           |        |
| g32493 | mhc class i antigen|                                                                          | 0.04   | 0.000  |           |        |
| g9182  | scn5a              | sodium channel protein type 5 subunit alpha                                  | 0.03   | 0.000  |           |        |
| g11847 | lyg                | lysozyme g                                                                   | 0.01   | 0.000  |           |        |
| g22125 | twhh               | tiggy-winkle hedgehog protein                                                | Inf    | Inf    | Inf       | Inf    |
| g2358  | noxo1              | nadph oxidase organizer 1                                                   | 11.27  | 0.000  | 25.87     | 0.000  |
| g14663 | cp1b1              | cytochrome p450 1b                                                          | 11.08  | 0.000  | 0.22      | 0.002  |
| g12811 | co3                | complement c3 contains:                                                     | 9.09   | 0.000  | 9.61      | 0.002  |
| g23617 | chia               | acidic mammalian chitinase                                                   | 6.21   | 0.000  | 16.49     | 0.000  |
| g7584  | cxc1               | c-x-c chemokine receptor type 1                                              | 5.74   | 0.000  | 23.70     | 0.000  |
| g14950 | co3                | complement c3 contains:                                                     | 5.44   | 0.000  | 4.68      | 0.000  |
| g20618 | il6rb              | interleukin-6 receptor subunit beta                                         | 4.78   | 0.000  | 6.26      | 0.001  |
| g605   | i17rb              | interleukin-17 receptor b                                                   | 4.17   | 0.000  | 7.52      | 0.001  |
| g3715  | lpar6              | lysophosphatidic acid receptor 6                                             | 4.02   | 0.002  | 6.88      | 0.000  |
| g513   | sem3c              | semaphorin-3c                                                                | 3.96   | 0.001  | 41.77     | 0.002  |
| g24625 | clm3               | cmrf35-like molecule 3                                                       | 3.92   | 0.000  | 9.47      | 0.001  |
| g24330 | dck2               | serine threonine-protein kinase dck2                                         | 3.56   | 0.002  | 8.15      | 0.000  |
| g34568 | muc5a              | mucin-5ac                                                                    | 3.02   | 0.002  | 24.74     | 0.000  |
| g16142 | tic1               | testican-1                                                                  | 0.21   | 0.000  | 16.89     | 0.000  |
| g23142 | hecw1              | e3 ubiquitin-protein ligase hecw1                                           | Inf    | 0.002  |           |        |
| g16672 | smoc1              | spac-related modular calcium-binding protein 1                              | Inf    | 0.003  |           |        |
| g26738 | hfe                | hereditary hemochromatosis protein                                           | 24.64  | 0.000  |           |        |

