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Potential disruptive effects of zoosporic parasites on peatland-based organic freshwater aquaculture: Case study from the Republic of Ireland

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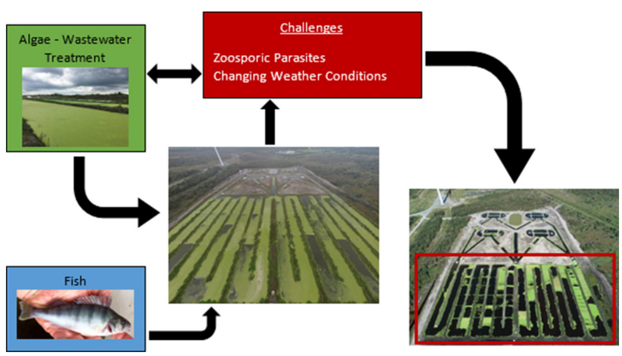
HIGHLIGHTS

- Novel aquaculture system offers sustainable fish production.
- System depends on microalgae for waste bioremediation and water quality.
- Algal presence is important for organic processes.
- Zoosporic parasites may cause collapse of algae population.
- Climate change may contribute to zoosporic parasite issues.

GRAPHICAL ABSTRACT



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ABSTRACT

Irish freshwater aquaculture holds great potential for aiding food security. However, its necessary expansion has been hampered by the adoption of important environmental EU directives. A novel peatland-based recirculating aquaculture multi-trophic pond system (RAMPS) was developed to assess its potential to assist in the sustainable development of industry whilst remaining aligned with environmental protection by adhering to organic aquaculture practices. Microalgae play a pivotal role in the farms' wastewater bioremediation. However, a collapse of the algal population within the system towards the end of the pilot study was observed. No relationship between physicochemical fluctuations and the collapse were indicated. Further investigations into the potential presence of biological agents were then conducted and fourteen species of zoosporic parasites from five different genera (*Labyrinthula, Vampyrella, Amoeboaphelidium, Paraphelidium* and *Aphelidium*) were identified after conducting next-generation sequencing (MinION). The presence of these species indicated the potential cause of algal collapse. Additionally, changes in weather conditions may have also contributed to the issue. Given the lack of data available on zoosporic parasites and their potential impact on organic aquaculture practices, additional research needs to be conducted. Developing a means to monitor and mitigate against these complex zoosporic parasites will inform food security, it will particularly help safeguard "organic" freshwater aquaculture where there is a reliance on using natural-based approaches to address disease mitigation. This information will in turn inform the replication of this RAMPs system in peatlands internationally creating local employment in green technologies, as communities' transition away from burning peat as fossil fuel. Also, zoosporic parasites may reduce important microalgae in peatland-based culture ponds that serve as exceptional sequesters of carbon. Findings of this study will inform related research that focus on the emergence of microbial pathogens in local aquatic ecosystems brought on by variances in climate.

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1. Introduction

Aquaculture has become the fastest growing food producing industry in the world (O'Neill et al., 2019, 2020; Houston et al., 2020) as a result of capture fisheries reaching the maximum sustainable yields, and in some instances surpassing it (Tsikliras and Froese, 2019; O'Neill and Rowan, 2022). It provides an important source for food security, both directly as a means of meeting the ever growing demand for food from an expanding global population and indirectly by providing a means of employment opportunities (Golden et al., 2021; Garlock et al., 2022). Fish produced *via* aquaculture is now available to countries that would have previously been limited or non-existent, often at cheaper prices, thus providing improved nutrition and food security (Rowan and Casey, 2021; Galanakis et al., 2021; O'Neill et al., 2022a, 2022b). However, growth of the industry has begun to slow down (Edwards et al., 2019; Mirto et al., 2022).

In Ireland, issues with licensing, environmental concerns and limited space / resources have hampered the growth of the industry (O'Neill et al., 2020, 2022b). To respond to these challenges, Bord Iascaigh Mhara (BIM), Ireland's state agency for developing the national marine fishing and aquaculture industries, developed Ireland's first trial peatland based, recirculating aquaculture multi-trophic pond systems (RAMPS) adhering to 'organic' principles, as peatlands now being listed as important habitats under the EU's Birds [79/409/EEC & 2009/147/EC] and Habitats [92/43/EC] directives. The peatland RAMPS system holds European perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiis*), common duckweed (*Lemna minor*) and gibbous duckweed (*Lemna gibba*) and exploits the use of microalgae for waste removal (Bord na Móna, 2019; O'Neill et al., 2022c; Rowan et al., 2022). The process differs from traditional aquaculture practices that use water from rivers and lakes where traditional systems must consider potential pollutants from agriculture, industry, wastewater treatments plants, *etc.* (Rowan, 2011; Tahar et al., 2017; Tiedeken et al., 2017; Tahar et al., 2018a, 2018b, 2018c).

