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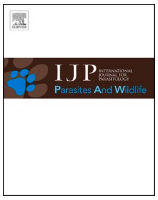

Subtle transcriptomic response of Eurasian perch (*Perca fluviatilis*) associated with *Triaenophorus nodulosus* plerocercoid infection

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ABSTRACT

Determining the physiological effects of parasites and characterizing genes involved in host responses to infections are essential to improving our understanding of host-parasite interactions and their ecological and evolutionary consequences. This task, however, is complicated by high diversity and complex life histories of many parasite species. The use of transcriptomics in the context of wild-caught specimens can help ameliorate this by providing both qualitative and quantitative information on gene expression patterns in response to parasites in specific host organs and tissues. Here, we evaluated the physiological impact of the widespread parasite, the pike tapeworm (*Triaenophorus nodulosus),* on its second intermediate host, the Eurasian perch (*Perca fluviatilis*). We used an RNAseq approach to analyse gene expression in the liver, the target organ of *T. nodulosus* plerocercoids, and spleen which is one of the main immune organs in teleost fishes. We compared perch collected from multiple lakes consisting of individuals with ($n = 8$) and without ($n = 6$) *T. nodulosus* plerocercoids in the liver. Results revealed a small number of differentially expressed genes (DEGs, adjusted p-value $\leq$0.05) in both spleen ($n = 22$) and liver ($n = 10$). DEGs in spleen consisted of mostly upregulated immune related genes (e.g., *JUN*, *SIK1*, *THSB1*), while those in the liver were often linked to metabolic functions (e.g., *FABP1*, *CADM4*, *CDAB*). However, Gene Ontology (GO) analysis showed lack of functional enrichment among DEGs. This study demonstrates that Eurasian perch displays a subtle response at a gene expression level to *T. nodulosus* plerocercoid infection. Given that plerocercoids are low-metabolic activity transmission stages, our results suggest that moderate *T. nodulosus* plerocercoid infection most likely does not provoke an extensive host immune response and have relatively low physiological costs for the host. Our findings illustrate that not all conspicuous infections have severe effects on host gene regulation.

1. Introduction

Mounting an appropriate response to a parasite infection is essential for host's fitness and survival prospects. The magnitude of such a response, involving immediate immune response, inflammation, or wound repair, is expected to be influenced by many factors, including the parasite, environmental variables, and individual host characteristics (Cox, 2001; Gopko et al., 2017; Turner et al., 2021). The use of next-generation sequencing (NGS) technologies improves our ability to understand the molecular basis of such complex host-parasite interactions, even in the wild (Brinker and Hamers, 2007; Greenwood et al., 2016; Sudhagar et al., 2018). For instance, by characterizing tissue-specific gene expression, we can gain novel insights into the genes that are likely involved in the immune responses or tissue repair of the host in response to parasite infection (Shanmugam et al., 2012; Hoy et al., 2014; Anderson et al., 2015; Shadab et al., 2019). Consequently, this provides targets for future investigation, and even discoveries of potential anti-parasitic drug targets for various ecologically or

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economically important species (Chandhini and Rejish Kumar, 2019; Ahmad et al., 2021a,b).

The Eurasian perch (*Perca fluviatilis*) is a widely distributed freshwater fish species native to Eurasia throughout Europe to Eastern Siberia (Behrmann-Godel and Brinker, 2016). It is a generalist feeder and has a rich parasite fauna (Kuperman, 1973; Balling and Pfeiffer, 1997; Valtonen et al., 2003; Lahnsteiner et al., 2009) hosting nearly 150 parasite species (Behrmann-Godel and Brinker, 2016). Despite a large and increasing body of research on perch biology, ecology, and genetics (Thorpe, 1977; Acerete et al., 2004; Vasemägi et al., 2023), relatively little is known about perch–parasite interactions at a molecular level; however, this situation is slowly changing, at least in part because of growing importance of Eurasian perch in aquaculture (Fontaine and Teletchea, 2019) and an increasing availability of molecular resources (Malmstrøm et al., 2017; Roques et al., 2020; Ozerov et al., 2022). Yet, the ecological relevance and physiological impact of the majority of parasite infections on perch are currently not known, which underlines the need for performing studies outside of a contained laboratory setting (Sasser and Weber, 2023).

