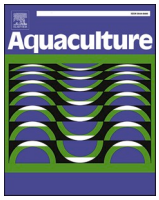
Aquaculture 574 (2023) 739648

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Microbiome structure of milt and ovarian fluid in farmed Arctic charr (*Salvelinus alpinus*)

Christos Palaiokostas [a,*], Daniyal Gohar [b], Khrystyna Kurta [a], Henrik Jeuthe [a,c], Mohammad Bahram [d]

[a] Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
[b] Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, Estonia
[c] Aquaculture Center North, Åvägen 17, 844 61 Kälarne, Sweden
[d] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

ARTICLE INFO

Keywords:
16S sequencing
18S sequencing
Sperm quality
Ovarian fluid
Arctic charr

ABSTRACT

Limited knowledge exists about the residing microbiome in gamete-related samples in fish. A potential effect between the seminal microbiome composition and sperm quality traits has been previously suggested in humans and livestock. Using a metabarcoding approach, we aimed to gain insights into the structure of the residing prokaryotes and microbial eukaryotes in ovarian fluid ($n = 10$) and milt ($n = 84$) from farmed Arctic charr - a species with highly variable reproductive success in captivity. In addition, sperm quality traits were recorded on the sampled males to investigate potential associations with the residing seminal microbiome. Higher microbial diversity was found in the ovarian fluid compared to the milt habitat. Even though the residing microbiome showed distinct differences between the two habitats, substantial overlap was observed, with >70% of the milt core microbiome being found in the ovarian fluid habitat. Statistically significant associations were found between the Shannon diversity index and sperm motility-related traits. Additionally, a fungal operational taxonomic unit (OTU) potentially belonging to the Leotiomycetes class was associated with sperm concentration and motility. Overall, our study documents the microbiome structure of gamete-related samples from Arctic charr. Even though some associations were obtained between sperm quality parameters and either microbiome diversity or with a fungal OTU, follow-up studies on a larger scale with more tank replicates are needed to confirm the robustness and causality of these relationships.

1. Introduction

Reproduction in captivity is an essential prerequisite for any animal production system. Aquaculture is the fastest-growing food sector, increasing on average by >6% per year since the early nineties, with a global production that exceeds 110 million tonnes (Tacon, 2019). To cover the protein needs of the expanding human population and if we are to reach the United Nations Sustainable Development Goals by 2030, a substantial boost of global aquaculture production will be required (Naylor et al., 2021). Nevertheless, the above goals can only be fulfilled if the farmed animals produce high-quality and fertile gametes under captive conditions (Mylonas et al., 2010).

A decline in reproductive success in captivity appears to be a common phenomenon for terrestrial and aquatic animals. Notably, a most striking decrease in reproductive performance is common in the case of farmed fish (Farquharson et al., 2018). Additionally, since farmed fish are characterised by exceptionally high fecundity, especially compared to livestock, many fish farms can achieve their short-term production goals using a low number of broodfish (Villanueva et al., 2022). Even though a low inbreeding accumulation can have positive effects on fitness (Kokko and Ots, 2006), the above practices run the risk of resulting to inbreeding depression (Hely et al., 2013). Current knowledge suggests that reproduction-related traits in livestock are amongst the first to be negatively affected by inbreeding depression (Doekes et al., 2021). However, no conclusive evidence exists regarding the underlying causes of the reduced reproductive success in farmed fish. Environmental parameters (most prominently water temperature), broodfish nutrition, and underlying genetic and epigenetic factors have been previously pinpointed as affecting fertility in farmed fish (Cabrita et al., 2014; Migaud et al., 2013). Yet an underexplored parameter

* Corresponding author.
E-mail address: christos.palaiokostas@slu.se (C. Palaiokostas).

https://doi.org/10.1016/j.aquaculture.2023.739648
Received 23 October 2022; Received in revised form 21 April 2023; Accepted 4 May 2023
Available online 5 May 2023

thought to affect male fertility in fish (Bates and Tiersch, 1998) is the bacterial composition of the milt.

In recent years, particular emphasis has been placed on deciphering the microbiome composition in humans and animals, aiming to identify beneficial or harmful associations with fitness or health-related traits (Foo et al., 2017). The advent of high-throughput sequencing technologies, in particular, has allowed unprecedented insights into both taxonomic and functional aspects of microbes, facilitated the study of host-microbiome interactions and opened avenues for microbiome bioengineering (Wei et al., 2019). Regarding the latter, due to the often tied connection between host genotypes and the microbiome's diversity and abundance (Li et al., 2019), selecting directly on them appears to be a promising bioengineering approach (Raaijmakers and Mazzola, 2016). Notably, production efficiency in livestock has been linked to the composition of the ruminal microbiome (Myer et al., 2015). Though no conclusive inference can be drawn yet, the host's microbiome has been suggested to play a role in reproductive success in humans and animals (Comizzoli et al., 2021; Farahani et al., 2021).

In general, we have minimal knowledge about specific traits directly affected by the composition of the host's microbiome in farmed fish. To date, most of microbiome research in fish has been centred on the gut focusing in many cases on diet effects and potential associations with fitness and health traits (Luna et al., 2022). To the best of our knowledge, no prior study ever attempted to investigate the impact of the host microbiome on the reproductive success of any farmed fish. Therefore, characterising and understanding the role of the microbiome in fish gamete fluids could provide valuable insights.

Arctic charr (*Salvelinus alpinus*) has high commercial value for the Nordic aquaculture industry. This species is particularly suitable for farming across the Holarctic region mainly due to its superior ability to grow at low water temperatures compared to some of the most popular cold-water aquaculture species like Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*) (Elliott and Elliott, 2010). Nevertheless, a highly variable reproductive success in captivity hampers the industry's expansion (Olk et al., 2019). Current knowledge suggests that reproductive success in Arctic charr is affected by water temperature even during months outside the reproductive season (Nilsson et al., 2016), physiological attributes like the age of each broodfish (Jeuthe et al., 2013), nutritional factors like fatty acid composition of the provided diet (Pickova et al., 2007) and underlying genetic factors (Olk et al., 2019). Recent studies demonstrated the existence of the latter as Arctic charr from different families, while being reared in identical environmental conditions and fed the same diet, displayed highly variable levels of reproductive success and gamete quality (Kurta et al., 2022; Palaiokostas et al., 2020). As microbiome breeding is a promising avenue for increasing productivity in farmed set-ups (Mueller and Linksvayer, 2022), it would be worth investigating whether the microbial composition and abundance in gamete-related host samples play a role in fertility (Mueller and Linksvayer, 2022). Furthermore, as no information is available about the residing core microbiome in gamete-related body fluids on any fish, novel knowledge can be gained.

In the current study, we performed 16S and 18S rDNA sequencing on milt and ovarian fluid samples from Swedish Arctic charr and profiled the residing communities of bacteria and microeukaryotes in both habitats. Furthermore, in the case of the milt samples, sperm quality parameters were recorded, and potential associations with the residing seminal microbiota were explored. As such, additional hypotheses were tested: To what extent, if any, do differences in the water temperature affect the milt microbiome structure? Does inbreeding accumulation affect the milt microbiome structure? Are there any links between sperm traits and the residing microbiome structure?

2. Methods

2.1. Collection of milt and ovarian fluid

Male and female Arctic charr from the Swedish breeding program were used in our study. Sampling took place in October 2020 at the facilities of Aquaculture Centre North (ACN, Kälarne, Sweden). The studied fish were reared in concrete tanks (12 m^3, ~1 m depth) and supplied with water from the nearby lake Ansjön. Animals were fed 2% of the body weight per day with Vitalis feed (Skretting, Tooele, UT, USA) with an automatic feeding system (Arvo-Tec Oy, Huutokoski, Finland). The sampled males were reared in two tanks with ambient ($n = 69$) or cooled ($n = 15$) water temperature. More specifically, in the latter case, a water-cooling system was used (from July to November), resulting in a drop in water temperature by $3°C$ compared to the ambient one. During the sampling period, the ambient water temperature ranged from $10.6°C$ to $4.8°C$, while in the water-cooling case, it ranged between 7.7 $°C$ and 4.3 $°C$. For females, ambient water temperature was used (Fig. 1). Stocking densities were similar across all three tanks, equal to approximately 15 kg/m^3.

During sampling, tricaine methanesulfonate (MS-222, Sigma Aldrich, St. Louis, MO, USA) was used to anaesthetise the fish (0.15 gl^{-1}). After that, ovarian fluid and milt were collected in 10 ml disposable cups through manual stripping. Overall, ovarian fluid from 10 females and milt from 84 males was collected. All sampled animals were of the same age (3+ years) and inbreeding coefficients were available for all males in the study (Kurta et al., 2022).