The potential beneficial roles of microalgae in aquaculture has only recently been confirmed (Gao et al., 2016; Ansari et al., 2017; Han et al., 2019; Nagarajan et al., 2021). For example, microalgae could efficiently assimilate nutrients providing a good method for wastewater remediation (Wang et al., 2015; Leng et al., 2018; Han et al., 2019) in aquaculture, having already demonstrated promising performances in the food and agriculture industries, and in municipal wastewater treatment (DeBashan et al., 2004; Lu et al., 2015, 2017; Han et al., 2019; Nagarajan et al., 2021; O'Neill et al., 2022b). As a result of this and other emerging benefits, the use of microalgae in aquaculture has recently emerged into the forefront. However, research into the role of microalgae in aquaculture is still lacking (Han et al., 2019; O'Neill et al., 2022a).

The development of microalgae derived products has greatly increased in recent years however, sustainable production faces biological challenges, such as the presence of zoosporic parasites (Höger et al., 2021). Zoosporic parasites are facultative parasites that produce motile spores as their infective propagules causing frequent epidemics in aquatic ecosystems (Scholz et al., 2016) as many of these parasites are known to infect vertebrates, invertebrates, vascular plants, macroalgae, phytoplankton, fungi and protists (Gleason et al., 2014a). Additionally, it is very difficult to differentiate between divergent species of zoosporic parasites as many exhibit very similar morphological characteristics. As such, it is therefore possible that the profile of zoosporic parasites present in aquatic habitats may be undescribed. Given these adversities, it is also possible that a general lack of research in this area may result in infection incidences within the susceptible algal populations of these aquatic habitats being underreported (Scholz et al., 2016). They are believed to be major drivers of phytoplankton succession, and as a consequence, infections can alter the composition of algal species in aquatic ecosystems (Scholz et al., 2016). Zoosporic parasites have received increased attention in recent years; however, research is still greatly lacking (Gleason et al., 2014b; Scholz et al., 2016).

The hypothesis behind this research evolved around the fact that dramatic losses in the microalgae populations within the peatland based trial fish farm could not be explained through use of traditional monitoring techniques and approaches. This intimated that there was other unexplained agent(s) potentially present in the RAMS system causing the observed disruptive issues. Therefore, the aim of this research was to determine whether issues associated with the loss of important algal and cyanobacterial populations within this unique peatland-based aquaculture farm may have been due to the presence and deleterious activities of zoosporic parasites and if so, what potential effects could their presence induce on this novel primary food production system and its connected aquatic environment.

2. Materials & methods

2.1. Study site

Oasis fish farm is an innovative trial peatland based recirculating aquaculture multi-trophic pond system (RAMPS) process set in the middle of Mount Lucas Wind Farm, Co. Offaly (53°17′3″ N - 7°11′45″ W). The RAMPS system combines elements from a recirculating aquaculture system (RAS) and an integrated multi-trophic aquaculture (IMTA) system). The RAMPS consists of four split (pill) ponds connected to a microalgae and duckweed lagoon with sixteen channels serving as an organic treatment system. See Fig. 1. Fish are kept at a density that does not exceed the organic farming standard (*e.g.*, <20 kg/m^{-3} for perch), using screens at the D-ends of each split pond. The space between two D-end fish culture areas is also used to treat waste with free living microalgae in suspension. Flow in each split pond is generated and water is circulated using an airlift. Each D-end fish culture area is equipped with oxygen and temperature probes connected to paddlewheels to provide extra oxygen when necessary. Flow in the lagoon is generated with paddle wheels. The farm is designed to hold a maximum of 32,000 Kg of fish. European perch (*Perca fluviatilis*) is cultured in one pond and rainbow trout (*Oncorhynchus mykiis*) is cultured in the other three. No effluent is released from the farm unless excessively high levels of rainfall is experienced (O'Neill et al., 2022b; Rowan et al., 2022).

2.2. Sample collection and preservation

Collection and preservation of samples were conducted as per O'Neill et al. (2022a, 2022b) with some modifications (samples were collected on different days to the previous studies conducted in parallel). Five litres (grab) water samples were collected from the trial RAMPS fish farm in octagonal carboy HDPE bottles (Lennox) and transported directly to the lab *via* car, 62 km away, in insulated boxes in order to maintain as close to *in-situ* conditions as possible. Samples were collected once a week between July 2020 and October 2020. Samples were taken from each of the culture ponds and, the entry and exit points of the treatment lagoon. See Fig. 1 for an overview of the farm sampling locations. One hundred mL of each sample was stored at -20 °C until molecular analysis was conducted. Physicochemical analysis was conducted immediately upon arrival from the fish farm.

2.3. Microalgae and cyanobacteria monitoring

The microalgae and cyanobacteria levels were monitored on the farm in real time using the AlgaeTorch® (ISO 10260:1992; DIN 38412-16). The hand help device measurement is based on fluorescence which is proportional to the chlorophyll present in the microalgae and cyanobacteria (Naughton et al., 2020). Measurements were recorded in the same locations where the environmental samples were taken (Fig. 1). The device was placed into the water for approximately 15 s and the readings indicated on the device's display were recorded.