One of the common parasites of Eurasian perch is the pike tapeworm (*Triaenophorus nodulosus*), a widespread parasitic helminth (*Cestoda*) which frequently uses *P. fluviatilis* as a second intermediate host (Kuperman, 1973) to infect its definitive host, the northern pike (*Esox lucius*) (Bregazzi, 2006). Commonly, perch are infected by *T. nodulous* as fry and juveniles by feeding on infected zooplankton (e.g., *Cyclops*, *Daphnia*, *Diaptomus*, and others (Lahnsteiner et al., 2009)). The ingested parasite migrates through the intestine of the perch and punctures the intestinal wall to reach the targeted liver tissue, where it encysts and develops into the plerocercoid phase. The parasite's migration through tissues can result of the lysis of host membranes, causing inflammation, atrophy, necrosis, and other potentially lethal consequences (Rosen, 1918; Scheuring, 1922; Kuperman, 1973; Hoffmann et al., 2006; Behrmann-Godel and Brinker, 2016). Damage to the host may be inflicted also at a later infection stage from the increased pressure on the surrounding tissues by large, encapsulated larvae which continue growth and development within the liver (Scheuring, 1922). The severity of *T. nodulosus* infection was shown to depend on parasite load, which past research has found to be between 2 and 3 plerocercoids per infected *P. fluviatilis* liver (Morley and Lewis, 2010, 2017), where the condition of the perch deteriorates with increasing parasite numbers (Behrmann-Godel and Brinker, 2016). This density-dependent effect has been also observed in pumpkinseeds (*Lepomis gibbosus*), an invasive teleost in Europe that is often occupying similar niches as the perch (Brinker and Hamers, 2000; Masson et al., 2015). Infection with *T. nodulosus* in adult perch has been also associated with a reduction in fertility (Brinker and Hamers, 2007) and ~10% reduction in growth rate (Kuperman, 1973; Brinker and Hamers, 2000; Lahnsteiner et al., 2009; Behrmann-Godel and Brinker, 2016). However, until now, the physiological effect of *T. nodulosus* on *P. fluviatilis* has been studied only using histological techniques and based on a single gene (proliferating cell nuclear antigen) (Dezfuli et al., 2014). Thus, despite high prevalence and wide distribution of *T. nodulosus* (Brinker and Hamers, 2007; Borvinskaya et al., 2019), we currently lack detailed information on the molecular pathways and processes involved in host responses to the parasite which may be currently attained using whole-transcriptome sequencing.

The aim of this study was to evaluate the response of perch to *T. Nodulosus* infection by comparing the expression profiles of naturally and moderately infected and uninfected individuals in two tissues (spleen, liver) at the whole transcriptome level. We hypothesized that (i) once *T. nodulosus* has encysted and given that plerocercoids are a relatively low-metabolic activity stage of development of a parasite (Liu et al., 2022), host responses at the level of transcript abundances are expected to be mild to moderate. This is supported by earlier work reporting relatively weak physiological effects of plerocercoid infections (Brinker and Hamers, 2007; Behrmann-Godel and Brinker, 2016), which, in less severe cases, may enable perch to survive and withstand infection by *T. nodulosus* plerocercoids for years in its natural environment. We also hypothesized that (ii) due to a vital role of liver in haematopoiesis, detoxification and nutritional metabolism, stronger transcriptomic response in liver compared to spleen would predominantly reflect the effect of parasite on host condition, feeding and response to physical damage. On the other hand, (iii) given the spleen's key role in infection response, a greater proportion of differentially expressed genes (DEGs) in the spleen compared to liver would indicate the importance of immune response of the host against the parasite.

2. Materials and methods

2.1. Fish collection and tissue preparation

In August 2021, fifteen adult *P. fluviatilis* individuals were caught using gillnets from four lakes in Estonia. Perch was euthanized with an overdose of benzocaine and measured for weight (±0.1 g) and length (total length; TL, mm) (Table 1). After opening the fish using a sterile scalpel, liver and spleen were dissected, snap-frozen on dry ice while in the field and subsequently stored at −80 °C. In addition, we recorded the maturation status (mature, immature) and sex of the individual. The national guidelines for the care and use of animals were followed per the Estonian Animal Protection Act, and fishing permits were obtained by the Estonian Ministry of the Environment (no. 10–1/21/18).

2.2. T. nodulosus molecular identification

Infection status was determined by visual identification of plerocercoids in fresh *P. fluviatilis* livers. Between 2 and 5 plerocercoids were detected on each infected fish liver (data not shown) and a single plerocercoid was dissected from each of the infected specimens for molecular conformation of the species. Plerocercoid samples were fixed in 96% ethanol and stored at −20 °C. DNA was extracted from whole plerocercoids using a Dneasy Tissue Kit (Qiagen) according to the manufacturer's instructions. The concentration of isolated DNA was measured with a NanoDrop ND-2000 spectrophotometer (Thermo Scientific). Amplification of the *rrnL* ribosomal subunit gene (Brabec et al., 2015) was performed in 10 μL reactions to confirm the occurrence of *T. nodulosus*. Each 10 μL polymerase chain reaction (PCR) consisted of 5 μL of 2*x* Typeit Buffer (Qiagen); 0.5 μL of each primer (500 nM); 2 μL of DNA (100–150 ng total) and 2 μL of nuclease-free water. The primer sequences used for *rrnL* amplification were following: Cest16Sfgen (5′-TRCCTTTTGCATCATG-3′) and Cest_16SRc (5′- AATAGATAAGAAC CGACCTGGC-3′) (Scholz et al., 2013). The thermocycler amplification protocol consisted of initial denaturation at 95 °C for 15 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 54 °C and 90 s at 72 °C; the final extension at 72 °C was 10 min. Sequencing of PCR product using Sanger method was performed at the Institute of Genomics Core Facility, University of Tartu (Estonia) from both directions, and the 22 sequences (forward and reverse) were successfully merged manually. BLAST analysis of 11 consensus sequences indicated that the infecting worms were *T. nodulosus* (GenBank ID: KR780832.1, highest sequence similarity: 97%). Sequences produced in this study can be accessed at GenBank (OR065063-OR065071).