(Continued)
| Gene | Name | Description | Yellow Fold cha. | pval | Silver Fold cha. | pval |
|------|------|-------------|------------------|------|------------------|------|
| g29481 | mk11 | mitogen-activated protein kinase 11 | 23.92 | 0.000 | 21.34 | 0.001 |
| g35363 | muc5a | mucin-5ac short = muc-5ac | 20.05 | 0.000 | 17.45 | 0.001 |
| g34977 | ffar2 | free fatty acid receptor 2 | 16.53 | 0.001 | 16.53 | 0.001 |
| g25084 | pnph | purine nucleoside phosphorylase | 15.96 | 0.000 | 15.96 | 0.000 |
| g30308 | dclk2 | serine threonine-protein kinase dclk2 | 15.11 | 0.001 | 15.11 | 0.001 |
| g11737 | noxo1 | nadph oxidase organizer 1 | 15.02 | 0.001 | 15.02 | 0.001 |
| g22925 | dclk2 | serine threonine-protein kinase dclk2 | 9.97 | 0.002 | 9.97 | 0.002 |
| g45517 | argn3 | non-hepatic 3 | 9.65 | 0.000 | 9.65 | 0.000 |
| g41010 | s1pr3 | sphingosine 1-phosphate receptor 3 | 8.25 | 0.004 | 8.25 | 0.004 |
| g36054 | s1pr4 | sphingosine 1-phosphate receptor 4 | 8.13 | 0.001 | 8.13 | 0.001 |
| g26998 | bpi | bactericidal permeability-increasing protein | 7.50 | 0.010 | 7.50 | 0.010 |
| g44943 | mk11 | mitogen-activated protein kinase 11 | 6.32 | 0.008 | 6.32 | 0.008 |
| g6564 | prg4 | proteoglycan 4 | 6.13 | 0.003 | 6.13 | 0.003 |
| g838 | ptx3 | pentraxin-related protein ptx3 | 5.92 | 0.004 | 5.92 | 0.004 |
| g5914 | dmbl1 | deleted in malignant brain tumors 1 protein | 5.17 | 0.002 | 5.17 | 0.002 |
| g11202 | crld2 | cysteine-rich secretory protein lcc domain-containing 2 flags: precursor | 5.11 | 0.004 | 5.11 | 0.004 |
| g14464 | aqp3 | aquaporin-3 | 4.44 | 0.002 | 4.44 | 0.002 |
| g20649 | arg12 | arginase- mitochondrial | 3.88 | 0.007 | 3.88 | 0.007 |
| g23306 | gima4 | gtpase imap family member 4 | 3.78 | 0.007 | 3.78 | 0.007 |
| g21455 | pnph | purine nucleoside phosphorylase | 3.72 | 0.001 | 3.72 | 0.001 |
| g30996 | cadm1 | cell adhesion molecule 1 | 0.28 | 0.004 | 0.28 | 0.004 |
| g9834 | cadm1 | cell adhesion molecule 1 | 0.26 | 0.002 | 0.26 | 0.002 |
| g35655 | cxt10 | c-x-c motif chemokine 10 | 0.23 | 0.003 | 0.23 | 0.003 |
| g16242 | cfab | complement factor b | 0.22 | 0.001 | 0.22 | 0.001 |
| g1090 | ileu | leukocyte elastase inhibitor | 0.22 | 0.003 | 0.22 | 0.003 |
| g8995 | scub2 | signal cub and egl-like domain-containing protein 2 | 0.20 | 0.004 | 0.20 | 0.004 |
| g18096 | ubc4 | probable bifunctional e2 e3 enzyme r795 includes: | 0.18 | 0.008 | 0.18 | 0.008 |
| g26753 | cxt11 | c-x-c motif chemokine 11 | 0.17 | 0.000 | 0.17 | 0.000 |
| g14172 | cadm2 | cell adhesion molecule 2 | 0.16 | 0.004 | 0.16 | 0.004 |
| g10119 | mb2 | mannose-binding protein c | 0.16 | 0.000 | 0.16 | 0.000 |
| g36679 | galt8 | probable polypeptide n-acetylglactosaminyltransferase 8 | 0.15 | 0.001 | 0.15 | 0.001 |
| g7564 | uch1 | ubiquitin carboxyl-terminal hydrolase isozyme l1 | 0.14 | 0.009 | 0.14 | 0.009 |
| g25543 | gima7 | gtpase imap family member 7 | 0.10 | 0.000 | 0.10 | 0.000 |
| g37466 | hmr1 | major histocompatibility complex class i-related gene protein | 0.10 | 0.000 | 0.10 | 0.000 |
| g4910 | s100b | protein s100-b | 0.09 | 0.005 | 0.09 | 0.005 |
| g24694 | mmp17 | matrix metalloproteinase-17 | 0.08 | 0.000 | 0.08 | 0.000 |
| g31085 | gbp5 | guanylate-binding protein 5 | 0.05 | 0.000 | 0.05 | 0.000 |
| g17364 | tecta | alpha-tectorin flags: precursor | 0.04 | 0.004 | 0.04 | 0.004 |
| g31792 | lv302 | ig lambda chain v-iii region loi | 0.02 | 0.000 | 0.02 | 0.000 |
| g1076 | cats | cathepsin s flags: precursor | 0.01 | 0.000 | 0.01 | 0.000 | (Continued)
proteins were modified in the expression level in infected silver eels gas gland cells, and, as already observed in infected yellow eels, the expression level of sodium potassium-transporting ATPase was largely reduced, but in contrast to yellow eels, in silver eels subunit gamma (atng) was affected. In infected silver eels, the amino acid transporters showed increased mRNA expression levels (sodium and chloride-dependent neutral and basic amino acid transporter b (0+), s6a14; excitatory amino acid transporter 2, eaa2).

**Extracellular matrix.** In infected silver eels, 8 genes related to the extracellular matrix were modified, but only two of these genes (acidic mammalian chitinase, chia, and mucin 5b, muc5b) were also affected in infected yellow eels (Table 5). In contrast to infected yellow eels, 4 additional mucin genes showed an increased expression level. In fact, in silver eels 5 out of 8 affected genes were mucin genes. Collagen alpha-1 (co5a1) was expressed at a 3-fold lower level in infected silver eel gas gland cells.

**Angiogenesis or vasculogenesis.** In infected silver eels, the number of genes modified with respect to angiogenesis or vasculogenesis was much smaller than in infected yellow eels (20 and 51 genes, respectively) (Table 6), and of these genes only tiggy-winkle hedgehog protein (twhh) and complement c3 (co3) were affected in yellow as well as in silver eels. Expression of prostaglandine2 receptor (pe2r1), of sphingosine receptors (s1pr3; s1pr4), and of roundabout homolog 2 (robo2) was elevated, and mRNA of complement proteins was increased (co3, co5). The expression level of angiopeitin was not affected by the nematode infection.

**Immune defense.** Compared to infected yellow eels, the immune related changes were much less pronounced in infected silver eels (Table 7). In infected silver eels only 64 genes were expressed at a different level, and 21 of these genes were downregulated. Only two of the interleukin genes were elevated in their expression level (il6rb, i17rb), and immunoglobulin genes were unaffected. As observed in infected yellow eels, two genes coding for complement proteins (co3; co5) were elevated in their expression level, but complement factor b (cfab) was more than 4-fold reduced in the expression level. Major histocompatibility complex class I related gene (hmr1) was even 10-fold decreased in the expression level.

**Maturation.** Of the genes related to maturation, 25 genes were modified in their expression level in infected silver eels, and 13 of these genes decreased (S2 Table). As observed in infected yellow eels, two copies of fer-1-like protein 4 (fr1l4) were elevated in their expression level (11-fold and 37-fold). The expression of three zona pellucida genes (zp1, zp2, zp3) was more than 100-fold reduced.