2.4. Physicochemical monitoring

Physicochemical analysis was conducted as per O'Neill et al. (2022a, 2022b) with some modifications. Temperature, pH, ammonium (NH_4^+),

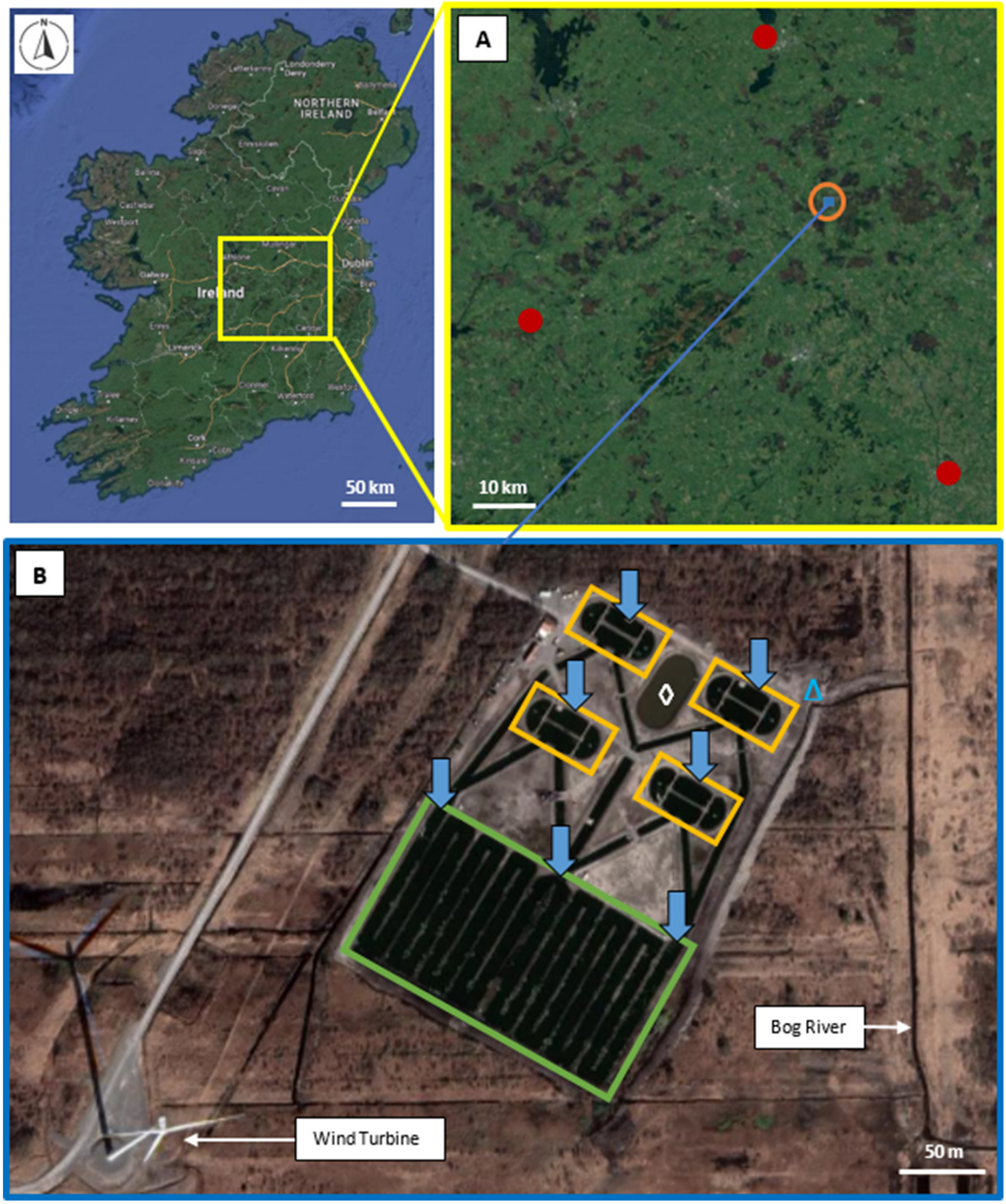

Fig. 1. A) Location of the three meteorological weather stations (yellow box); Gurteen – 53°02′24″ N, 08°00′36″ W; Oak Park– 52°51′36″ N, 06°55′36″ W; Mullingar – 53°33′ 36″ N, 07°20′24″ W, (red •) surrounding the trial fish farm (orange **O**). Meta data from these three stations were used to analyse any potential impacts changing weather conditions may have had on the system. B) Ariel view of the trial fish farm (Oasis) at Mount Lucas. Culture ponds (orange box), algae and duckweed lagoon (green box), reservoir (white ◊) and water intake location (blue **Δ**) displayed. Blue arrows indicate location of sampling points. Wind turbine which generates all electricity for the farm and the bog river which supplies water for the farm are also included. Figures adapted from O'Neill et al., 2022a. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), dissolved oxygen (DO), biochemical oxygen demand (BOD), total suspended solids (TSS) and total dissolved solids (TDS) were investigated in the laboratory within 24 h of collection to prevent the need for preservation. Temperature, pH and TDS were analysed using a benchtop pH and conductivity meter (VWR pHenomenal™ MU 6100 L meter, VWR 111662–1157 pH probe, VWR CO11 conductivity probe). DO and BOD_{5day} were analysed using a benchtop DO_2 meter and probe (Jenway). Photometric kits were used to assess the NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} (Merck), as per the manufacturer's (Spectroquant®) instructions. The TSS were analysed *via* filtration using a Buchner flask, Buchner funnel and 0.20 μm pore membrane filter (Whatman).