Moreover, in the case of milt, sperm quality parameters related to motility were recorded the same day as stripping at the on-site laboratory of ACN, as described in (Kurta et al., 2022). Specifically, sperm motility-related parameters were recorded using a computer-aided sperm analysis (CASA) system equipped with the SCA® Motility imaging software v6.5 (Microptic, Barcelona, Spain). CASA measurements for each sample were taken 2–3times using 20 μm-depth slides with two counting chambers (CellVision, Heerhugowaard, Netherlands). Before loading the sample, the slides were pre-cooled at $8°C$. Sperm motility parameters were recorded at 15 s after activation with water, using a frame rate of 100 fps with recordings every 5 s (50 frames). The recorded parameters were: total motility (TM, %); total medium motility (%); total rapid motility (%); sperm velocity: average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s) and straight-line velocity (VSL, μm/s). The minimum velocity for sperm cells to be considered motile was set to VCL $\geq$ 20 μm/s (Kurta et al., 2022).

Finally, the sperm concentration of each sample was measured using a NucleoCounter® SP-100™ (Chemometec, Allerod, Denmark). Sperm samples were diluted at 1:1000 with a lysis buffer (20 μl sample and 20 ml buffer; Reagent S-100, Chemometec, Allerod, Denmark). After that, the built-in setting for analysing bull sperm was used; shown to be suitable for estimating sperm concentration in fish as well (Nynca and Ciereszko, 2009).

2.2. Library preparation and sequencing

DNA from 94 samples (84 milt; 10 ovarian fluid) was extracted with the Power Soil kit (Qiagen) according to the manufacturer's instructions using a Kingfisher Flex extraction robot (ThermoFisher). Each sample was PCR amplified in triplicates using Ready-To-Go (RTG) PCR beads (Cytiva) with the 18S-EUK581-F and 18S-EUK1134-R primers. For 18S sequencing, the following cycling conditions were used: 95 °C for 5 min, 40 PCR cycles of 95 °C for 30 s, 51 °C for 30 s incubation, 72 °C for 45 s and then a final elongation step at 72 °C for 8 min.

In the case of 16S sequencing, the 16S—341F and 16S-805R primers were used. The cycling conditions, in this case, were: 95 °C for 5 min, 40 PCR cycles of 95 °C for 20 s, a gradual drop from 56 to 46 °C with 20 s incubation (0.25 °C drop per cycle), 72 °C for 15 s and then 72 °C for 8 min. The PCR products were pooled and sent for library preparation

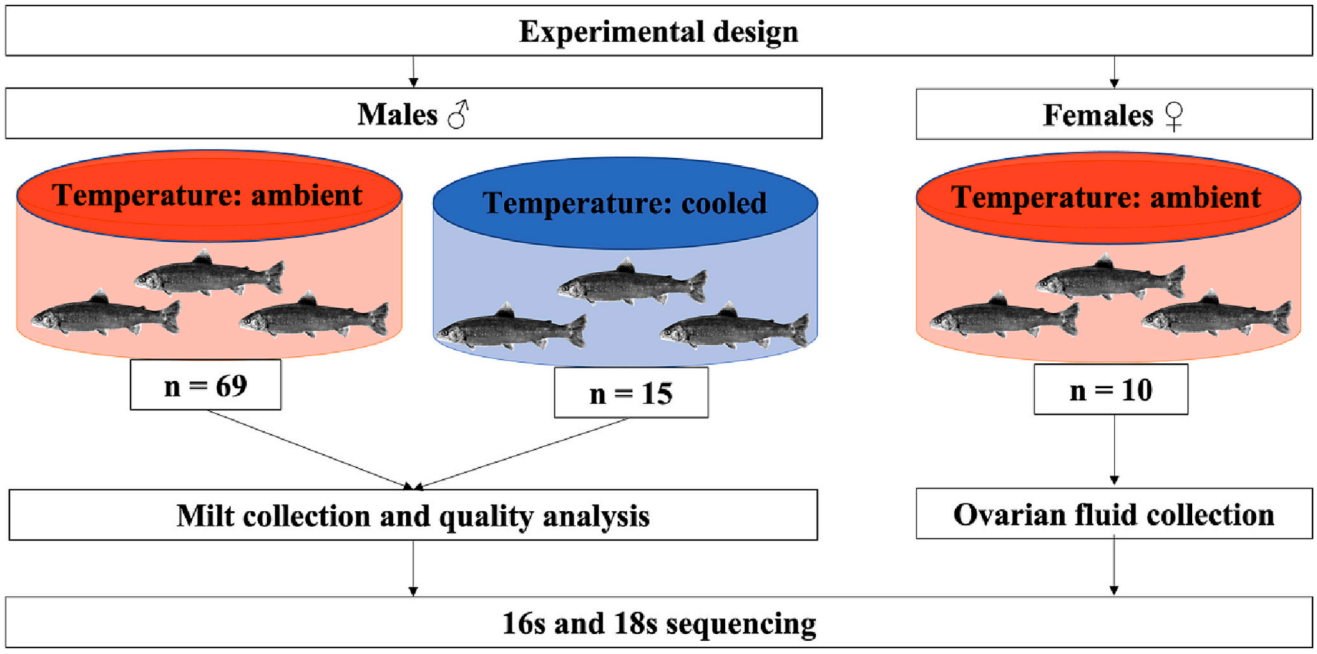


Fig. 1. Rearing conditions of the sampled Arctic charr (*Salvelinus alpinus*). Stocking densities were approximately 15 kg/m^{3}in all three tanks.

(https://ngisweden.scilifelab.se/methods/illumina-amplicon-sequencing/) and sequencing to the Swedish national genomics infrastructure (SciLifeLab; Uppsala Sweden). Libraries were sequenced in an Illumina MiSeq using v3 chemistry with a read setup of 2 × 300 cycles. We included negative controls for DNA extractions and verified the lack of contamination based on PCR reactions.

2.3. Bioinformatic analysis

Following sequencing, the data were quality-filtered and clustered into operational taxonomic units (OTUs). This was done using the LotuS2 pipeline v2.19 (Özkurt et al., 2021) as described earlier (Bahram et al., 2018). Briefly, demultiplexed paired-end reads were quality filtered using the sdm read filtering software v1.95 (Özkurt et al., 2021) based on the default settings for Illumina MiSeq read processing and assembled using FLASH (Magoč and Salzberg, 2011). Quality-filtered sequences were clustered using a 97% similarity threshold, followed by de novo and reference-based chimera filtering using Vsearch (Rognes et al., 2016). Taxonomic identification was made based on a BLAST search of representative sequences per OTU against the SILVA database (version 138; (Quast et al., 2013)).

2.4. Descriptive and statistical analysis

All analyses of the obtained sequenced data following taxonomic identification were performed in R v4.2.0 (R Core Team, 2022) unless otherwise stated. A descriptive analysis was performed for the recorded sperm traits using R/tidyverse v1.3.2 (Wickham et al., 2019). To check whether statistically significant differences existed between the sperm traits of the animals that were reared in ambient or cooled water temperature, the non-parametric Wilcoxon test was used. Moreover, as already mentioned, inbreeding coefficients were available for the sampled males (Kurta et al., 2022).

Prior to statistical analysis, the sequencing depth of each sample was adjusted to the overall mean value. The Richness and Shannon diversity indices were calculated using the functions *specnumber* and *diversity,* respectively from the R/vegan package (Oksanen et al., 2020). A pairwise comparison of the Richness and Shannon diversity indices between microbial habitats (milt and ovarian fluid) was performed using a *t*-test. The core microbiome for both habitats (milt and ovarian fluid) was identified using the function *Core* from the R/microbiome package (Leo Lahti, 2017). Considering the number of samples from both habitats, we applied stringent threshold criteria to define the core OTUs. More specifically, OTUs were classified as core taxa if they were consistently present in at least 50% of the studied samples and had abundances >0.01% in the case of milt and > 1% in the ovarian fluid samples. The relative abundance of OTUs was calculated using the R/phyloseq package (McMurdie and Holmes, 2013). Moreover, to test whether the microbiome's composition differed significantly between the ovarian fluid and semen samples, we performed a permutational ANOVA (PERMANOVA) analysis with the function *Adonis* from the R/vegan using 999 permutations.

Furthermore, the relationship between the alpha diversity indices and the recorded sperm traits was tested through Pearson correlation and fitting linear regression models. Both univariate and multivariate regression models were fitted, including each of the sperm quality variables in succession. At the same time, the effect of rearing at ambient versus cool water temperature and the inbreeding coefficient of each male was evaluated. To account for multiple comparisons, the obtained *P* values were adjusted with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). The variance inflation factor (VIF) was also used to assess multicollinearity between the fitted variables (Fox and Weisberg, 2019). To further test whether the sperm quality traits (motility, concentration etc.) were related to the identified OTUs, we performed an environmental fit and variation partition analysis using the R/vegan package (Oksanen et al., 2020). Finally, the beta-diversity was visualised with nonmetric multidimensional scaling (NMDS) using the *metaMDS* function with a Bray-Curtis distance dissimilarity matrix from the R/vegan package.