2.3. RNA extraction

Frozen liver tissue samples were mechanically crushed in liquid nitrogen using a steel mortar and pestle to produce a homogenized powder, while frozen spleen tissue was mechanically crushed using a Retsch Mixer Mill MM 400 (Retsch) (the tissue consistency of spleen samples was not conducive to mortar and pestle homogenization). Total RNA was extracted using a NucleoSpin RNA extraction kit (MACHEREY-NAGEL, Duren, Germany). RNA sample concentrations were measured with a NanoDrop 2000 (ThermoFisher), and sample quality was evaluated using a TapeStation 2200 (Agilent Technologies).

Table 1
Summary information of analysed *P. fluviatilis* samples and tissues used for this study. Sex (F for female, M for male) and infection status, location, and total length are provided.

Sample ID	Infection status	Lake name	Lake type	Latitude	Longitude	Tissue used	Sex	Total length (mm)
1	Infected	Kasaritsa	Clear	57.781	27.03	Liver	M	126
2	Infected	Kasaritsa	Clear	57.781	27.03	Liver	F	144
3	Infected	Lasa	Humic	57.919	25.789	Liver	M	141
4	Infected	Lasa	Humic	57.919	25.789	Liver, spleen	F	161
5	Uninfected	Lasa	Humic	57.919	25.789	Liver, spleen	M	167
6	Uninfected	Lasa	Humic	57.919	25.789	Liver, spleen	F	163
7	Uninfected	Lasa	Humic	57.919	25.789	Spleen	M	140
8	Uninfected	Lasa	Humic	57.919	25.789	Spleen	M	140
9	Infected	Saadjärv	Clear	58.538	26.656	Liver, spleen	F	134
10	Infected	Saadjärv	Clear	58.538	26.656	Spleen	F	129
11	Uninfected	Saadjärv	Clear	58.538	26.656	Liver, spleen	F	103
12	Uninfected	Saadjärv	Clear	58.538	26.656	Liver, spleen	M	103
13	Uninfected	Udriku	Humic	59.296	26.064	Liver	M	152
14	Infected	Udriku	Humic	59.296	26.064	Liver, spleen	F	143
15	Infected	Udriku	Humic	59.296	26.064	Spleen	F	133

2.3.1. Library preparation, sequencing, and quality assessment

Sequencing libraries were prepared at the Novogene Cambridge Science Park (Cambridge, United Kingdom) from 300 ng total RNA. After fragmentation and end repair, poly-A tail attachment, adapter ligation, size selection, amplification, and purification, sequencing (2 × 150 bp) was performed via NovaSeq 6000 (Illumina).

The raw sequence files in fastq format returned from Novogene were run through FastQC v0.11.9 (Andrews, 2010) both before and after trimming to check the overall quality of the reads. The trimming was performed by fastp v.0.20 (Chen et al., 2018). Only reads which were both longer than 50 bp and were of mean quality of 25 were processed further.

2.3.2. Alignment and mapping

The reads which passed the quality control were mapped on the reference genome of *Perca fluviatilis* (Roques et al., 2020) using hisat2 v.2.1.1 (Kim et al., 2019) using default parameters. The produced. sam files were subsequently processed with samtools v.1.10 (Li et al., 2009). Lastly, the mapped reads were extracted in bam format for subsequent differential expression analysis.

2.4. Differential expression

Analysis for differentially expressed genes (DEGs) was performed in R v.4.2.2 (R Core Team, 2022) using the DESeq2 v.1.36.0 (Love et al., 2014) package. When designing the DESeq dataset object, we considered infection status and population as fixed factors, and the DESeq model internally corrects for potential differences in library sizes. Genes with an adjusted p-value (*p-adj*) ≤ 0.05 (Benjamini and Hochberg, 1995) were considered as significantly differentially expressed (DEGs) between infected and uninfected individuals. Human orthologue genes were obtained via Ozerov et al. (2022), and DEGs without human orthologues were manually searched using BLAST.

2.4.1. Gene ontology (GO) analysis

GO enrichment was performed using Gorilla (Eden et al., 2009) separately for both liver and spleen samples. DEGs were compared with all expressed genes from each respective tissue.

3. Results

Sequencing resulted in an average of 45.1 and 47.8 million raw reads for every spleen and liver sample, respectively. After trimming and filtering, a total of 432 million clean reads were retained for spleen samples, with the average number of 39.3 million reads per sample (Supplementary file 1). A total of 465 million clean reads were retained for liver samples, with the average number of reads of 42.4 million per sample (Supplementary file 2).