2.5. Molecular analysis

Isolation of DNA, 18S sequencing and bioinformatics were conducted as per O'Neill et al. (2022b) with some modifications. The frozen water samples were first thawed, then centrifuged and the resulting pellet re-suspended in 2 mL of dH_2O, before sonication at 40 kHz in a sonicator bath (Cuyson) at 45 °C for 20 min. DNA was isolated using the Roche High Pure PCR Template Preparation kit (Merck). Between 100 and 150 ng of DNA was used in a PCR reaction for the amplification of an 1800 base pair segment of the 18S ribosomal DNA sequence that was modified with adapter sequences (O'Neill et al., 2022b) for subsequent sequencing on the Oxford Nanopore MinION platform (adaptor sequences in red and underlined):

(F): 5′- TTTCTGTTGGTGCTGATATTGCGGTGATCCTGCCAGTAGTCATATGCTTG -3′.

(R): 3′- ACTTGCCTGTCGCTCTATCTTCGATCCTTCCGCAGGTTCACCTACGGAAACC -5′.

Sequencing was conducted using the Ligation Sequencing Kit SQK-LSK114 (Oxford Nanopore Technologies) protocol, as per the manufacturer's instructions. The quality of the sequencing was assessed using the FastQC tool (v0.11.9). The adapters were removed using the Porechop adaptor trimmer tool (v0.2.4). The FastQ tool (v0.14.0) was then used to eliminate low quality reads, followed by quality assessment once again to confirm its success. The resulting high-quality reads were then grouped together using the isONclust software (v0.0.4). Each group, representing all reads that came from an individual gene, was then aligned against the National Center for Biotechnology Information (NCBI) nucleotide database using the BLASTn tool (v2.13.0) with an *E*-value of 0.05.

2.6. Statistical analysis

Statistical analysis were performed on GRAPHPAD PRISM 9.3. The data generated were grouped and subject to normality tests (Anderson-Darling), to determine if samples were from a normal distribution ($P > 0.05$ = normal distribution). Parametric testing was then applied as a normal distribution was indicated. Grubbs test was used to determine if any outliers were indicated. *t*-tests and ANOVA (one-way with Tukey and two-way with Sidak) were used to determine if any significant differences were observed between the variables ($P < 0.05$ = significant difference). Unpaired tests were used as different sets of samples were analysed. Pearson's coefficient was applied to determine whether any relationship correlations (r) were indicated.

3. Results

In order to obtain a more comprehensive analysis of the water quality conditions, ideally a composite sampler would be used to collect approximately 100 mL of water every hour for 24 h. However, due to technical difficulties and limitations in obtaining composite samplers, grab samples were collected. A larger volume (5 L) was collected as slowly as possible in order to aid in compensating for the lack of access to composite samples. This should be noted when interpreting these results.

3.1. Microalgae and cyanobacteria

Microalgae and cyanobacteria readings across the entire system indicated no statistically significant difference (microalgae $P = 0.8976$, cyanobacteria $P = 0.9380$). Therefore, results were grouped together for ease of reporting. Microalgae levels fluctuated between 170 μg L^{-1} and 280 μg L^{-1} between December 2019 and August 2020, as shown in Fig. 2. However, levels dropped dramatically between August 2020 and September 2020 (Fig. 2), falling to 31.65 μg L^{-1} before rising slightly to 95.83 μg L^{-1} the following month (October 2020). Cyanobacteria levels remained consistently between 20 μg L^{-1} and 40 μg L^{-1} from January 2020 to April 2020 before spiking in May to 140 μg L^{-1} (Fig. 2), as would be expected given the time of year. Levels dropped back to 37.45 by July 2020, as shown in Fig. 2. However, similarly with the microalgae, cyanobacteria levels dropped to 1.45 μg L^{-1} during September 2020 before making a slight increase tom 8.83 μg L^{-1} in October 2020 (Fig. 2). Similar but not identical trends were observed in a separate study conducted in parallel