3. Results

3.1. Prokaryotic microbiota differences between milt and ovarian fluid samples

Following quality control filtering, 1,265,341 and 20,999,151 high-quality 16S sequence reads were retained from the milt and ovarian fluid samples and clustered into 1688 and 2976 operational taxonomic units

(OTUs), respectively. Taxonomic annotation of these OTUs showed that bacterial communities in the ovarian fluid samples were dominated by Gammaproteobacteria (33.9%) Actinobacteriota (25.4%), Alphaproteobacteria (15.1%), Firmicutes (8.9%), Bacteroidota (7.6%) and Cyanobacteria (2.6%). Similarly, bacterial communities in the semen samples were dominated by Gammaproteobacteria (31.4%), Actinobacteriota (27%), Alphaproteobacteria (21.2%), Firmicutes (10.7%), Bacteroidota (5.1%) and Cyanobacteria (1.6%).

Alphaproteobacteria were more abundant in the milt than in the ovarian fluid samples, whereas Gammaproteobacteria were slightly more abundant in the ovarian fluid samples. Finally, 6.4% and 2.9% of the detected OTUs in ovarian fluid and milt samples were classified as "others" (Fig. 2).

The conducted alpha-diversity-based analysis revealed a statistically significant difference between the ovarian fluid and the semen samples for both Richness and Shannon diversity indices (Fig. 3). The ovarian fluid samples exhibited significantly higher richness (569.8 ± 74.4 SE) and higher Shannon diversity (5.06 ± 0.12) as compared to the milt samples (Richness index: 70.94 ± 7.9; Shannon index 3.27± 0.09).

Furthermore, non-metric multidimensional scaling (NMDS), based on the Bray-Curtis distance matrix, revealed that microbial communities in milt formed a distinct cluster indicating their dissimilarity to the ovarian fluid samples (PERMANOVA with Adonis test: $F = 5.12$, $R^2 = 0.05$, $P = 0.001$) (Fig. 4).

3.2. Core microbiome of milt and ovarian fluid samples

Altogether, 11 and 7 core OTUs belonging to 11 genera were identified in the ovarian and seminal fluid microbiome. Despite their relatively low number, these OTUs accounted for 38% and 26% of the relative microbial abundance of those two habitats. The genera of the core OTUs included Bradyrhizobium (2 OTUs), Pelomonas, Curvibacter, Mycobacterium (2 OTUs), Pseudomonas, Staphylococcus, Micrococcus, Streptococcus, Sphingomonas, Acidibacter, and Afipia. Five OTUs were found common in both habitats (Fig. 5).

3.3. Descriptive analysis of sperm quality parameters

Arctic charr males from the tank with ambient water temperature exhibited lower mean sperm concentration ($3.04 \pm 1.54 \times 10^9$/ml) than the ones held in the cooled water ($3.55 \pm 1.98 \times 10^9$/ml). However, the difference was not statistically significant (Wilcoxon, $P > 0.05$). Motility-related parameters in animals reared in ambient temperature were, on average higher (total motility: 83 ± 15%, total rapid motility: 9 ± 9%, total medium motility: 27 ± 14%, VCL: 74 ± 18 μm/s, VAP: 49 ± 18 μm/s, VSL: 35 ± 15 μm/s), as compared to the cold water group (total motility: 69 ± 21%, total rapid motility: 5 ± 6%, total medium motility: 19 ± 12%, VCL: 65 ± 16 μm/s, VAP: 39 ± 15 μm/s, VSL: 27 ± 11 μm/s). In addition, all motility parameters, except for the total rapid motility, had significantly higher means in the ambient group (Wilcoxon, $P < 0.05$) (Fig. 6). However, it should be pointed that the genetic composition of the two groups in terms of used families was different.

3.4. Milt microbiota in animals reared in cool versus ambient water temperature

Both the Richness and the Shannon diversity indices did not significantly differ between semen samples from the animals that were reared in the cool and ambient water temperature ($P > 0.3$; Fig. S1). Furthermore, PERMANOVA analysis did not reveal any significant differences regarding the bacterial community composition between the two groups (Adonis: $F = 1.1$, $R^2 = 0.01$, $P = 0.24$).

3.5. Associations between OTUs, inbreeding, and sperm quality traits

No statistically significant associations were found between the relative abundance of any of the most abundant taxa and the recorded sperm traits. This also agreed with the output from the univariate linear regression models. Furthermore, no statistically significant associations were found between inbreeding levels and the bacterial community composition of the tested semen samples. Additionally, no statistically significant associations were found between the milt microbiota Richness index and any of the recorded sperm quality traits ($P > 0.05$).

Nevertheless, the Shannon diversity index was found to be negatively correlated with total medium motility ($r = -0.23$, $P = 0.033$), VCL ($r = -0.26$, $P = 0.017$), VAP ($r = -0.27$, $P = 0.014$), and VSL ($r = -0.24$, $P = 0.029$) (Fig. 7).

Similarly, there were no statistically significant associations in either univariate or multivariate linear regression models between Richness and the sperm quality traits ($P > 0.4$). However, with the exception of total motility and sperm concentration the rest of the recorded traits were found to be significantly associated with the Shannon diversity index (Fig. 7; Table 1).

In the case of multivariate regression models when water

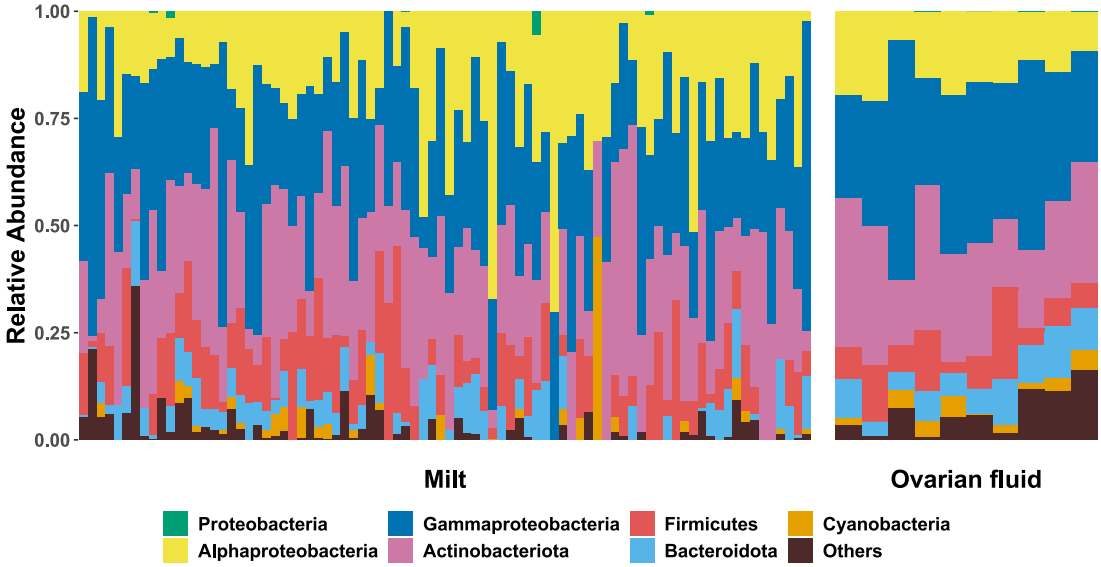


Fig. 2. Relative abundance of main prokaryotic taxa across milt and ovarian fluid samples from farmed Arctic charr (*Salvelinus alpinus*).

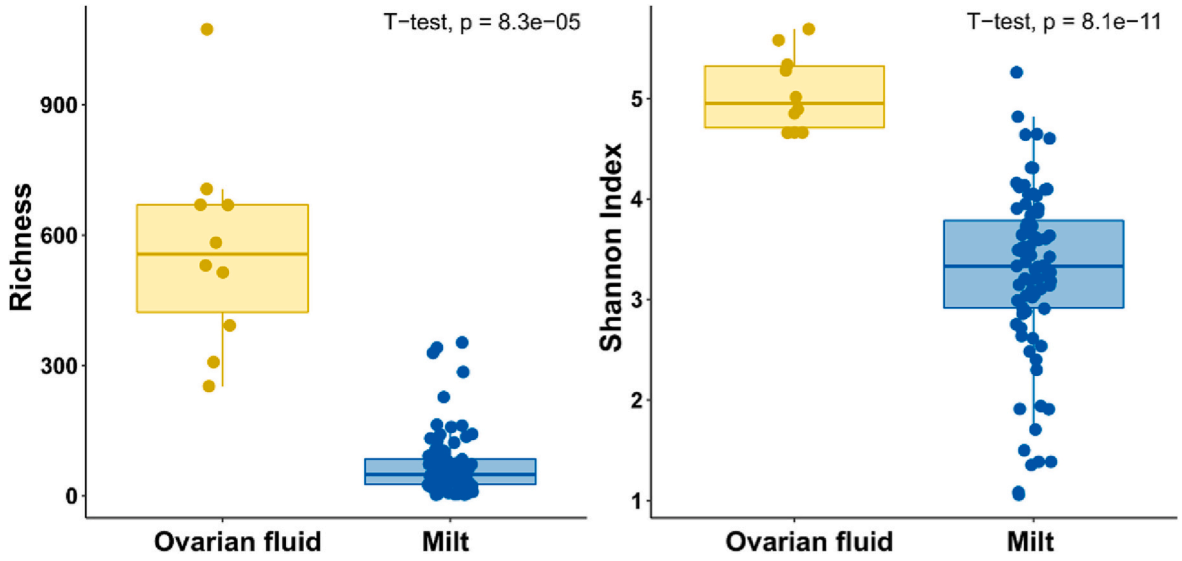


Fig. 3. Comparison of alpha diversity indices amongst ovarian fluid and milt samples from Arctic charr (*Salvelinus alpinus*). To have comparable sequencing depth across all samples, the raw read numbers were rarified to the mean number of reads across all samples (1,914,287reads) before calculating the diversity indices.