RNAseq analysis for spleen samples revealed 17, 246 genes being expressed (>1 counts per million), with only 22 genes being differentially expressed between infected and uninfected fish (*p-adj* ≤ 0.05; Table 2). A heatmap generated from DEGs revealed that samples were segregating based on infection status, except for a single infected sample which clustered among uninfected fish (Fig. 1A). All except one DEG showed upregulation in infected fish (2 × 2 contingency table, Fisher's exact test $P = 0.0015$). Identified DEGs were primarily encoding for membrane-bound proteins such as adhesion molecules and receptors (*GPIA*, *SYTL3*, *BTN2A1*), kinase binding (*MIDN*, *AP1B1*, *DUSP4*), cell death and tumorigenesis (*SIK1*, *THBS1*) as well as were uncharacterized or unknown genes (LOC120571416, LOC120570956, LOC120573523, LOC120557941, si:ch73-335l21.4, LOC120555327) (Table 2). Tumour suppression and related oncogenes (*JUN*, *JUND*, *RDH12*) were also observed. The majority of DEGs in the spleen relate to host immune response, metabolism, stress response, and membrane-associated proteins (Table 2). However, formal Gene Ontology (GO) analysis showed lack of functional enrichment among DEGs, most likely because of limited number of DEGs (see Fig. 2).

RNAseq analysis for liver samples revealed 15, 377 expressed genes (>1 counts per million), with only ten genes being differentially expressed (DE) between infected and uninfected fish (*p-adj* ≤ 0.05; Table 3). Like the spleen, a heatmap generated from DE genes in liver revealed incomplete segregation of samples based on infection status (Fig. 1B). Lymphocyte and cell differentiation (*ACSF2*), galactose-specific lectin nattectin, as well as binding proteins and cell adhesion molecules and their catalysts (*LMNA, FABP1, CADM4, CDAB, APBB1IP, FTCD*) were among DEGs. Two DEGs (*BTN3A2*, *NRG3B*) were related to transcription factors involved in the host adaptive immune response. In contrast to spleen, up- and downregulation of DEGs in liver occurred evenly (2 × 2 contingency table, Fisher's exact test $P = 1$). Similar to the spleen, GO analysis showed lack of functional enrichment among DEGs, most likely because of limited number of DEGs.

4. Discussion

Parasitic infections can have severe effects on host physiology, immune system function, survival, and performance (Bienvenu et al., 2010). Many populations of fish are at risk to a wide range of parasites (Dezfuli et al., 2014; Behrmann-Godel and Brinker, 2016; Gopko et al., 2017; Vollset et al., 2018) which can have broad implications on the health and well-being of the host. However, not all infections have a severe or detrimental effect on the host and its fitness. In this study, we found that *T. nodulosus* plerocercoid infection evoked very subtle host response on both spleen and liver transcriptomes of adult *P. fluviatilis*. This suggests that moderate *T. nodulosus* plerocercoid load likely has

Table 2
Summary statistics and gene information for 22 DEGs (*p-adj* ≤ 0.05) in spleen between infected and uninfected *P. fluviatilis* individuals. N/A indicates unknown protein.

Genbank ID	Base mean (Infected)	Base mean (Uninfected)	log2 Fold Change	p-value	p-adj	Gene name	Gene ID
PFLUV_G00160980	222.75	99.89	1.97	1.32E-11	1.23E-07	uncharacterized LOC120571416	N/A
PFLUV_G00160960	163.54	17.56	3.38	1.43E-11	1.23E-07	uncharacterized LOC120570956	N/A
PFLUV_G00137210	242.75	54.2	2	1.60E-10	9.15E-07	midnolin-like	*MIDN*
PFLUV_G00146270	1608.68	460.33	1.61	3.43E-10	1.48E-06	salt-inducible kinase 1	*SIK1*
PFLUV_G00160970	89.23	6.63	4.17	1.66E-09	5.69E-06	uncharacterized protein LOC120571417 isoform *X1*	N/A
PFLUV_G00169820	178.7	23.66	3.01	6.68E-09	1.91E-05	uncharacterized protein LOC120573523	N/A
PFLUV_G00042630	250.63	79.28	1.9	3.10E-08	7.60E-05	probable nuclear hormone receptor HR38	N/A
PFLUV_G00114330	2619.87	884.36	1.68	4.11E-08	8.82E-05	transcription factor AP-1	*JUN*
PFLUV_G00234600	3792.66	1154.08	1.75	3.92E-07	0.000748084	thrombospondin-1-like	*THBS1*
PFLUV_G00093590	1039.83	845	0.41	5.16E-07	0.000885785	glucose-6-phosphate isomerase a	*GPIA*
PFLUV_G00165910	197.7	59.92	1.77	7.91E-07	0.001235261	E3 ubiquitin-protein ligase-like	N/A
PFLUV_G00223220	208.77	86.83	1.05	3.05E-06	0.004362188	arginine vasopressin-induced protein 1-like	*AVPO1*
PFLUV_G00214770	289.01	151.81	1.01	4.10E-06	0.005413646	synaptotagmin-like protein 3	*SYTL3*
PFLUV_G00257880	609.91	415.74	0.48	9.00E-06	0.01017912	retinol dehydrogenase 12: like	*RDH12L*
PFLUV_G00277620	458.37	200.03	1.88	9.19E-06	0.01017912	uncharacterized protein LOC120555327	N/A
PFLUV_G00132110	484.34	248.1	0.88	9.48E-06	0.01017912	transcription factor AP-1-like	*AP-1*
PFLUV_G00054610	435.84	132.77	1.71	1.53E-05	0.015501048	nuclear receptor subfamily 4, group A, member 1	*NR4A1*
PFLUV_G00004570	2519.13	1093.24	1.03	2.37E-05	0.022595861	Unknown	N/A
PFLUV_G00139600	1160.74	524.36	1.17	3.04E-05	0.027506318	transcription factor jun-D-like	*JUND*
PFLUV_G00051240	10,006.48	19,495.98	−0.95	3.82E-05	0.032055553	butyrophilin subfamily 2 member A1-like	*BTN2A1*
PFLUV_G00199970	1436.69	747.55	0.87	3.95E-05	0.032055553	LOW QUALITY PROTEIN: dual specificity protein phosphatase 4	*DUSP4*
PFLUV_G00008200	176.2	76.43	1.08	4.10E-05	0.032055553	low density lipoprotein receptor b	*LDLRB*