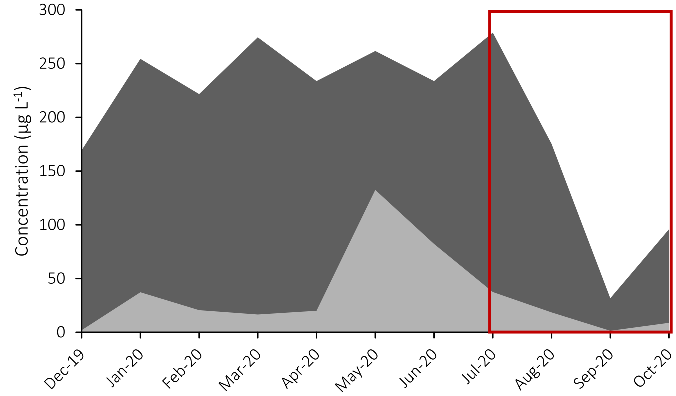

Fig. 2. Concentrations of algae (dark grey) and cyanobacteria (light grey) in μg L^{-1} detected on the trial fish farm between December 2019 and October 2020 using the AlgaeTorch®. Red box indicates when issues were observed within the system resulting in the loss of both algal and cyanobacterial levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with this research at the same site where direct algal and cyanobacterial counts were established using flow cytometry (O'Neill et al., 2022a).

3.2. Weather conditions

Research conducted by O'Neill et al. (2022a) at the same location indicated changing weather conditions, most likely as a result of climate change, played a contributing factor in issues associated with fish mortalities. Therefore, Met Éireann (The Irish Meteorological Service) meta data was subsequently investigated during the time period where issues with the loss of algal and cyanobacteria populations were experienced to determine whether any relationships existed. Meta data from the three closest Met Éireann weather stations surrounding the fish farm (Mullingar, Co. Westmeath 53°33′36″ N, 07°20′24″ W; Oak Park, Co. Carlow 52°51′36″ N, 06°55′36″ W; Gurteen, Co. Tipperary 53°02′24″ N, 08°00′36″ W) were investigated. See Fig. 1 for the location of all weather stations with respect to the trial fish farm. The average monthly rainfall and temperature between July 2020 and October 2020 were included, as well as rainfall and temperature long term averages (LTA) for those months (Met Éireann, 2022), as shown in Fig. 3.

The average rainfall for the time period remained between 104 mm and 111 mm with the exception of the month of September 2020 where an average of only 59.33 mm was recorded. When data from the 2020 averages were compared to the LTA, rainfall was found to increase for the months of July, August and October but decrease for the month of September (Fig. 3). However, this was not statistically significant ($P = 0.3124$). The average temperature for the time period ranged between 15.43 °C and 9.57 °C. When the 2020 average data was compared to the LTA, similar trends were observed ($P = 0.9830$), as shown in Fig. 3. A correlation study then was conducted to determine whether the issues with algal and cyanobacterial populations (Fig. 2) were as a result of changing temperature or rainfall levels. Although moderate to strong relationships were observed for the microalgae and cyanobacteria with regards to both the rainfall and temperature data, none were statistically significant, as shown in Fig. 4 (microalgae v rainfall $r = 0.647$, $P = 0.3527$; microalgae v temperature $r = 0.534$, $P = 0.4664$; cyanobacteria v rainfall $r = 0.583$, $P = 0.4165$; cyanobacteria v temperature $r = 0.517$, $P = 0.4827$).

3.3. Physicochemical analysis

The physicochemical results were compared to water quality parameters set out by the Irish Environmental Protection Agency (EPA) and the Irish Statutory Office [SI 272/2009 and SI 77/2019] for guidance

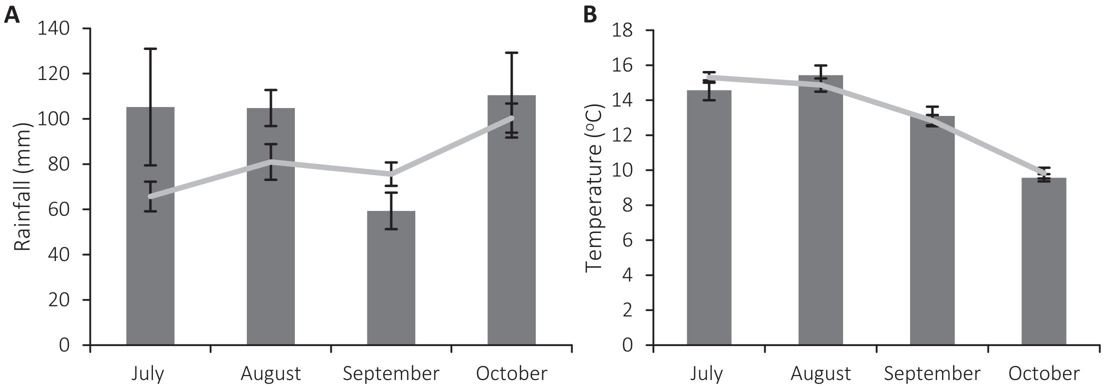

Fig. 3. A) Average rainfall (mm) and B) average temperature (°C) for 2020 (bar chart) and the long term average or LTA (line chart) records across the three meteorological weather stations (Gurteen; Oak Park; Mullingar) surrounding the Mount Lucas trial fish farm (Oasis) during the period (July 2020 – October 2020) where issues were observed within the system. SD indicated.