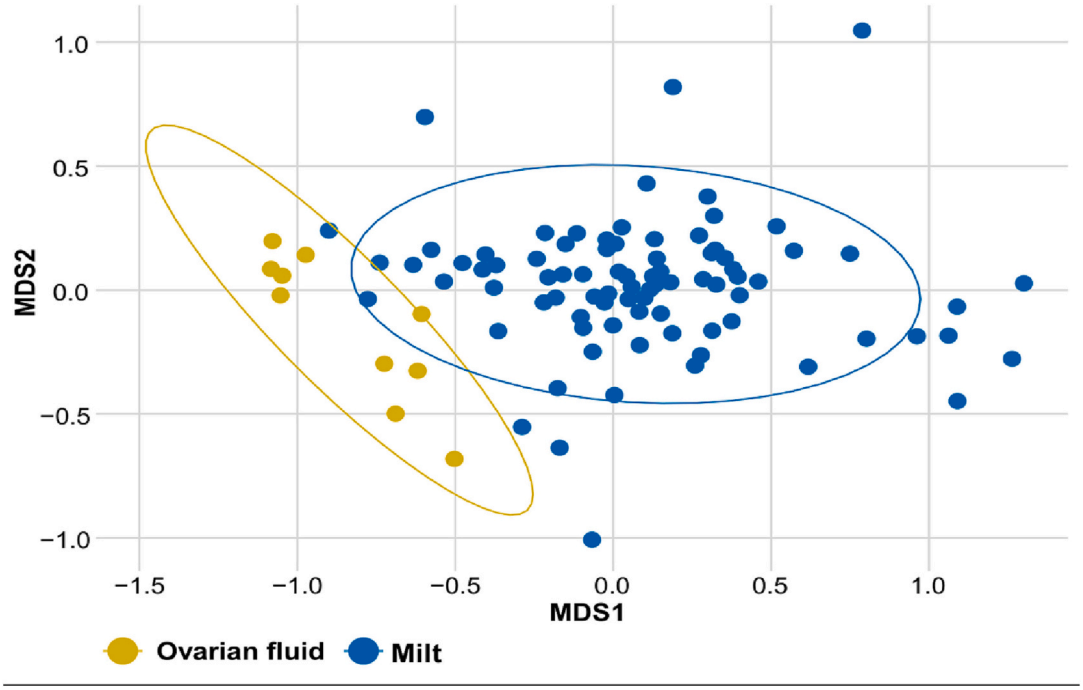


Fig. 4. Non-metric multidimensional scaling of bacterial community composition in milt and ovarian fluid samples from farmed Arctic charr (*Salvelinus alpinus*). Relative dissimilarities or distances between samples were calculated based on the Bray-Curtis distance matrix.

temperature and inbreeding levels were included as fixed effects, the corresponding *P*-values of the tested sperm quality parameters were also affected. More specifically, the following statistically significant associations were found between the Shannon diversity index and total rapid or total medium motility ($R^2 = 0.09$; $P = 0.01$), VCL or VAP ($R^2 = 0.11$; $P = 0.004$) and VSL ($R^2 = 0.10$; $P = 0.006$). Finally, the conducted variation partition analysis did not detect any statistically significant associations between the recorded sperm traits and the residing microbial communities ($P > 0.05$).

3.6. Microeukaryotes – 18S sequencing

Compared to the detected prokaryotic community, the eukaryotic composition was less diverse. Overall, 23 OTUs (18S) were detected in both milt and ovarian fluid samples. Amongst these, only four OTUs belonged to the Protista kingdom. All four Protista OTUs were detected in only two ovarian fluid samples where the most abundant OTU was OTU131_Conoidasida (51 reads), followed by OTU263_Apicomplexa (27 reads), OTU577_Histiobalantium (8 reads), and OTU648_Cercozoa (6 reads).

On the other hand, the detected fungal community was more abundant in both habitats. OTU19 (*Rhodotorula sphaerocarpa*) and OTU9 (*Cordyceps javanica*) were the dominant OTUs in milt, whereas OTU7 (Polyporales), OTU12 (*Rhexocercosporidium carotae*), OTU46 (Pezizaceae) and OTU78 (Agaricomycetes) were abundant in the ovarian fluid (Fig. 8). Since OTU12 and OTU9 were represented in most samples, we

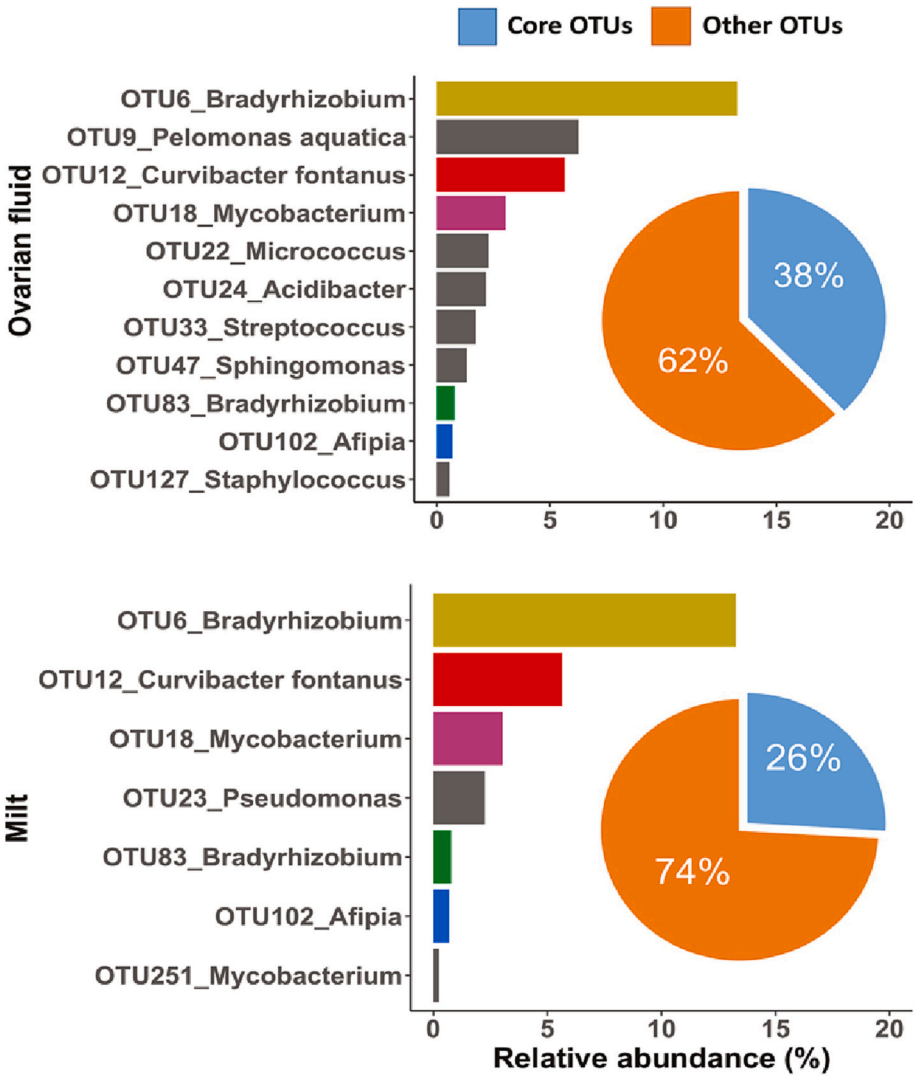


Fig. 5. Core microbiome of ovarian fluid and milt samples from farmed Arctic charr (*Salvelinus alpinus*). Bar charts represent the relative abundance of individual members of the core microbiome of each habitat (ovarian fluid and milt). Pie charts depict the total abundance of core microbiome members, and other OTUs detected from their respective habitats. Coloured bars represent the same respective OTUs detected in the core microbiome of both habitats. In contrast, grey bars indicate the unique association of that OTU with the core microbiome of the habitat.

estimated the Pearson correlation between their relative abundance and the recorded sperm quality traits. A strong negative correlation ($r = -0.78$, $P = 0.004$) was detected between the relative abundance of OTU12 (*Rhexocercosporidium carotae)* and sperm motility (Fig. 8). At the same time, a moderate positive correlation was found between the same OTU and sperm concentration ($r = 0.62$, $P = 0.04$) On the other hand, no statistically significant correlation was found for OTU9 (*Cordyceps javanica*).