**Table 8** summarizes the number of genes related to specific physiological functions expected to be important for swimbladder function and modified in their expression level in infected yellow and silver eels. The comparison clearly showed that in infected yellow eels, many more genes were affected, compared to infected silver eels. Furthermore, the number of genes affected in both, infected yellow and silver eels, was very small, indicating that, depending on the developmental stage, different sets of genes were affected.

| Gene | Name  | Description | Yellow | Silver |
|------|------|-------------|--------|--------|
| g1271| h2b3 | Histone     | 0.00   | 0.002  |

Fold cha. = Fold change; pval = 0.000 indicates P values < 0.0005

https://doi.org/10.1371/journal.pone.0183128.t007
Transcriptional changes observed in infected yellow eel gas gland tissue

In a previous study we addressed the transcriptional changes related to silvering in uninfected European eels, and at a significance level of $P < 0.01$, 646 genes were found to be transcribed at a different level [26]. The present study showed that the influence of an infection of the yellow eel swimbladder with the nematode *Anguillicola crassus* on transcriptional activity in gas gland cells by far exceeded the effect of silvering. In infected yellow eel gas gland tissue, 1675 genes were modified in their mRNA expression level. As expected, GO enrichment analysis revealed that the most prominent category was immune response with 143 genes expressed at a higher level and only 24 genes expressed at a lower level. The large fraction of genes with elevated expression level included various inflammatory components, complement proteins, and immunoglobulins, indicating a strong defense reaction of the eel. An extensive non-specific immune response has been reported in response to juvenile nematodes/parasites entering the swimbladder [50], and Nimeth et al. [51] demonstrated that even glass eels can be infected by feeding on copepods. An activation of the immune system in infected eels has previously been suggested by presence of macrophages in swimbladder tissue [52–54]. Experimental infections of the swimbladder have also been reported to cause a humoral response [55]. An infection of the swimbladder with the histophagous nematode results in severe histological modifications of the swimbladder epithelium [27,29–31,56]. The single layered epithelium of the eel becomes severely thickened and multilayered. Signs of tissue degeneration appear, and the lumen is filled with eggs, larvae, and exudate. Ultimately, these effects can lead to a total loss of swimbladder function [29]. The elevated expression of acidic mammalian chitinase among the extracellular matrix components also can be interpreted as an immune response to the nematode infection. Chitin is a surface component of parasites and induces the expression of chitinase in the host [57]. MMP9 expression is also elevated in infected eels, and this protein has been shown to be an essential component of the innate immune system [58].

More recent observations suggest that the infection rate may stabilize [59], and eels with thickened swimbladder wall, but with very few or even no nematode inside the bladder indicate that the mechanical barrier, combined with the inflammatory response, may be successful in defending the nematode [37].

Thickening of the tissue in response to the infection results in larger diffusion distances. The elevated expression levels of glucose transporters and of monocarboxylate transport proteins, and in particular of fructose-bisphosphate aldolase suggested a stimulation of
glycolytic activity. Fructose-bisphosphate aldolase is known as a key enzyme for glycolytic flux. Glucokinase, in turn, was found with largely reduced copy numbers in infected yellow eel swimbladder. In swimbladder tissue of cod, hexokinase appears to be the key enzyme for phosphorylation of glucose taken up from the blood [60]. Therefore, the reduced expression rate of glucokinase, an enzyme of crucial importance in liver tissue, may not compromise glycolytic flux in gas gland tissue.

The elevated expression level of a number of genes related to the extracellular matrix, including collagen alpha, versican, and two thrombospondins, appeared to be connected to the thickening of the swimbladder tissue. Collagen is a typical component of the extracellular matrix. The proteoglycan versican has been reported to be expressed by vascular smooth muscle cells [61], and the glycoprotein thrombospondin has been shown to inhibit angiogenesis and neovascularization [62]. The thickening of the gas gland epithelium obviously coincided with an increase in extracellular matrix in infected eels.

The induction of Angiopoietin-related protein in infected eels also appeared to be connected to tissue thickening. In contrast to thrombospondin, which inhibits angiogenesis, angiopoietin-related protein 7 has been shown to induce sprouting in endothelial cells [63], which would reduce diffusion distances and therefore improve nutrient and oxygen supply to the tissue.

Ion regulation and in particular acid secretion is crucial for swimbladder functioning [64–66], and in infected yellow eels a number of ion transporters were modified in their expression level. Several Na⁺, K⁺, and Cl⁻ transport proteins were affected, but the expression changes were not consistent. While 7 mRNA species showed elevated levels, 6 were significantly reduced. V-ATPase and Na⁺/H⁺ exchange proteins were not affected, suggesting that acid secretion in particular was not seriously modified [64,65]. Interestingly, sodium-potassium ATPase subunit beta-2 was more than 8-fold reduced in the expression level. As many ion transport processes require Na⁺/K⁺-ATPase activity as a second step, this suggested that overall ion transport activity was not enhanced by the infection.

ROS and ROS defense play a special role in swimbladder tissue due to the high oxygen partial pressures encountered [32], and several genes related to the GO term ROS defense were affected in their expression level. Genes particularly important for the degradation of ROS like glutathione reductase, glutathione peroxidase and superoxide dismutase were not among the modified genes, but a number of transcription factors like fos, fosb, and junb were affected by the infection. These transcription factors may be involved in a number of different physiological functions and signaling cascades, so that this result may not be indicative of a special enhancement of ROS defense in infected yellow eels. Jun and Fos family members heterodimerize to form Activator Protein 1 (AP1), which has a major role in tissue regeneration. Some of the observed expression changes may thus be secondary effects due to the formation of the AP1 complex [67–69]. The elevated expression levels of two cytochromes may, however, again reveal a connection to a defense reaction of the host, since cytochrome b245 has been connected to superoxide production and phagocyte activity [70], and cytochrome p450 is involved in detoxification [71]. Accordingly, the elevated expression levels of these enzymes again provide a strong indication for the defense reaction of the host against the infection.