only a weak effect on host metabolism and condition.

Given that the liver is a major metabolic hub and the target tissue for *T. nodulosus* plerocercoids enduring physical abrasion during encysting, we anticipated that a pronounced transcriptome-wide response to liver infection would predominantly reflect the effect of parasite on host condition, feeding and response to physical damage. Instead, *T. nodulosus* infection was associated only with a subtle transcriptomic response in perch (number of DEGs = 10), which most likely reflects a weak effect of the parasite infection on host metabolism and physiological condition. Yet, several identified DEGs in liver (e.g., *FABP1*, *BT3A2*, *LMNA*, *CADM4*, *APBB1IP*) have been shown to associate with various parasite infections and liver ailments in other host-parasite systems; for example, Alvarez Rojas et al. (2015) found an increase in immune- and fibrosis-related genes as a result of trematode *Fasciola hepatica* infection in sheep liver. Similarly, one of the upregulated genes in perch liver, *LMNA* (LOC120572191), provides increased structural integrity for the cell as component of the fibrous nuclear lamina, and has been shown to be involved with liver fibrosis (Wang et al., 2016). We also found down-regulation of inhibitors associated with wound healing and cell death (*APBB1IP*, *CADM4*) and upregulation of neutrophil chemotaxis genes and transcription factors (*CDAB*, *Galactose-specific lectin nattectin*) associated with host immune response.

Due to the spleen's key role in immune defence, we anticipated that substantially higher proportion of DEGs in the spleen compared to liver would reflect stronger immune response of the host against the parasite. Yet, we found similar, very small number of DEGs in the spleen (n = 22) among which there were several genes without known human orthologs (n = 8). The lack of strong transcriptome-wide response may stem from a couple of processes. One such possibility is that *T. nodulosus* infection has an overall low physiological effect which does not provoke a strong host immune response and fish are able to cope with low to moderate levels of parasite burden. In contrast, many parasite infections often cause major changes in specific host tissues involving hundreds or thousands of genes reflecting a major systemic response; for example, over 200 DEGs and a strong immune functioning GO process enrichment was observed in perch eye tissues in association with *Diplostomidae* infections (Noreikiene et al., 2020). It is also possible that *T. nodulosus* is able to evade triggering the host immune system, such as the spleen or