4. Discussion

Despite the importance of microbes in host performance and fitness (Wallace et al., 2019), scarce information yet exists about their role in shaping critical biological functions in fish. Notably, regarding fertility, limited knowledge exists even in more well-studied organisms (Farahani et al., 2021; Swanson et al., 2020). Gaining insights into the core microbiome composition of gamete-related samples from farmed fish and investigating for associations between the residing microbiome and fertility proxies could open bioengineering avenues aiming to increase fertility. Particularly in the case of the Scandinavian Arctic charr, where a highly variable reproductive success is often observed in captivity, microbiome-focused studies could reveal underlying associations that remained undetected in previous studies that focused solely on rearing conditions, nutrition, or host genetics (Kurta et al., 2022; Olk et al., 2019; Pickova et al., 2007).

4.1. Microbiota differences amongst gamete-related habitats and rearing conditions

Overall, the diversity of the residing prokaryotic microbial communities was significantly higher in ovarian fluid compared to the milt habitat (Fig. 3). Even though we need to account for the substantial difference in terms of sample size between the ovarian fluid ($n = 10$) and the milt samples ($n = 84$), all the estimated microbial diversity-related indices supported this finding. As no prior study has attempted to gain insights into the microbiome abundance in gamete-related samples in fish, no reference is available to compare our results. Although direct comparisons with mammals are of limited value, the milt microbiome diversity, in that case, appears to be higher compared to gamete-related habitats in females (Rowe et al., 2020; Wang et al., 2020).

Despite the fact that separate clusters of the residing bacterial communities were obtained for the two habitats, common taxa were shared between them. As both sexes were communally reared until the onset of the reproductive season (usually between September and November in Sweden), the identified shared taxa amongst the two habitats appear to be in line with previous studies showing that the rearing environment has a drastic effect on shaping the residing microbiota communities in fish (Deng et al., 2021a). In accordance with the above, distinct microbial communities were found both in the skin and intestine of wild Arctic charr sampled in fresh- or marine water environments (Hamilton et al., 2019). However, it is worth mentioning that in this study,

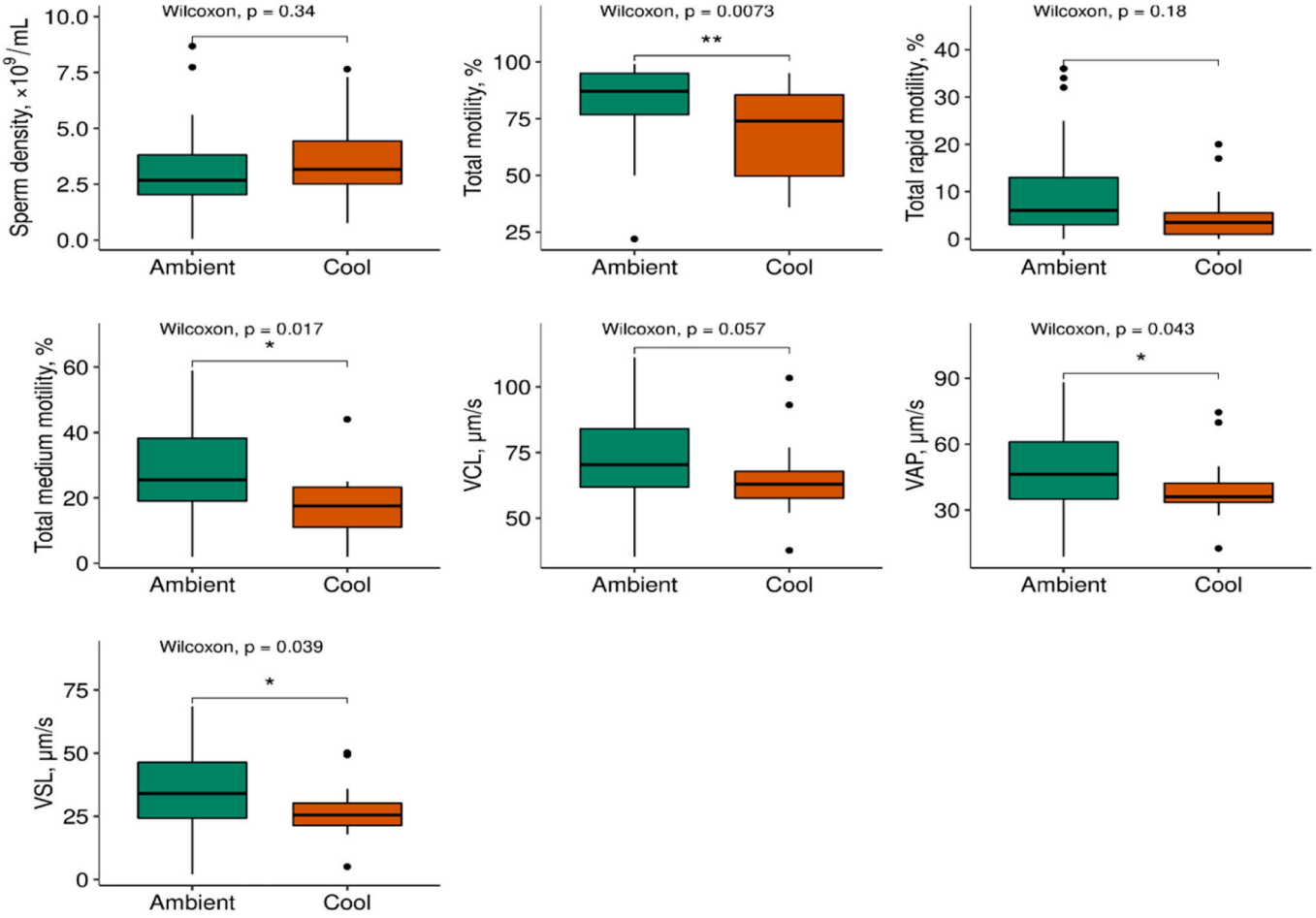


Fig. 6. Statistical analysis of sperm quality parameters in farmed Arctic charr (*Salvelinus alpinus*). The y-axis shows the recorded parameter, and the x-axis shows the water temperature of the two groups. Horizontal lines with asterisks indicate sperm concentration means that were significantly different (Wilcoxon test, $*P < 0.05$, $** P < 0.01$, $*** P < 0.001$).

Cyanobacteria and Proteobacteria were also amongst the most abundant taxa. Whether this is merely due to the fact that those two taxa are highly abundant in aquatic environments or they play a functional role in the fitness of the fish is not clear. Notably, even though the residing fish microbiome appears to be species-specific (Minich et al., 2018), amongst the most abundant taxa found in both ovarian fluid and milt, the Actinobacteriota and Proteobacteria have also been found previously in high abundance in Nile tilapia (*Oreochromis niloticus*) gut (Deng et al., 2021b), a species with strikingly different rearing conditions compared to Arctic charr. More specifically, *Nile tilapia* is usually reared in water temperatures above 25 °C (Moses et al., 2021), while Arctic charr farming ideally is performed at temperatures below 15 °C (Jobling et al., 1998). It should be noted that the water temperature during sampling in our study was ~5 °C. As mentioned above, we could not determine whether those taxa play a role in fish health or other critical biological functions, which would be worth exploring further in future studies.

In terms of detected eukaryotic taxa, far less diversity was observed in both habitats compared to the prokaryotic taxa. More specifically, only four OTUs from protists were found. Moreover, high variability was obtained, especially amongst the ovarian fluid samples, with only two of them containing all four protistan OTUs. On the other hand, more reliable results were obtained in the case of the residing fungal community, where a higher fungal diversity was found in the ovarian fluid habitat. Nevertheless, we need to consider that we did not account for genetic relationships in the case of the sampled females. Therefore, it is possible that those animals were not fully representative of the entire farmed population, and validation of the results on a larger sample size would be needed. Moreover, as our study was conducted in an industrial set-up, future studies using replicate tanks would be needed to validate the above findings.

4.2. Core microbiome in gamete-related samples

Deciphering the core microbiome of a specific habitat could reveal microbial communities tightly interconnected to relevant host traits, which explains why identifying it is often a key focus of microbiome studies in animals (Tarnecki et al., 2017). To the best of our knowledge, the composition of the core microbiome in fish has been characterised only for the gut so far (Givens et al., 2015; Kokou et al., 2020; Sharpton et al., 2021). A relatively higher number of OTUs (11 vs 7) present in the ovarian fluid microbiome compared to the milt is in agreement with the higher diversity of the residing microbial community in the former habitat. Furthermore, the putative core microbiome in ovarian fluid comprised overall approximately 50% higher relative microbial abundance than that of the milt. Nevertheless, a substantial overlap between the two habitats was found, with approximately 70% of the milt core microbiome OTUs found also in ovarian fluid samples. Even though limited comparisons with prior studies are possible, the *Pseudomonas* OTU classified as a member of the milt core microbiome in our study is an abundant generalist genus found in the gut of several farmed fish, e.g., Atlantic salmon, Nile tilapia, European seabass, and rainbow trout (Kokou et al., 2019).