(EPA, 2001; Irish Statutory Office, 2009, 2019) as no physicochemical values specifically for Irish aquaculture were available (O'Neill et al., 2022a), as shown in Table 1. No issues were observed after physicochemical analysis, with the exception of NO_2^-, PO_4^{3-}, DO and TSS. Both NO_2^- and PO_4^{3-} were consistently above the guidance value of 0.03 mg L^{-1} which may be due to the reducing levels of microalgae and cyanobacteria present in the system and the nutrients were not therefore being consumed by the organisms. The BOD levels were consistently above the guidance value of 6 mg O_2 L^{-1}. This was an on-going problem and mitigation techniques were applied (additional aeration and filtration) to aid in reducing this issue. The TSS levels were also consistently above the guidance value of 25 mg L^{-1}. However, once additional filtration was applied TSS dropped below the guidance value. Fluctuations in oxygen were continually observed however, paddle wheels and airlifts were present in the farm to ensure oxygen levels remained above the guidance values. Oxygen levels may have also dropped during the transfer of environmental samples from the farm to the lab. Results were consistent with physicochemical analysis observed in studies conducted in parallel with this research at the same site during 2020. A correlation study was then conducted to determine whether fluctuations in the physicochemical parameters may have contributed to the loss of microalgae and cyanobacteria populations. No statistically significant correlations were observed between any of the physicochemical parameters and the microalgae or cyanobacteria results, as shown in Fig. 4.

3.4. Zoosporic parasites

Samples for each month and for each location were pooled together. After analysis of the MinION data, fourteen species from five genera (*Labyrinthula, Vampyrella, Amoeboaphelidium, Paraphelidium* and *Aphelidium*) of zoosporic parasites were identified across all of the sampling locations within the system (Fig. 5). All fourteen species were identified in every month (July to October).

4. Discussion

The novel RAMPS, which follows organic aquaculture principles, was initially designed to aid in alleviating issues surrounding the development of the Irish freshwater aquaculture industry as a result of the adoption of important environmental EU directives that have delayed the licensing process, as well as highlighted limitations in space and resources for the industry. Part of the organic process is the use of microalgae for wastewater bioremediation making it an important factor within the farm. This is additionally important as peatlands are now protected habitats given their increasing scarcity. Therefore, the unforeseen and unexpected loss of microalgal populations within the system, particularly around the month of September 2020 was highly problematic. Physicochemical results suggested that the fluctuations in the parameters were unlikely the cause of

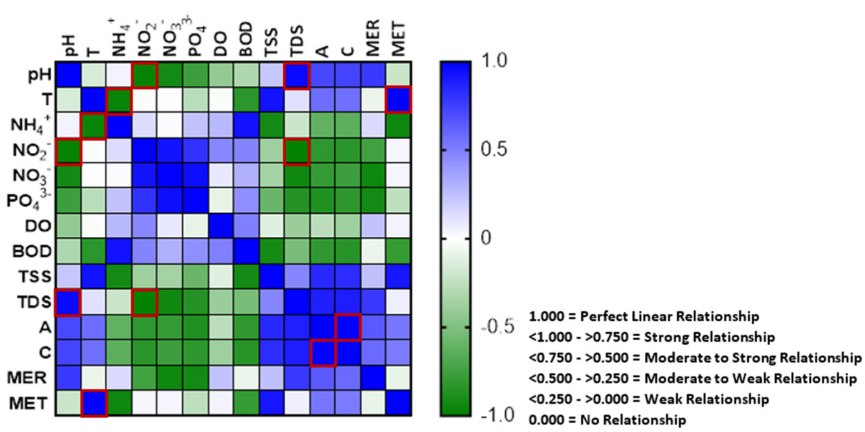

Fig. 4. Correlation matrix used to determine whether any relationships existed between physicochemical analysis, algal levels and cyanobacterial levels observed in the trial fish farm (Oasis) and, the average rainfall and the average temperatures recorded in the surrounding meteorological weather stations between July 2020 and October 2020. Positive correlations indicated by blue, inverse correlations indicated by green. Red box indicates statistically significant results where $P < 0.05$. T = temperature, NH_4^+ = ammonium, NO_2^- = nitrite, NO_3^- = nitrate, PO_4^{3-} = orthophosphate, DO = dissolved oxygen, BOD = biochemical oxygen demand, TSS = total suspended solids, TDS = total dissolved solids, A = algae, C = cyanobacteria, MER = Met Eireann Rainfall Data, MET = Met Eireann Temperature Data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Breakdown of the physicochemical analysis conducted on the trial fish farm (Oasis), during the period between July 2020 and October 2020, where issues within the system were observed. Values from the Irish Environmental Protection Agency and the Irish Statutory Office with regards to water quality were included for guidance as no physicochemical values specifically for Irish aquaculture were available. * indicates when results were beyond the guidance values. S.D. indicated, $n = 6$.