As already observed in a previous study focusing on the effect of silvering on transcriptional activity [26], an infection with the nematode caused modifications in the expression level of genes related to maturation in swimbladder tissue. Several of these proteins were also listed under different GO terms, like transporters (gtr5, cfr) and a transcription factor (fos), so that a specific connection to maturation may not be obligatory in this tissue. Noteworthy was the elevation of fer-like proteins, which have previously been connected to vesicle fusion and membrane trafficking [72]. Ferlins represent an ancient protein family and appear to be of general importance for these membrane processes.
Transcriptional changes observed in infected silver eel gas gland tissue

An initial comparison of the transcriptional effects observed in infected yellow eels with the effects detected in infected silver revealed large scale differences: while 1675 genes were differentially expressed in infected yellow eels, only 291 genes were affected in infected silver eels. Only a third of the genes modified in silver eels was also affected in yellow eels. Twenty-two of these genes, however, showed the opposite response in yellow compared with silver eels, supporting the impression that the nematode infection provoked quite different responses in yellow and silver eels.

Expressed at elevated levels in yellow eels but reduced in silver eels were zinc binding proteins. Zinc metalloenzymes are, for example, carboanhydrase, superoxide dismutase, collagenase, and elastase, enzymes that are important for the acidification of blood during passage of the swimbladder, for ROS defense and reconstruction of the extracellular matrix [18,65]. The elevated expression level of these enzymes in yellow eels would support swimbladder function, and thus could indicate that, in addition to the strong immune defense reaction, yellow eels attempted to retain a functional swimbladder. In infected silver eels, in turn, Ca\(^{2+}\) binding enzymes showed elevated expression levels. Ca\(^{2+}\) is a pivotal signaling component [73], but with respect to swimbladder function the role of Ca\(^{2+}\) does not appear to be crucial.

The conclusion that in infected silver eels transcriptional changes were not supportive for swimbladder function was underlined by the observation that in contrast to infected yellow eels, in infected silver eels, genes involved in glycolysis were not affected, and in addition, there was almost no response in genes involved in ROS defense. Both, glycolysis and ROS defense, however, are crucial for swimbladder function [18,19,32].

In infected silver eel gas gland tissue, the compared to infected yellow eels reduced responses of inflammatory components, of complement proteins and the reduced expression level of major histocompatibility complex revealed a very much reduced immune defense reaction. Silverying requires severe physiological reorganization, not only in gas gland cells [26], but also in terms of ion regulation to prepare for the transition to the marine environment. In addition, maturation is prepared [38]. These modifications require a lot of energy, which could result in reduced capacities for the immune response.

In line with these considerations, only few genes related to the GO term ‘ion regulation’ were differentially expressed in infected silver eels. Only two genes related to Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) transport were modified, and a subunit of Na\(^{+}\)/K\(^{+}\)-ATPase was reduced in the mRNA expression level, indicating that ion transport activity overall was reduced.

In a previous study we detected that at least in some uninfected silver eels, zona pellucida genes showed a significantly elevated expression level compared to uninfected yellow eels [26]. The present results revealed a significant reduced expression level in infected silver eels, as compared to uninfected one’s. These results supported the conclusion that silverying does include the onset of sexual maturation, and an elevation in plasma steroid concentrations [44] may have induced expression changes of maturation connected genes not only in gonads, but in other tissues as well.

The results of the present study revealed a very strong effect of the *Anguillicola crassus* infection on gas gland tissue of yellow eels, and compared to these changes in the mRNA expression the changes observed in infected silver eel gas gland tissue were very small, almost negligible. The largest difference in the response was observed in the immune response. In addition, some of the expression changes in infected yellow eels indicated an attempt to keep the swimbladder functional, but this was totally absent in infected silver eels. A possible explanation for this difference could be the silverying process. Silverying not only includes an improvement of swimbladder function [22–25,74], but also a total rearrangement of ion regulation to prepare
for the switch to the marine environment, and the onset of maturation or puberty [38,41,75]. This could require so much energy and so many resources that there is not much scope to cope with the additional challenge of a nematode infection.

Another possible explanation is related to swimbladder function. The silvering event has been shown to improve swimbladder function [23], and this appears essential to prepare the swimbladder for the excessive changes in hydrostatic pressure, encountered during the vertical migrations taking place during the spawning migration [13,76]. On the other hand, theoretical considerations [19] demonstrated that it is impossible that the swimbladder can keep a constant volume throughout a six month journey to the Sargasso Sea (perhaps even longer; [14]) with daily vertical migrations between 200 or 300 m depth at night time, and 600–800 m depth at day time. Therefore, it is expected that the swimbladder provides neutral buoyancy near the upper level of the daily migrations, and is compressed during the descent to lower levels. If this is correct, the swimbladder volume must be adjusted to the upper level, and then the volume should be kept constant, which could be achieved by reducing gas loss through the swimbladder wall. Permeability of the swimbladder is in fact reduced during silvering [24], and this was supported by changes in the mRNA levels of genes related to the extracellular matrix in silver eels, as compared to yellow eels [26]. In this situation, gas-secreting activity of the bladder could be largely reduced, which could coincide with a down-regulation of metabolic activity and a reduced responsiveness to other challenges, like a nematode infection.

Supporting information
S1 Table. Differentially transcribed genes (fold change > 3) based on GO term “ion transport” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.
(DOCX)

S2 Table. Differentially transcribed genes (fold change > 3) based on GO terms related to “maturation” in infected yellow and infected silver eels as compared with uninfected yellow and uninfected silver eels, respectively.
(DOCX)

Acknowledgments
We would like to thank Marko Freese, Reinhold Hanel, Jan-Dag Pohlmann, and the Thünen Institute of Fisheries Ecology, Hamburg, Germany, for providing eels.