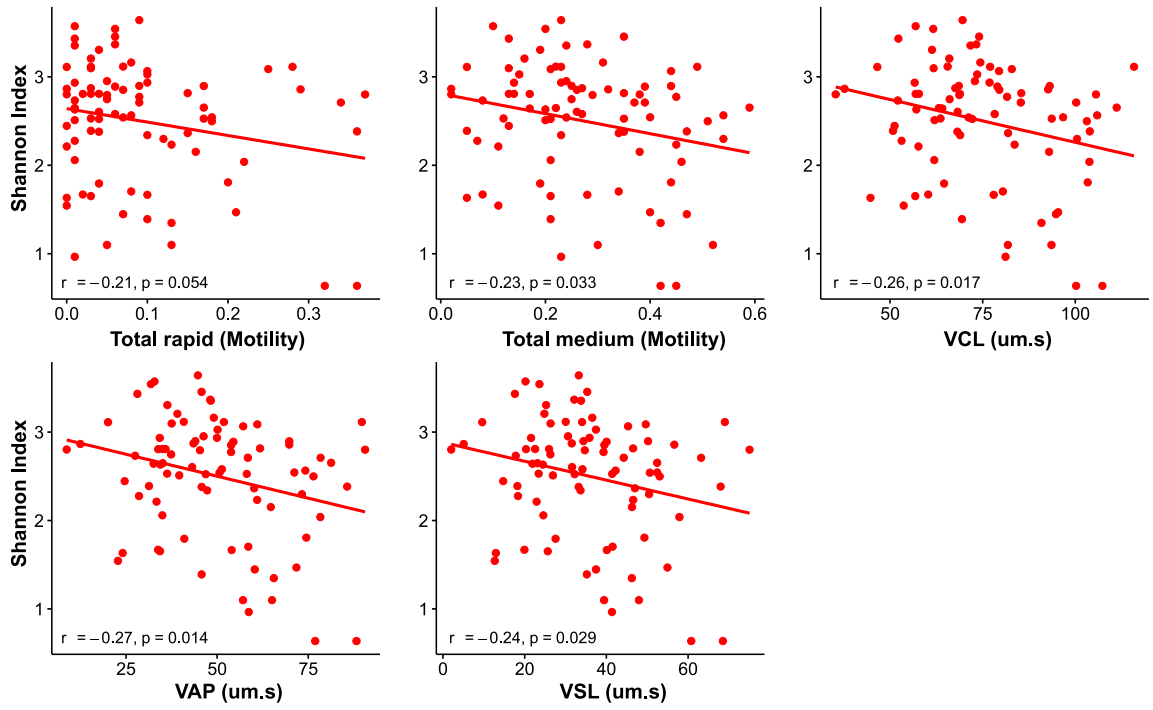


Fig. 7. Pearson's correlation between Shannon diversity and sperm quality traits of farmed Arctic charr (*Salvelinus alpinus*). The number of raw reads per sample was rarified to the mean number of reads across all sperm samples before calculating the Shannon diversity index to allow for comparable sequencing depth (15,063 reads) across all samples.

Table 1
Summary from fitting univariate linear regression models.

Recorded traits	Richness		Shannon Index		
	R squared	*P*-value	R squared	P-value	P adjusted*
Sperm concentration	−0.01	0.95	−0.008	0.57	0.57
Total Motility	0.002	0.26	0.0002	0.31	0.36
Total medium motility	0.002	0.23	0.05	0.028*	0.04*
Total rapid motility	0.010	0.17	0.05	0.024*	0.04*
VCL	0.006	0.22	0.06	0.013*	0.04*
VAP	0.006	0.21	0.067	0.0098*	0.04*
VSL	0.008	0.19	0.05	0.019*	0.04*

* Adjusted *P*-values with the Benjamini-Hochberg method.

4.3. Associations between the milt residing microbiome, inbreeding and sperm quality parameters

Associations were found between the microbial diversity (Shannon diversity index based on the 16S data) and several recorded traits (total medium and rapid motility, VCL, VAP, VSL). At the same time, no associations were found between the Richness index and any of the sperm quality traits. Nevertheless, we need to point out that the latter index only considers the presence of a species. On the contrary, the Shannon index also includes information about the corresponding relative abundances. However, no statistically significant associations were obtained between the identified bacterial OTUs and the recorded sperm traits. Additionally, as the sampled animals of our study originated from a breeding program with accompanying pedigree records, we were also able to obtain preliminary results regarding the association between inbreeding accumulation and the residing seminal microbiome. Overall, the inbreeding levels could not be connected to the residing seminal microbiome.

The hypothesis that the residing milt microbiome might affect fertility has been previously formulated and studied in humans and livestock with no uniform results across studies (Comizzoli et al., 2021; Wang et al., 2022). Furthermore, probiotics have been suggested to positively affect the reproductive performance of teleost fish (Gioacchini et al., 2014). As already mentioned, a considerable knowledge gap exists regarding the role of microbiota in fish reproductive success in commercial aquaculture settings (Parata et al., 2021). Although the presence of particular bacterial taxa in the milt can affect male fertility, whether the overall microbiome structure plays a role or not is still debatable, at least in the case of humans (Baud et al., 2019; Farahani et al., 2021). Notably, potential associations between milt microbiota and fertility were recently suggested in bulls (Cojkic et al., 2021). In our study, the only statistically significant associations between individual OTUs and any of the sperm quality traits concerned a member of the fungal community (similar to *Rhexocercosporidium carotae*). The OTU above is potentially a pathogen belonging to the Leotiomycetes class and has been previously found in the gut of both wild and domesticated zebrafish (Siriyappagouder et al., 2018).

The suspected pathogenicity of OTU12 may, to some extent, explain its negative association with sperm motility. Nevertheless, the same OTU showed a positive association with sperm concentration. We speculate that owing to a higher sperm concentration, a richer niche is provided, allowing the suspected pathogenic OTU to thrive, consistent with the species-area relationship (Horner-Devine et al., 2004). Alternatively, it is possible that an unaccounted confounding factor could be present, resulting in a spurious association. Additionally, we need to point out that a potential limitation of our study is that even though the sampled males encompass most of the families of the breeding population, only one or two individuals were used from each family, which probably is not sufficient to allow for in-depth host genetic-related inferences. Future studies using a larger number of sampled males with sperm quality recordings could shed additional light and allow for investigating the existence of potential interactions between them. Furthermore, the fact that we could not use tank replicates would require validation of our findings through a more concise experimental set-up.

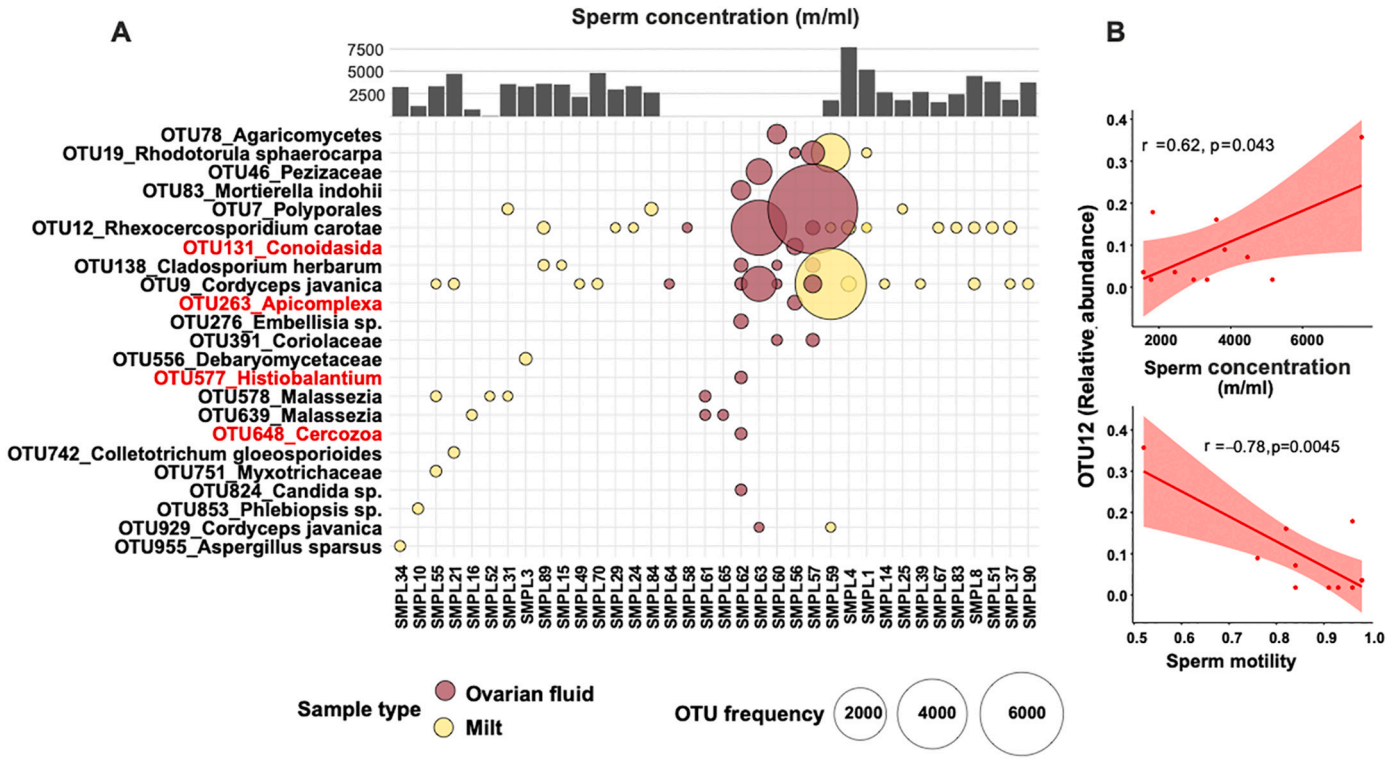


Fig. 8. (A) Abundance (raw read counts) of eukaryotic communities detected in milt and ovarian fluid from farmed Arctic charr (*Salvelinus alpinus*). Each bubble point represents a sample and the size of the point represents the frequency of each particular OTU in that sample. Bar charts show sperm concentration (million per millilitre) for each sample. Y-axis label colours represent the OTUs from Fungi (black) and Protista (red). (B) Pearson correlation between the relative abundance of OTU12 (Rhexocercosporidium carotae) and sperm motility and sperm concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