	July	August	September	October	Guidance Values
pH	8.11 ± 0.08	7.03 ± 0.01	7.48 ± 0.06	7.70 ± 0.04	>6 - < 9
Temperature (°C)	18.00 ± 0.46	17.46 ± 0.39	13.42 ± 0.16	12.16 ± 0.10	<25
Ammonium (mg NH_4^+ L^{-1})	0.01 ± 0.01	0.12 ± 0.03	0.10 ± 0.01	0.33 ± 0.22	<1
Nitrite (mg NO_2^- L^{-1})	0.05 ± 0.01*	0.13 ± 0.01*	0.10 ± 0.01*	0.08 ± 0.01*	<0.03
Nitrate (mg NO_3^- L^{-1})	1.39 ± 0.12	2.72 ± 0.34	4.88 ± 0.30	2.60 ± 0.77	<50
Orthophosphate (mg PO_4^{3-} L^{-1})	1.11 ± 0.07*	1.81 ± 0.06*	1.72 ± 0.07*	1.68 ± 0.11*	<0.03
Dissolved Oxygen (mg O_2 L^{-1})	5.13 ± 0.20*	7.07 ± 0.19	5.59 ± 0.17*	6.49 ± 0.11*	>7 (C) >9 (S)
BOD (mg O_2 L^{-1})	21.98 ± 1.50*	28.87 ± 2.32*	22.42 ± 2.26*	26.05 ± 1.94*	<6 (C) <3 (S)
Suspended Solids (mg L^{-1})	72.33 ± 7.53*	40.00 ± 2.53*	23.68 ± 5.28	14.00 ± 5.51	<25
Dissolved Solids (mg L^{-1})	170.00 ± 2.77	169.00 ± 4.33	172.00 ± 4.51	171.00 ± 2.47	<300

C = Cyprinids, S = Salmonids, BOD = Biochemical Oxygen Demand.

the problem especially given the fact the fluctuations were consistently observed previously without the loss of microalgal (microalgae and cyanobacteria) numbers (O'Neill et al., 2022b). The changes in weather conditions may be potentially contributing to the issues, as moderately strong relationships were indicated, although not statistically significant. This in turn led the authors to consider that an unforeseen deleterious agent may have been the cause of the previously unexplained disruption and reduction in algal in this RAMPs system. Zoosporic parasites were subsequently investigated as they have been previously reported to cause sudden and considerable cellular death in microalgae in both natural and industrial settings (Höger et al., 2021). The presence of five known genera of zoosporic parasites genera (*Labyrinthula, Vampyrella, Amoeboaphelidium, Paraphelidium* and *Aphelidium*) was confirmed.

Labyrinthula are a fungal like aquatic protist found in a diverse range of habitats (both freshwater and marine) and are opportunistic zoosporic parasites known to infect diatoms (Popova et al., 2020), which were routinely observed in the system (O'Neill et al., 2022b). *Vampyrella*, which generally occur in freshwater systems, are naked amoebae that are known as 'vampire' amoebae. They puncture hole in the host's cell wall and 'suck up' the cytoplasm causing deterioration and collapse of the cell, most

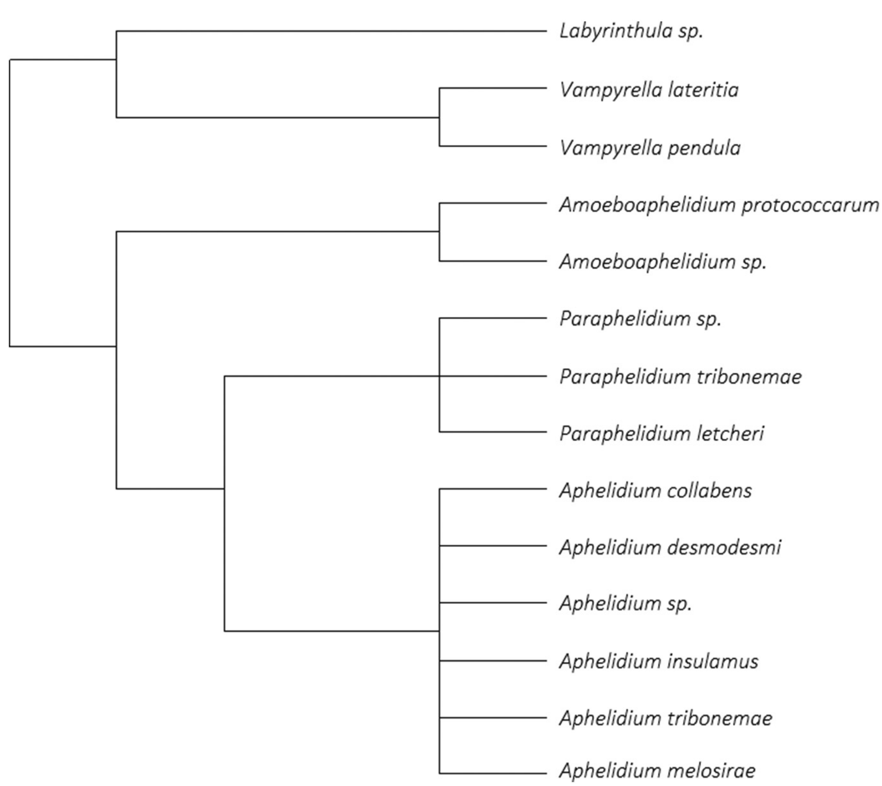

Fig. 5. Phylogenetic tree indicating all zoosporic parasites identified in the trial fish farm (Oasis) between July 2020 and October 2020. See supplementary data for breakdown.