Author Contributions
Conceptualization: Bernd Pelster.
Data curation: Gabriel Schneebauer, Ron P. Dirks.
Formal analysis: Gabriel Schneebauer, Ron P. Dirks.
Funding acquisition: Bernd Pelster.
Investigation: Gabriel Schneebauer.
Methodology: Ron P. Dirks.
Project administration: Bernd Pelster.
Resources: Bernd Pelster.
Supervision: Bernd Pelster.

Validation: Gabriel Schneebauer, Ron P. Dirks.

Visualization: Gabriel Schneebauer.

Writing – original draft: Gabriel Schneebauer, Bernd Pelster.

Writing – review & editing: Gabriel Schneebauer, Ron P. Dirks, Bernd Pelster.

References

1. Rousseau K, Aroua S, Dufour S. Eel Secondary Metamorphosis: Silvering. In: Dufour S, Rousseau K, Kapoor BG, editors. Metamorphosis in Fish. Boca Raton, FL: CRC Press, Taylor & Francis Group; 2012. pp. 216–249.

2. Schmidt J. Breeding Places and Migrations of the Eel. Nature. 1923; 111: 51–54. https://doi.org/10.1038/11051a0

3. Miller MJ, Bonhommeau S, Munk P, Castonguay M, Hanel R, McCleave JD. A century of research on the larval distributions of the Atlantic eels: a re-examination of the data. Biol Rev. 2015; 90: 1035–1064. https://doi.org/10.1111/brv.12144 PMID: 25291986

4. Dekker W. What caused the decline of the Lake IJsselmeer eel stock after 1960? ICES J Mar Sci. 2004; 61: 394–404. https://doi.org/10.1016/j.icesjms.2004.01.003

5. Kettle AJ, Vøllestad LA, Wibig J. Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa? Fish Fish. 2011; 12: 380–411. https://doi.org/10.1111/j.1467-2979.2010.00400.x

6. Geeraerts C, Belpaire CGJ. The effects of contaminants in European eel: a review. Ecotoxicology. 2010; 19: 239–266. https://doi.org/10.1007/s10646-009-0424-0 PMID: 19806452

7. Baltazar-Saeres M, Biastoch A, Harrod C, Hanel R, Marohn L, Prigge E, et al. Recruitment collapse and population structure of the European eel shaped by local ocean current dynamics. Curr Biol. 2014; 24: 104–108. https://doi.org/10.1016/j.cub.2013.11.031 PMID: 23473406

8. Bonhommeau S, Chassot E, Planque B, Rivot E, Knap AH, Le Pape O. Impact of climate on eel populations of the Northern Hemisphere. Mar Ecol Prog Ser. 2008; 373: 71–80. https://doi.org/10.3354/meps07696

9. Lefebvre F, Fazio G, Crivelli AJ. Anguillicoloides crassus. In: Woo P, Buchmann K, editors. Fish Parasites: Pathobiology and Protection. London: CAB International; 2012. pp. 310–326. http://www.uoguelph.ca/~pwoo/FPFP.descripti.pdf

10. Bandín I, Souto S, Cutrín JM, López-Vázquez C, Oliveira JG, Esteve C, et al. Presence of viruses in wild eels Anguilla anguilla L, from the Albufera Lake (Spain). J Fish Dis. 2014; 37: 597–607. https://doi.org/10.1111/jfd.1392 PMID: 24846700

11. ICES 2016. Report of the Working Group on Eels (WGEEL), 15–22 September 2016, Cordoba, Spain. ICES CM 2016/ACOM:19. 107 pp.

12. Jacoby D, Golloc M. Anguilla anguilla. IUCN Red List Threat Species 2014. The IUCN Red List of Threatened Species 2014; 2014; e.T60344A4.

13. Aarestrup K, Okland F, Hansen MM, Righton DA, Gargan P, Castonguay M, et al. Oceanic Spawning Migration of the European Eel (Anguilla anguilla). Science. 2009; 325: 1660. https://doi.org/10.1126/science.1178120 PMID: 19779192

14. Righton DA, Westerberg H, Feunteun E, Okland F, Gargan P, Amliah E, et al. Empirical observations of the spawning migration of European eels: The long and dangerous road to the Sargasso Sea. Sci Adv. 2016; 2: e1501694. https://doi.org/10.1126/sciadv.1501694 PMID: 27713924

15. Schabetsberger R, Miller MJ, Dall’Olmo G, Kaiser R, Økland F, Watanabe S, et al. Hydrographic features of anguillid spawning areas: potential signposts for migrating eels. Mar Ecol Prog Ser. 2016; 554: 141–155. https://doi.org/10.3354/meps11824

16. Fänge R. Gas exchange in fish swim bladder. Rev Physiol Biochem Pharmacol. 1983; 97: 111–158. Available: http://www.ncbi.nlm.nih.gov/pubmed/6408725 PMID: 6408725

17. Pelster B. Buoyancy at Depth. In: Randall DJ, Farrel AP, editors. Deep Sea Fishes. San Diego, USA: Academic Press; 1997. pp. 195–237.

18. Pelster B. The swimbladder. In: Trischitta F, Takei Y, Sebert P, editors. Eel Physiology. Boca Raton, FL: CRC Press; 2013. pp. 44–67.