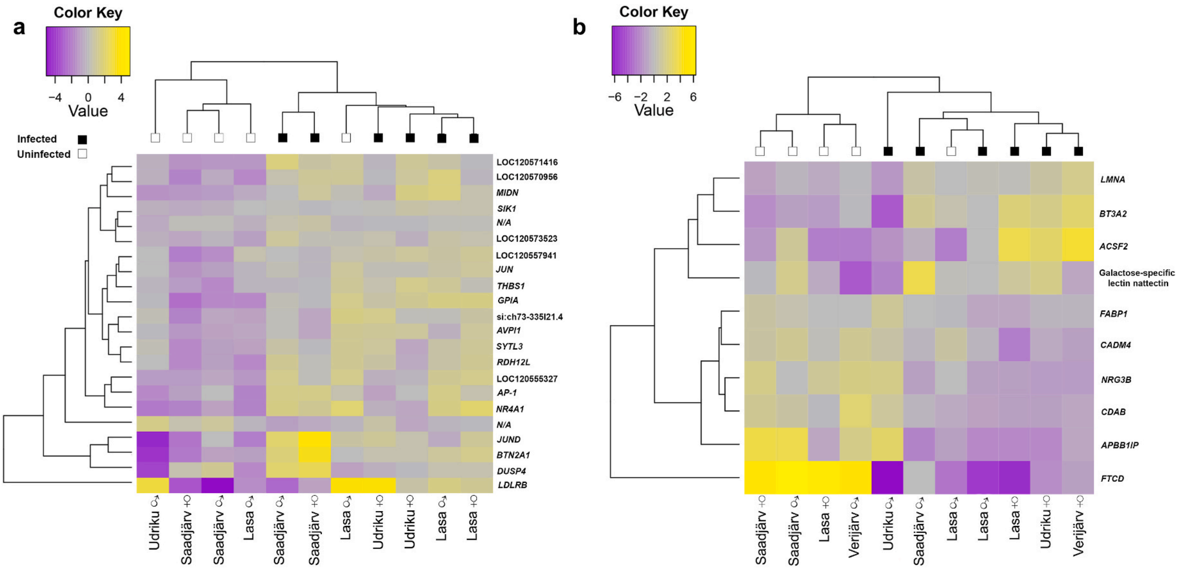

Fig. 1. Differentially expressed genes between infected and uninfected *P. fluviatilis* in a) spleen and b) liver tissues. Filled-in and empty boxes on the top of each plot represent infected and uninfected individuals, respectively. N/A indicates unknown protein.

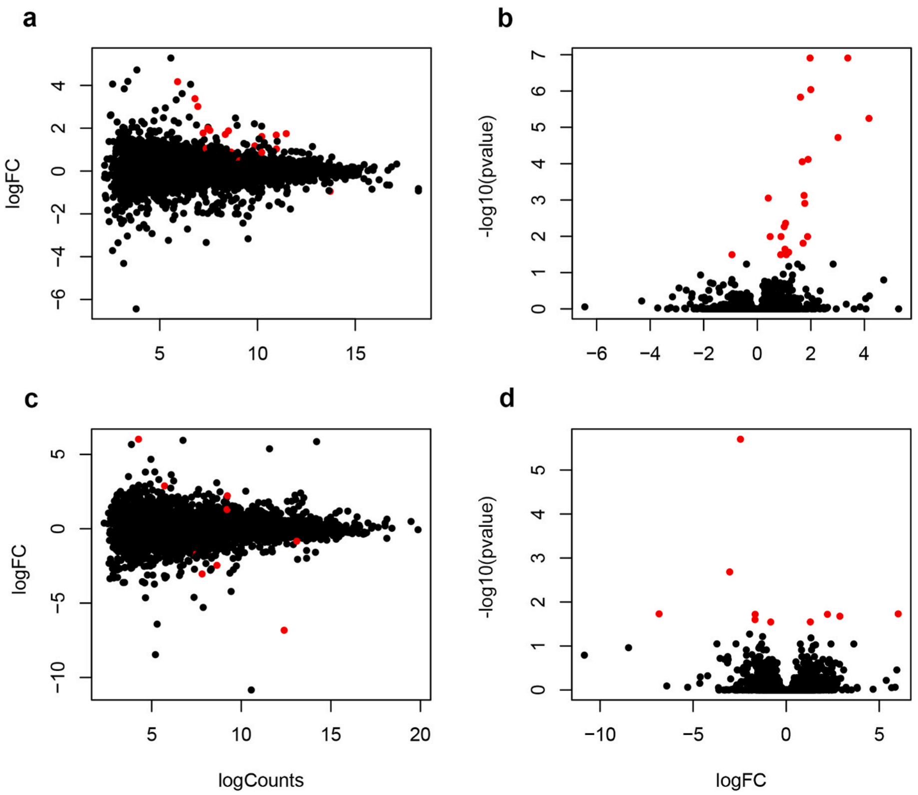

Fig. 2. MA and volcano plots comparing infected and uninfected spleen samples (a & b) and liver samples (c & d). In the MA plots (a & c), the log counts and the log fold change are represented on the x- and y-axis, respectively. For each volcano plot (b & d), log fold change is represented on the x-axis and the –log10 p-value on the y-axis, respectively. Positive fold change corresponds to upregulated genes in infected individuals.

even other immune-privileged sites (Chulanetra and Chaicumpa, 2021). For example, trematodes which can coat their surfaces in a complex which degrades host antibodies and thus allows the parasite to avoid the host's immune response (Cortés et al., 2017; Chulanetra and Chaicumpa, 2021). *Fasciola hepatica* has also been shown to evade host immunity, despite drugs and other procedures such as vaccination and chemotherapy (Flynn and Musah-Eroje, 2020). Finally, the subtle gene response in both liver and spleen is consistent with low-metabolic activity plerocercoid infections after host has been able to encapsulate and isolate the parasite. Therefore, it is likely that the major interactions between the host and *T. nodulosus* takes place at the time of early invasion, rather than during the established resident plerocercoid stage. Future studies should therefore focus on early infection stages of *T. nodulosus*, when the effects of parasite migration and establishment is likely more severe.

Despite this low number of DEGs, they indicate some degree of

Table 3
Summary statistics and gene information for ten DEGs (p-$adj \leq 0.05$) in liver between infected and uninfected *P. fluviatilis* individuals. N/A indicates unknown protein.