often targeting green microalgae such as *Scenedesmus* (Gong et al., 2015) which was one of the most common green microalgae in the system (O'Neill et al., 2022b). The three remaining genera identified (*Aphelidium, Paraphelidi*um and *Amoeboaphelidium*) are all aphelids which are intracellular parasitoids of many groups of microalgae (Karpov et al., 2017). *Aphelidium* form cysts that penetrate the host cell and migrate the cysts contents into the cell causing deterioration and subsequent death (Karpov et al., 2020) and are commonly found to infect *Scenedesmus* (Gleason et al., 2014c). *Parapheilidium* penetrate and digest the cell wall by both mechanical and enzymatic means, targeting a range of different algal groups (Torruella et al., 2018). *Amoeboaphelidium* attaches itself to the host cell wall then encapsulates it and ultimately causes cell death by engulfing it (Höger et al., 2021). They have been found to infect *Ankistrodesmus, Desmodesmus, Chlorella* and *Scenedesmus* (Gleason et al., 2014c), all of which were routinely observed within the farm, with *Chlorella* being the most common and potentially most beneficial to the system (Ahmad et al., 2020; Arguelles, 2021; Arteaga Quico et al., 2021; O'Neill et al., 2022b). Although it cannot be inferred directly from the results, this suggested that the collapse of the microalgal populations may potentially be linked to the presence of the reported zoosporic parasites as they have the capacity to and are known to infect algal cells. Therefore, additional research is required from both an aquaculture and environmental point of view (as indicated in the future work). Not only would the presence of zoosporic parasites have a huge impact on organic aquaculture that strongly relies on microalgae, but they could also have knock on effects on surrounding aquatic ecosystems. Although this would unlikely be an issue with this system as water is very rarely released, should such parasites accidently be released from other aquaculture facilities that are directly connected to freshwater systems, the loss of microalgae (primary producers) within an aquatic ecosystem could instigate the collapse of the aquatic food chain. These findings also align with related research that considers a relations between climate variance and the emergence of infectious agents and vectors (El-Sayed and Kamel, 2020).

5. Conclusion

Research from this pilot study has indicated that the present of zoosporic parasites may, in part, have great adverse effects on the organic aquaculture system given their ability to demolish microalgal populations so quickly. Additionally, the changes in weather conditions, most likely due to climate change, may also be potentially contributing. The identification and real-time profiling of zoosporic parasites is significantly underappreciated for sustainable-food based systems given that this also has implications for green-industries and entrepreneurs who are focusing on developing large pond-based systems for the growth of microalgae as new biomass with a view to the bio-refining of bioactive-products for potential food, feed and health applications. Also, the microalgae in ponds serve as exceptional sequesters of carbon that could be disrupted. Ultimately, more focused research of the potential effects the presence of zoosporic parasites on organic aquaculture processes needs to be conducted, as well as an increased focus on their effects on the surrounding environments and the potential effects climate change may contribute. As such, during the next phase of the project, the following research will be conducted;

6. Future work

- Given that the collapse of the algal population was not consistently observed, research into the origin of the zoosporic parasites presence in the system needs determined in order to aid in the prevention of future occurrences.
- As molecular analysis could not be conducted on a week-to-week basis, additional research is needed to determine what time period throughout the year zoosporic parasites are most prevalent. This will include an extended monitoring period (12 months). Additionally, research needs to be conducted as to determine which species, and at what quantity (Real-Time PCR / qPCR), are occurring *i.e.*, are different species more prevalent throughout different periods of the year.
- Given that some species of microalgae are more beneficial than other, analysis on which species of microalgae are predominantly affected needs to be determined in order aid in preventing their entire collapse.
- Mitigation measures needs to be investigated in order to reduce and eliminate the zoosporic parasites presence without causing adverse effects on the microalgae, the fish or the surrounding system in general.
- Although the novel RAMPS is ultimately a closed system in so far as no discharge is released from the system unless excessive levels of rainfall is experienced causing the lagoon to overflow; given that the surrounding peatlands are a very important ecosystem, analysis into the potential effects zoosporic parasites on the surrounding peatland environment needs to be conducted in order to ensure all systems are protected.
- Finally, additionally research on any potential links with zoosporic emergence and changes in weather conditions needs to be considered, especially given the fact that climate change has increased the occurrence of more changing and erratic weather conditions.

CRediT authorship contribution statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Science of the Total Environment.

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Supervision	N. J. Rowan
Validation	E. A. O'Neill
Visualization	E. A. O'Neill
Roles/Writing – Original Draft	E. A. O'Neill, N. J. Rowan
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Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that there are no competing interests or conflicts of interest with respect to the publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.161495.

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