19. Pelster B. Swimbladder function and the spawning migration of the European eel Anguilla anguilla. Front Physiol. 2015; 5: 1–10. https://doi.org/10.3389/fphys.2014.00486 PMID: 25646080
20. Tesch FW, Bartsch P, Berg R, Gabriel O, Henderson IW, Kamstra A, et al. The Eel. 3rd ed. Thorpe JE, editor. Oxford, UK: Blackwell Science Ltd; 2003.

21. van Ginneken VJT, Maes GE. The European eel (Anguilla anguilla, Linnaeus), its Lifecycle, Evolution and Reproduction: A Literature Review. Rev Fish Biol Fish. 2005; 15: 367–398. https://doi.org/10.1007/s11160-006-0005-8

22. Righton DA, Aarestrup K, Jellyman D, Sébert P, van den Thillart GEEJM, Tsukamoto K. The Anguilla spp. migration problem: 40 million years of evolution and two millennia of speculation. J Fish Biol. 2012; 81: 365–386. https://doi.org/10.1111/j.1095-8649.2012.03373.x PMID: 22803715

23. Kleckner RC. Swim bladder volume maintenance related to initial oceanic migratory depth in silver-phase Anguilla rostrata. Science. 1980; 208: 1481–1482. Available: http://www.ncbi.nlm.nih.gov/pubmed/7384792 PMID: 7384792

24. Kleckner RC. Swim bladder wall guanine enhancement related to migratory depth in silver phase Anguilla rostrata. Comp Biochem Physiol Part A Physiol. 1980; 65: 351–354. https://doi.org/10.1016/0300-9629(80)90041-9

25. Yamada Y, Zhang H, Okamura A, Tanaka S, Horie N, Mikawa N, et al. Morphological and histological changes in the swim bladder during maturation of the Japanese eel. J Fish Biol. 2001; 58: 804–814. https://doi.org/10.1046/j.1095-8649.2000.1486

26. Pelster B, Schneeberger G, Dirks RP. Anguillicola crassus Infection Significantly Affects the Silvering Related Modifications in Steady State mRNA Levels in Gas Gland Tissue of the European Eel. Front Physiol. 2016; 7: 1–13.

27. Kirk RS. The impact of Anguillicola crassus on European eels. Fish Manag Ecol. 2003; 10: 385–394. https://doi.org/10.1111/j.1365-2400.2003.00355.x

28. Lefebvre F, Fazio G, Mouaibid P, Crivelli AJ. Is the continental life of the European eel Anguilla anguilla affected by the parasitic invader Anguillicoloides crassus? Proc R Soc B Biol Sci. 2013; 280: 20122916. https://doi.org/10.1098/rspb.2012.2916 PMID: 23325776

29. Würtz J, Taraschewski H, Pelster B. Changes in gas composition in the swimbladder of the European eel (Anguilla anguilla) infected with Anguillicola crassus (Nematoda). Parasitology. 1996; 112: 233–238. Available: http://www.ncbi.nlm.nih.gov/pubmed/8851864 PMID: 8851864

30. Würtz J, Taraschewski H. Histopathological changes in the swimbladder wall of the European eel Anguilla anguilla due to infections with Anguillicola crassus. Dis Aquat Organ. 2000; 39: 121–134. https://doi.org/10.3354/dao039121 PMID: 10715817

31. Barry J, McLeish J, Dodd JA, Turnbull JF, Boylan P, Adams CE. Introduced parasite Anguillicola crassus infection significantly impedes swim bladder function in the European eel Anguilla anguilla (L.). J Fish Dis. 2014; 37: 921–924. https://doi.org/10.1111/jfd.12215 PMID: 24422641

32. Schneeberger G, Hanel R, Pelster B. Anguillicola crassus impairs the silvering-related enhancements of the ROS defense capacity in swimbladder tissue of the European eel (Anguilla anguilla). J Comp Physiol B. Springer Berlin Heidelberg; 2016; 186: 867–877. https://doi.org/10.1007/s00360-016-0994-0 PMID: 27146148

33. Fazio G, Sasal P, Mouahid G, Lecomte-Finiger R, Moné H. Swim bladder nematodes (Anguillicoloides crassus) disturb silverying in European eels (Anguilla anguilla). J Parasitol. 2012; 98: 695–705. https://doi.org/10.1645/GE-2700.1 PMID: 22404329

34. Morris SM, Albright JT. Superoxide dismutase, catalase, and glutathione peroxidase in the swim bladder of the physoclistous fish, Opsanus tau L. Cell Tissue Res. 1981; 220: 739–752. Available: http://www.ncbi.nlm.nih.gov/pubmed/7296650 PMID: 7296650

35. Morris SM, Albright JT. Catalase, glutathione peroxidase, and superoxide dismutase in the rete mirabile and gas gland epithelium of six species of marine fishes. J Exp Zool. 1984; 232: 29–39. https://doi.org/10.1111/j.1365-294X.2010.05011.x PMID: 21299662

36. Lushchak VI, Semchysyn HM. Oxidative stress-Molecular mechanisms and Biological effects. Lushchak V, Halyna MS, editors. Rijeka, Croatia: InTech; 2012.

37. Lefebvre F, Fazio G, Palstra AP, Székely C, Crivelli AJ. An evaluation of indices of gross pathology associated with the nematode Anguillicoloides crassus in eels. J Fish Dis. 2011; 34: 31–45. https://doi.org/10.1111/j.1365-2761.2010.01207.x PMID: 21118268

38. Dufour S, Burzawa-Gerard E, Le Belle N, Sbaihi M, Vidal B. Reproductive endocrinology of the European eel, Anguilla anguilla. In: Aida K, Sukamoto K, Yamauchi K, editors. Eel Biology. Tokyo: Springer Japan; 2003. pp. 373–383.