Our study provides novel information about the ovarian fluid and milt microbiome structure from farmed Arctic charr. Despite a more diverse microbial community in the case of the ovarian fluid, there was substantial overlap between the two habitats indicating that the rearing environment significantly shapes the residing microbiome. No effects of inbreeding levels or water temperature were found in the composition of the milt microbiome. We further found some evidence pointing to links between the sperm quality parameters and the microbiome composition– particularly a fungal OTU. Further investigation is needed to validate our findings and establish cause-and-effect relationships with fertility.

Ethics declarations

The current study was performed in accordance with the Swedish Animal Welfare Act 2018:1192 under the ethics permit: 5.2.18–09859/2019 issued by the Swedish Board of Agriculture Jordbrukverket.

Authors' contributions

CP and MB conceived the study. CP, KK and HJ collected and recorded the phenotypic data. MB performed the bioinformatic analysis. DG performed the statistical analysis. CP drafted the initial manuscript, which was further edited by all the authors.

Funding

This study was funded by the SLU aquaculture platform. Fish were provided through the FORMAS funded project "Temperature optimization of Arctic charr hatcheries" (2019–02091). Sequencing was performed by the SNP&SEQ Technology Platform in Uppsala. The facility is part of the National Genomics Infrastructure (NGI) Sweden and Science for Life Laboratory. The SNP&SEQ Platform is also supported by the Swedish Research Council and the Knut and Alice Wallenberg Foundation. Finally, we thank ACN personnel for assisting with the fish handling.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

All raw sequences were deposited at the NCBI's Sequence Read Archive under accession number PRJNA883606 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA883606).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739648.

References

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6.

Bates, M.C., Tiersch, T.R., 1998. Preliminary studies of artificial spawning of channel catfish as male-female pairs or all-female groups in recirculating systems. J. World Aquacult. Soc. 29, 325–334. https://doi.org/10.1111/j.1749-7345.1998.tb00654.x.