Genbank ID	Base mean (Infected)	Base mean (uninfected)	log2 Fold Change	p-value	p-adj	Gene name	Gene ID
PFLUV_G00169300	111.07	636.76	−2.46	1.32E-10	1.99E-06	lamin-A-like	*LMNA*
PFLUV_G00169310	20.67	393.12	−3.04	2.75E-07	0.00207	butyrophilin subfamily 3 member A2-like isoform *X*2	*BT3A2*
PFLUV_G00177580	37.58	2.26	6.02	4.19E-06	0.018592	medium-chain acyl-CoA ligase ACSF2: mitochondrial-like isoform *X*1	*ACSF2*
PFLUV_G00150020	99.55	9776.31	−6.83	4.93E-06	0.018592	galactose-specific lectin nattectin-like isoform *X*1	N/A
PFLUV_G00072130	982.28	273.76	2.22	6.51E-06	0.019055	fatty acid-binding protein, liver-type-like	*FABP1*
PFLUV_G00087640	63.21	258.32	−1.67	7.58E-06	0.019055	cell adhesion molecule 4	*CADM4*
PFLUV_G00243290	95.47	15	2.88	9.77E-06	0.021038	neuregulin 3 b	*NRG3B*
PFLUV_G00041980	116.83	390.21	−1.68	1.34E-05	0.025163	cytidine deaminase b	*CDAB*
PFLUV_G00257980	870.56	350.03	1.3	1.69E-05	0.028373	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	*APBB1IP*
PFLUV_G00277100	5813.36	11,217.62	−0.83	1.89E-05	0.028451	formimidoyltransferasecyclodeaminase	*FTCD*

impact on the host. For example, one of the uncovered DEGs (*JUN*) is an oncogenic transcription factor which has been associated with anti-apoptotic properties, immune response, and cell death (Wisdom, 1999; Lukey et al., 2016). Genes belonging to this family of transcription factors have been upregulated in mice, when infected by a helminth parasite (*Taenia crassiceps* or *Taenia solium*) (Morales-Montor et al., 2004). This present study has found both an increased expression of *AP-1*, *NR4A1,* and *THBS1* orthologs in infected perch. Infection in bovines by the tick-borne *Thallia annulata* and *Thallia parva* parasites has also been shown to result in an increased expression of the *AP-1* transcription factor and an associated upregulation of *JUN* protein. Recently it has been reported that mice infected with the liver parasite *Entamoeba histolytica* showed an upregulation of *NR4A1*, a transcription factor responsible for regulation macrophages in the host (Hoenow et al., 2022). Additionally, it has been discovered that when three-spined stickleback (*Gasterosteus aculeatus*) are infected by the helminth parasites *Diplostomum pseudospathaceum* and *Schistocephalus solidus*, one of the few genes that was upregulated because of the infection was the *THBS1* (Haase et al., 2016), which is associated with both cellular matrix and cell-cell interactions (Isenberg and Roberts, 2020). Finally, upregulation of *SIK1* in infected perch coincides with an analysis by Kang et al. (2020), who found a significant correlation between *SIK1* and a negative prognosis in patients suffering from diffuse large B-cell lymphoma. While speculative, this may be relevant for other parasites, such as various liver and blood flukes (*Opisthorchis viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis*) that have been categorized as Group 1 carcinogens by the World Health Organization (Feng and Cheng, 2017). Taken together, these studies demonstrate that several identified DEGs in spleen are likely involved in host-pathogen responses in diverse systems.

Prior research has shown that perch can survive and withstand infection by *T. nodulosus* plerocercoids for years in its natural environment (Brinker and Hamers, 2007). Our findings support this by identification of only a small total number of DEGs across two tissues and corroborates earlier work by Masson et al. (2015) which demonstrated a relatively weak physiological effect (hepatosomatic index, body condition index, and others) of *T. nodulosus* plerocercoid infection on adult *P. fluviatilis*. However, our results are to some extent at odds with Brinker and Hamers (2000), who found a stronger physiological effect (on growth) which was linked to the severity of *T. nodulosus* infection. A potential reason for this difference may be related to the varying infection tolerance across perch ontogeny coupled with the selective disappearance of the weakest individuals. Here, we sampled mature Eurasian perch individuals that have already survived their most vulnerable and challenging stages of development. Thus, it is entirely possible we would see a more pronounced effect of *T. nodulosus* infection in juveniles that initially suffer plerocercoid formation and are expected to have competing resource demands between immune function (current survival) and growth (future survival). Considering the described effects during reproduction (Brinker and Hamers, 2007), it is also possible that a stronger response to infection would be observed during the breeding season, which is especially demanding for egg producing females (Šimková et al., 2005). Due to the random nature of catching fish in the wild, we were not able to control for the degree of infection status, and it may be the case that more heavily infected perch would exhibit more substantial response against *T. nodulosus*. However, prior research on *T. nodulosus* in the liver of *P. fluviatilis* has shown a mean intensity of 2.9 parasites (Morley and Lewis, 2017), which falls comfortably into the observed range of parasite load within the present study (between 2 and 5 plerocercoids per infected liver). The findings of Morley and Lewis (2017) are in agreement with other research which has found mean infection intensities of 3.87 plerocercoids per live (SD = 1.79) (Morozińska-Gogol, 2013), as well as having a 'normal' infection range being defined as 1–3 plerocercoids per liver, with severe infections with >3 plerocercoids (Brinker and Hamers, 2007).