39. Als TD, Hansen MM, Maes GE, Castonguay M, Riemann L, Aarestrup K, et al. All roads lead to home: pannmixia of European eel in the Sargasso Sea. Mol Ecol. 2011; 20: 1333–1346. https://doi.org/10.1111/j.1365-290X.2011.05011.x PMID: 21299662
40. Pujolar JM, Jacobsen MW, Als TD, Flydenberg J, Munch K, Jónsson B, et al. Genome-wide single-geration signatures of local selection in the panmictic European eel. Mol Ecol. 2014; 23: 2514–2528. https://doi.org/10.1111/mec.12753 PMID: 24750353

41. Durif CMF, Dufour S, Elie P. The silverying process of Anguilla anguilla: a new classification from the yellow resident to the silver migrating stage. J Fish Biol. 2005; 66: 1025–1043.

42. Pankhurst NW. Relation of visual changes to the onset of sexual maturation in the European eel Anguilla anguilla (L.). J Fish Biol. 1982; 21: 127–140. https://doi.org/10.1111/j.1095-8649.1982.tb03994.x

43. Dirks RP, Burgerhout E, Britsijn SA, de Wijze DL, Ozupek H, Tuinhof-Koelma N, et al. Identification of molecular markers in pectoral fin to predict artificial maturation of female European eels (Anguilla anguilla). Gen Comp Endocrinol. Elsevier Inc.; 2014; 204: 267–276. https://doi.org/10.1016/j.ygcen.2014.06.023 PMID: 24992558

44. Burgerhout E, Minegishi Y, Britsijn SA, de Wijze DL, Henkel C V, Jansen HJ, et al. Changes in ovarian gene expression profiles and plasma hormone levels in maturing European eel (Anguilla anguilla); Biomarkers for broodstock selection. Gen Comp Endocrinol. Elsevier Inc.; 2016; 225: 185–196. https://doi.org/10.1016/j.ygcen.2015.08.006 PMID: 26255685

45. Henkel C V, Burgerhout E, de Wijze DL, Dirks RP, Minegishi Y, Jansen HJ, et al. Primitive duplicate Hox clusters in the European eel’s genome. PLoS One. 2012; 7: e32231. https://doi.org/10.1371/journal.pone.0032231 PMID: 22384188

46. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009; 25: 1105–1111. https://doi.org/10.1093/bioinformatics/btp120 PMID: 19289445

47. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009; 25: 2078–2079. https://doi.org/10.1093/bioinformatics/btp352 PMID: 19505943

48. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015; 31: 166–169. https://doi.org/10.1093/bioinformatics/btu638 PMID: 25260700

49. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010; 11: R106. https://doi.org/10.1186/gb-2010-11-10-r106 PMID: 20979621

50. van Banning P, Haenen OLM. Effects of the swimbladder nematode Anguillicola crassus in wild and farmed eel, Anguilla anguilla. Pathology in Marine Science. Elsevier; 1990. pp. 317–330. https://doi.org/10.1016/B978-0-12-550755-4.50037-9

51. Nimeth K, Zwerger P, Würtz J, Salvenmoser W, Pelster B. Infection of the glass-eel swimbladder with the nematode Anguillicola crassus. Parasitology. 2000; 121: 75–83. Available: http://www.ncbi.nlm.nih.gov/pubmed/11085227 PMID: 11085227

52. Beregi A, Molnár K, Békési L, Székely C. Radiodiagnostic method for studying swimbladder inflammation caused by Anguillicola crassus (Nematoda: Dracunculoidea). Dis Aquat Organ. 1998; 34: 155–160. https://doi.org/10.1016/S0169-7784(98)90097-9

53. Würtz J, Knopf K, Taraschewski H. Distribution and prevalence of Anguillicola crassus (Nematoda) in eels Anguilla anguilla of the rivers Rhine and Naab, Germany. Dis Aquat Organ. 1998; 32: 137–143. https://doi.org/10.1016/S0169-7784(98)90097-9

54. Knopf K, Madrilles Helm A, Lucas R, Bleiss W, Taraschewski H. Migratory response of European eel (Anguilla anguilla) phagocytes to the eel swimbladder nematode Anguillicola crassus. Parasitol Res. 2008; 102: 1311–1316. https://doi.org/10.1007/s00436-008-0910-y PMID: 18311570

55. Knopf K, Naser K, van der Heijden MHT, Taraschewski H. Humoral immune response of European eel Anguilla anguilla experimentally infected with Anguillicola crassus. Dis Aquat Organ. 2000; 42: 61–69. https://doi.org/10.1016/S0169-7784(00)042061 PMID: 10986646

56. Kennedy CR. The pathogenic helminth parasites of eels. J Fish Dis. 2007; 30: 319–334. https://doi.org/10.1111/j.1365-2761.2007.00821.x PMID: 17498176

57. Zhu Z, Zheng T, Homer RJ, Kim Y-K, Chen NY, Cohn L, et al. Acidic Mammalian Chitinase in Asthmatic Th2 Inflammation and IL-13 Pathway Activation. Science. 2004; 304: 1678–1682. https://doi.org/10.1126/science.1095336 PMID: 15192232