Baud, D., Pattaroni, C., Vulliemoz, N., Castella, V., Marsland, B.J., Stojanov, M., 2019. Sperm microbiota and its impact on semen parameters. Front. Microbiol. 10.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological) 57, 289–300.
Cabrita, E., Martínez-Páramo, S., Gavaia, P.J., Riesco, M.F., Valcarce, D.G., Sarasquete, C., Herráez, M.P., Robles, V., 2014. Factors enhancing fish sperm quality and emerging tools for sperm analysis. Aquaculture 432, 389–401. https://doi.org/10.1016/j.aquaculture.2014.04.034.
Cojkic, A., Niazi, A., Guo, Y., Hallap, T., Padrik, P., Morrell, J.M., 2021. Identification of bull semen microbiome by 16S sequencing and possible relationships with fertility. Microorganisms 9, 2431. https://doi.org/10.3390/microorganisms9122431.
Comizzoli, P., Power, M.L., Bornbusch, S.L., Muletz-Wolz, C.R., 2021. Interactions between reproductive biology and microbiomes in wild animal species. anim microbiome 3, 87. https://doi.org/10.1186/s42523-021-00156-7.
Deng, Y., Borewicz, K., van Loo, J., Olabarrieta, M.Z., Kokou, F., Sipkema, D., Verdegem, M.C.J., 2021a. In-situ biofloc affects the Core prokaryotes community composition in gut and enhances growth of Nile Tilapia (*Oreochromis niloticus*). Microb. Ecol. https://doi.org/10.1007/s00248-021-01880-y.
Deng, Y., Kokou, F., Eding, E.H., Verdegem, M.C.J., 2021b. Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. Animal Microbiome 3, 1–17. https://doi.org/10.1186/S42523-021-00145-W.
Doekes, H.P., Bijma, P., Windig, J.J., 2021. How depressing is inbreeding? A meta-analysis of 30 years of research on the effects of inbreeding in livestock. Genes 12, 926. https://doi.org/10.3390/GENES12060926/S1.
Elliott, J.M., Elliott, J.A., 2010. Temperature requirements of Atlantic salmon Salmo salar, brown trout Salmo trutta and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. J. Fish Biol. 77, 1793–1817. https://doi.org/10.1111/J.1095-8649.2010.02762.X.
Farahani, L., Tharakan, T., Yap, T., Ramsay, J.W., Jayasena, C.N., Minhas, S., 2021. The semen microbiome and its impact on sperm function and male fertility: a systematic review and meta-analysis. Andrology 9, 115–144. https://doi.org/10.1111/andr.12886.
Farquharson, K.A., Hogg, C.J., Grueber, C.E., 2018. A meta-analysis of birth-origin effects on reproduction in diverse captive environments. Nat. Commun. 9, 1055. https://doi.org/10.1038/s41467-018-03500-9.
Foo, J.L., Ling, H., Lee, Y.S., Chang, M.W., 2017. Microbiome engineering: current applications and its future. Biotechnol. J. 12, 1600099. https://doi.org/10.1002/biot.201600099.
Fox, J., Weisberg, S., 2019. CAR - An R Companion to Applied Regression, 2016. Sage, Thousand Oaks CA.
Gioacchini, G., Giorgini, E., Vaccari, L., Carnevali, O., 2014. Can probiotics affect reproductive processes of aquatic animals?. In: Aquaculture Nutrition. John Wiley & Sons, Ltd, pp. 328–346. https://doi.org/10.1002/9781118897263.ch12.
Givens, C.E., Ransom, B., Bano, N., Hollibaugh, J.T., 2015. Comparison of the gut microbiomes of 12 bony fish and 3 shark species. Mar. Ecol. Prog. Ser. 518, 209–223. https://doi.org/10.3354/meps11034.
Hamilton, E.F., Element, G., van Coeverden Groot, P., Engel, K., Neufeld, J.D., Shah, V., Walker, V.K., 2019. Anadromous Arctic char microbiomes: bioprospecting in the high Arctic. Frontiers in Bioengineering and Biotechnology 7.
Hely, F.S., Amer, P.R., Walker, S.P., Symonds, J.E., 2013. Optimised parent selection and minimum inbreeding mating in small aquaculture breeding schemes: a simulation study. Animal 7, 1–10. https://doi.org/10.1017/S1751731112001371.
Horner-Devine, M.C., Lage, M., Hughes, J.B., Bohannan, B.J.M., 2004. A taxa-area relationship for bacteria. Nature 432, 750–753. https://doi.org/10.1038/nature03073.
Jeuthe, H., Brännäs, E., Nilsson, J., 2013. Effects of egg size, maternal age and temperature on egg, viability of farmed Arctic charr. Aquaculture 408–409, 70–77. https://doi.org/10.1016/J.AQUACULTURE.2013.05.034.
Jobling, M., Tveiten, H., Hatlen, B., 1998. REVIEW cultivation of Arctic charr: an update. Aquac. Int. 6, 181–196. https://doi.org/10.1023/A:1009246509657.
Kokko, H., Ots, I., 2006. When not to avoid inbreeding. Evolution 60, 467–475. https://doi.org/10.1111/j.0014-3820.2006.tb01128.x.
Kokou, F., Sasson, G., Friedman, J., Eyal, S., Ovadia, O., Harpaz, S., Cnaani, A., Mizrahi, I., 2019. Core gut microbial communities are maintained by beneficial interactions and strain variability in fish. Nature Microbiology 4, 2456–2465. https://doi.org/10.1038/s41564-019-0560-0.
Kokou, F., Sasson, G., Mizrahi, I., Cnaani, A., 2020. Antibiotic effect and microbiome persistence vary along the European seabass gut. Sci. Rep. 10, 10003. https://doi.org/10.1038/s41598-020-66622-5.
Kurta, K., Jeuthe, H., de Koning, D.J., Palaiokostas, C., 2022. Evaluating the potential of improving sperm quality traits in farmed Arctic charr (*Salvelinus alpinus*) using selective breeding. Aquaculture Reports 25, 101234. https://doi.org/10.1016/J.AQREP.2022.101234.
Leo Lahti, S.S., 2017. Introduction to the Microbiome R Package (WWW Document].).
Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., Fitzsimmons, C., Plastow, G., Guan, L. L., 2019. Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle. Microbiome 7, 1–17. https://doi.org/10.1186/S40168-019-0699-1/FIGURES/7.
Luna, G.M., Quero, G.M., Kokou, F., Kormas, K., 2022. Time to integrate biotechnological approaches into fish gut microbiome research. Curr. Opin. Biotechnol. 73, 121–127. https://doi.org/10.1016/j.copbio.2021.07.018.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/JOURNAL.PONE.0061217.
Migaud, H., Bell, G., Cabrita, E., Mcandrew, B., Davie, A., Bobe, J., Herráez, M.P., Carrillo, M., 2013. Gamete quality and broodstock management in temperate fish. Rev. Aquac. 5, S194–S223. https://doi.org/10.1111/raq.12025.
Minich, J.J., Zhu, Q., Xu, Z.Z., Amir, A., Ngochera, M., Simwaka, M., Allen, E.E., Zidana, H., Knight, R., 2018. Microbial effects of livestock manure fertilization on freshwater aquaculture ponds rearing tilapia (*Oreochromis shiranus*) and north African catfish (*Clarias gariepinus*). MicrobiologyOpen 7, e00716. https://doi.org/10.1002/mbo3.716.
Moses, M., Chauka, L.J., de Koning, D.J., Palaiokostas, C., Mtolera, M.S.P., 2021. Growth performance of five different strains of Nile tilapia (Oreochromis niloticus) introduced to Tanzania reared in fresh and brackish waters. Sci. Rep. 11, 11147. https://doi.org/10.1038/s41598-021-90505-y.
Mueller, U.G., Linksvayer, T.A., 2022. Microbiome breeding: conceptual and practical issues. Trends Microbiol. https://doi.org/10.1016/J.TIM.2022.04.003.
Myer, P.R., Smith, T.P.L., Wells, J.E., Kuehn, L.A., Freetly, H.C., 2015. Rumen microbiome from steers differing in feed efficiency. PLoS One 10, e0129174. https://doi.org/10.1371/journal.pone.0129174.
Mylonas, C.C., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal manipulations of fish reproduction. Gen. Comp. Endocrinol. 165, 516–534. https://doi.org/10.1016/J.YGCEN.2009.03.007.
Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D. C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.
Nilsson, J., Backström, T., Stien, L.H., Carlberg, H., Jeuthe, H., Magnhagen, C., Brännäs, E., 2016. Effects of age and rearing environment on genetic parameters of growth and body weight and heritability of skin pigmentation in Arctic charr (*Salvelinus alpinus* L.). Aquaculture 453, 67–72. https://doi.org/10.1016/J.AQUACULTURE.2015.11.035.
Nynca, J., Ciereszko, A., 2009. Measurement of concentration and viability of brook trout (*Salvelinus fontinalis*)spermatozoa using computer-aided fluorescent microscopy. Aquaculture 292, 256–258. https://doi.org/10.1016/J.AQUACULTURE.2009.04.020.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Maintainer, H.W., 2020. Package "vegan" title community ecology package version 2.5-7.
Olk, T.R., Jeuthe, H., Thorarensen, H., Wollebæk, J., Lydersen, E., 2019. Brood-stock management and early hatchery rearing of Arctic charr (*Salvelinus alpinus* (Linnaeus)). Rev. Aquac. https://doi.org/10.1111/raq.12400 raq.12400.
Özkurt, E., Fritscher, J., Soranzo, N., Ng, D.Y.K., Davey, R.P., Bahram, M., Hildebrand, F., 2021. LotuS2: an ultrafast and highly accurate tool for amplicon sequencing analysis. bioRxiv. https://doi.org/10.1101/2021.12.24.474111, 2021.12.24.474111.
Palaiokostas, C., Jeuthe, H., De Koning, D., 2020. Assessing the potential of improving growth and survival to the eyed stage in selectively bred Arctic charr (*Salvelinus alpinus*). J. Anim. Breed. Genet. https://doi.org/10.1111/jbg.12509 jbg.12509.
Parata, L., Sammut, J., Egan, S., 2021. Opportunities for microbiome research to enhance farmed freshwater fish quality and production. Rev. Aquac. 13, 2027–2037. https://doi.org/10.1111/raq.12556.
Pickova, J., Brännäs, E., Andersson, T., 2007. Importance of fatty acids in broodstock diets with emphasis on Arctic char (*Salvelinus alpinus*) eggs. Aquac. Int. 15, 305–311. https://doi.org/10.1007/S10499-007-9084-3/TABLES/2.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
R Core Team, 2022. R: A Language and Environment for Statistical Computing.
Raaijmakers, J.M., Mazzola, M., 2016. Soil immune responses soil microbiomes may be harnessed for plant health. Science 352, 1392–1393. https://doi.org/10.1126/SCIENCE.AAF3252/ASSET/D65C8EED-FD4D-4B9F-8F8E-AB5BFACED03C/ASSETS/GRAPHIC/352_1392_F1.JPEG.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.
Rowe, M., Veerus, L., Trosvik, P., Buckling, A., Pizzari, T., 2020. The reproductive microbiome: an emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. Trends Ecol. Evol. 35, 220–234. https://doi.org/10.1016/j.tree.2019.11.004.
Sharpton, T.J., Stagaman, K., Sieler, M.J., Arnold, H.K., Davis, E.W., 2021. Phylogenetic integration reveals the zebrafish Core microbiome and its sensitivity to environmental exposures. Toxics 9, 10. https://doi.org/10.3390/toxics9010010.
Siriyappagouder, P., Kiron, V., Lokesh, J., Rajeish, M., Kopp, M., Fernandes, J., 2018. The intestinal Mycobiota in wild zebrafish comprises mainly Dothideomycetes while Saccharomycetes predominate in their laboratory-reared counterparts. Front. Microbiol. 9, 387. https://doi.org/10.3389/fmicb.2018.00387.
Swanson, G.M., Moskovtsev, S., Librach, C., Pilsner, J.R., Goodrich, R., Krawetz, S.A., 2020. What human sperm RNA-Seq tells us about the microbiome. J. Assist. Reprod. Genet. 37, 359–368. https://doi.org/10.1007/s10815-019-01672-x.
Tacon, A.G.J., 2019. Trends in Global Aquaculture and Aquafeed Production: 2000–2017, 28, pp. 43–56. https://doi.org/10.1080/23308249.2019.1649634.
Tarnecki, A.M., Burgos, F.A., Ray, C.L., Arias, C.R., 2017. Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. J. Appl. Microbiol. 123, 2–17. https://doi.org/10.1111/jam.13415.

Villanueva, B., Fernández, A., Peiró-Pastor, R., Peñaloza, C., Houston, R.D., Sonesson, A. K., Tsigenopoulos, C.S., Bargelloni, L., Gamsız, K., Karahan, B., Gökçek, E., Fernández, J., Saura, M., 2022. Population structure and genetic variability in wild and farmed Mediterranean populations of gilthead seabream and European seabass inferred from a 60K combined species SNP array. Aquaculture Reports 24, 101145. https://doi.org/10.1016/J.AQREP.2022.101145.

Wallace, R.J., Sasson, G., Garnsworthy, P.C., Tapio, I., Gregson, E., Bani, P., Huhtanen, P., Bayat, A.R., Strozzi, F., Biscarini, F., Snelling, T.J., Saunders, N., Potterton, S.L., Craigon, J., Minuti, A., Trevisi, E., Callegari, M.L., Cappelli, F.P., Cabezas-Garcia, E.H., Vilkki, J., Pinares-Patino, C., Fliegerová, K.O., Mrázek, J., Sechovcová, H., Kopečný, J., Bonin, A., Boyer, F., Taberlet, P., Kokou, F., Halperin, E., Williams, J.L., Shingfield, K.J., Mizrahi, I., 2019. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Science Advances 5, eaav8391. https://doi.org/10.1126/sciadv.aav8391.

Wang, H., Chen, T., Chen, Y., Luo, T., Tan, B., Chen, H., Xin, H., 2020. Evaluation of the inhibitory effects of vaginal microorganisms on sperm motility in vitro. Exp Ther Med 19, 535–544. https://doi.org/10.3892/etm.2019.8237.

Wang, N., Chen, L., Yi, K., Zhang, B., Li, C., Zhou, X., 2022. The effects of microbiota on reproductive health: a review. Crit. Rev. Food Sci. Nutr. 0, 1–22. https://doi.org/10.1080/10408398.2022.2117784.

Wei, Z., Gu, Y., Friman, V.P., Kowalchuk, G.A., Xu, Y., Shen, Q., Jousset, A., 2019. Initial soil microbiome composition and functioning predetermine future plant health. Sci. Adv. 5 https://doi.org/10.1126/SCIADV.AAW0759.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. Journal of Open Source Software 4, 1686. https://doi.org/10.21105/joss.01686.