Evidence across different host-parasite systems indicates that infection can increase the likelihood of predation (Furey et al., 2021; Turner et al., 2021), but this phenomenon is far from universal (Masson et al., 2015). To maximize the likelihood for reaching its final host (northern pike), the pike flatworm may benefit from weakening perch making it more susceptible to predation and thus completing its life cycle, but this has yet to be shown conclusively (Dezfuli et al., 2014). However, if the Eurasian perch is unable to sustain and survive given parasitic burden and dies before being eaten by the pike, it does not represent an optimal reproductive outcome for *T. nodulosus*. Rather, it is in the parasite's interest that the intermediate host can cope with the infection and live until being predated by pike. Therefore, it makes sense *prima facie* that the more optimal strategy for the parasitic flatworm would be to induce only mild response in the intermediate host and our transcriptomic analysis corroborates this. Recent research into the parasitic nematode *Contracaecum osculatum* revealed that fish with high infection densities of *C. osculatum* had decreased nutritional condition as evidenced by reduced standard metabolic rate, reduction in the digestive organ masses, and changes in the plasma, body and liver composition, and fish energy source (Ryberg et al., 2020). This effect on metabolism, immune function, and growth are in broad agreement with our own study and may suggest that liver-parasites induce the same general host response, despite differences in parasite species. Therefore, in a similar vein to *T. nodulosus*, it may be advantageous for *C. osculatum* to target growth and immune functions in Baltic cod (*Gadus morhua*) as a means to reach the final stage of its life cycle within its definitive host, the grey seal (*Halichoerus grypus*).

To our knowledge, this study provides first transcriptomic insights into the Eurasian perch response to pike tapeworm plerocercoid infection. Future investigation into this host-parasite system may benefit from an increase in sample size, and to also include analysis of severely infected individuals of different ages. RNAseq studies until now typically have employed a strategy of sequencing 3–5 individuals per treatment, while using an alternative library preparation methods (such as 3′-end sequencing, also known as QuantSeq) (e.g., Moll et al., 2014; Ahmad et al., 2021) are able to screen hundreds of samples; therefore, given the ongoing rapid development of next-generation sequencing technologies and dropping sequencing costs, future studies are likely able to carry out more comprehensive screening across different stages of infection. In this study our recording system was binary, in that a given liver tissue, when examined by eye, is recorded as either being infected or uninfected and the exact degree of infection severity was not taken into account. Therefore, subsequent research should include an explicit analysis of infection intensity as well as physiological condition, as for example in Baltic cod infected by the parasitic nematode *Contracaecum osculatum* (Marnis et al., 2019; Ryberg et al., 2020; Behrens et al., 2023). Such studies may provide additional insights into how parasite load is related to *P. fluviatilis* gene expression and immune response.

While the definition of 'parasite' commonly implies that one organism benefits at the expense of another organism (Olano et al., 2006), the actual cost of the parasitism may vary from lethal to essentially negligible. Hosts may harbour dozens to many hundreds of parasites, including many different parasite species. Therefore, it has been suggested that it is more fitting to describe the impact of being parasitized as range of effects which exist on a spectrum, whose ranges can extend from mild and virtually asymptomatic, to severe or even lethal (Ebert, 1998; Abernathy et al., 2011; Bracamonte et al., 2019; Barrett and Bartholomew, 2021; Hargitai et al., 2021; Hierweger et al., 2017; Sasser and Weber, 2023). However, it is likely that there exists a bias among current transcriptomic studies focusing on highly virulent or harmful parasites, as improving our understanding of most dangerous pathogens is often prioritized in medical, veterinary, or ecological research. Yet, given the incredible diversity of parasitic life-forms, it is likely that many parasites have only a small effect on host fitness at least during a certain period. Our study therefore represents one of such examples where a parasite, despite its conspicuous nature, evokes only a subtle physiological response from the host.

Note

Supplementary data associated with this article.

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Availability of data and materials

Sequence data will be made available upon publication.

Authors' contributions

KT was responsible for laboratory work, data analysis, manuscript writing, figure and table construction, and data interpretation. KN contributed to the original study design, sample collection, manuscript writing, and data interpretation. AV contributed to the original study design, sample collection, manuscript writing, and data interpretation. SK contributed to sample collection. MO contributed to data analysis and data interpretation. RG contributed to manuscript editing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

None.

Consent for publication

All authors give consent for publication.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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Glossary

DEG: Differentially expressed gene
PCR: Polymerase chain reaction
RNAseq: RNA Sequencing
TL:: Total length
GO: Gene ontology