58. Wang X, Yu YY, Lieu S, Yang F, Lang J, Lu C, et al. MMP9 regulates the cellular response to inflammation after skeletal injury. Bone. 2013; 52: 111–119. https://doi.org/10.1016/j.bone.2012.09.018 PMID: 23010105

59. Schabuss M, Kennedy CR, Konceny R, Grillitsch B, Reckendorfer W, Schiemer F, et al. Dynamics and predicted decline of Anguillicola crassus infection in European eels, Anguilla anguilla, in Neusiedler See, Austria. J Helminthol. 2005; 79: 159–167. https://doi.org/10.1079/JOH2005281 PMID: 15946398
60. Clow KA, Short CE, Hall JR, Gendron RL, Driedzic WR. High rates of glucose utilization in the gas gland of Atlantic cod (Gadus morhua) are supported by GLUT1 and HK1b. J Exp Biol. 2016; 219: 2763–2773. https://doi.org/10.1242/jeb.141721 PMID: 27401755

61. Lemire JM, Braun KR, Maurel P, Kaplan ED, Schwartz SM, Wight TN. Versican/PG-M Isoforms in Vascular Smooth Muscle Cells. Arterioscler Thromb Vasc Biol. 1999; 19: 1630–1639. https://doi.org/10.1161/1.ATV.19.7.1630 PMID: 10397680

62. Morris AH, Kyriakides TR. Matricellular proteins and biomaterials. Matrix Biol. International Society of Matrix Biology; 2014; 37: 183–191. https://doi.org/10.1016/j.matbio.2014.03.002 PMID: 24657843

63. Kim I, Moon S-O, Koh KN, Kim H, Uhm C-S, Kwak HJ, et al. Molecular Cloning, Expression, and Characterization of Angiopoietin-related Protein: Angiopoietin-Related Protein Induces Endothelial Cell Sprouting. J Biol Chem. 1999; 274: 26523–26528. https://doi.org/10.1074/jbc.274.37.26523 PMID: 10473614

64. Pelster B. Mechanisms of acid release in isolated gas gland cells of the European eel Anguilla anguilla. Am J Physiol. 1995; 269: R793–R799. Available: http://www.ncbi.nlm.nih.gov/pubmed/7485595 PMID: 7485595

65. Pelster B, Niederstätter H. pH-dependent proton secretion in cultured swim bladder gas gland cells. Am J Physiol. 1997; 273: R1719–R1725. Available: http://www.ncbi.nlm.nih.gov/pubmed/9374815 PMID: 9374815

66. Pelster B. pH regulation and swimbladder function in fish. Respir Physiol Neurobiol. 2004; 144: 179–190. https://doi.org/10.1016/j.resp.2004.03.019 PMID: 15556101

67. Stepniak E. c-Jun/AP-1 controls liver regeneration by repressing p53/p21 and p38 MAPK activity. Genes Dev. 2006; 20: 2306–2314. https://doi.org/10.1101/gad.390506 PMID: 16912279

68. Neub A, Houdek P, Ohnemus U, Moll I, Brandner JM. Biphasic Regulation of AP-1 Subunits during Human Epidermal Wound Healing. J Invest Dermatol. Elsevier Masson SAS; 2007; 127: 2453–2462. https://doi.org/10.1038/sj.jid.57.00864 PMID: 17495958

69. Shaulian E. AP-1—The Jun proteins: Oncogenes or tumor suppressors in disguise? Cell Signal. Elsevier Inc.; 2010; 22: 894–899. https://doi.org/10.1016/j.cellsig.2009.12.008 PMID: 20060892

70. Dinauer MC, Pierce EA, Bruns GAP, Curnutte JT, Orkin SH. Human neutrophil cytochrome b light chain (p22-phox). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. J Clin Invest. 1990; 86: 1729–1737. https://doi.org/10.1172/JCI114898 PMID: 2243141

71. Guengerich FP. Reactions and significance of cytochrome P-450 enzymes. J Biol Chem. 1991; 266: 10019–10022. Available: http://www.ncbi.nlm.nih.gov/pubmed/2037557 PMID: 2037557

72. Lek A, Evesson FJ, Sutton RB, North KN, Cooper ST. Ferlins: Regulators of Vesicle Fusion for Auditory Neurotransmission, Receptor Trafficking and Membrane Repair. 2012; 13: 185–194. https://doi.org/10.1111/j.1600-0854.2011.01267.x PMID: 21838746

73. Yáñez M, Gil-Longo J, Campos-Toimil M. Calcium Binding Proteins. In: Islam MS, editor. Calcium Signaling. Dordrecht: Springer Netherlands; 2012. pp. 461–482. https://doi.org/10.1007/978-94-007-2888-2_19 PMID: 22453954

74. Sébert P, Vettier A, Moisan C. High pressure resistance and adaptation of European eels. In: van den Thillart G, Dufour S, Rankin JC, editors. Spawning Migration of the European Eel. New York: Springer Science; 2009. pp. 99–127.

75. van Ginneken VJT, Durf CMF, Balm SP, Boot R, Verstegen M, Antonissen E, et al. Silvering of European eel (Anguilla anguilla L.): seasonal changes of morphological and metabolic parameters. Anim Biol. 2007; 57: 63–77. https://doi.org/10.1163/157075607780002014

76. Wysujack K, Westerberg H, Aarestrup K, Trautner J, Kurwie T, Nagel F, et al. The migration behaviour of European silver eels (Anguilla anguilla) released in open ocean conditions. Mar Freshw Res. 2015; 66: 145–157. https://doi.org/10.1071/MF14023