Potential use of peatlands as future locations for the sustainable intensification of freshwater aquaculture production – a case study from the Republic of Ireland

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Abstract

There has been an increasing interest in enhancing freshwater aquaculture processes without hindering the progress of the Water Framework Directive. Bord Iascaigh Mhara (Ireland's national seafood development organisation), undertook a feasibility study to assess the potential use of peatlands (bogs) for sustainable aquaculture diversification. AquaMona, a new concept in integrated multitrophic aquaculture (IMTA), uses cutaway bogs to farm rainbow trout and Eurasian perch with associated organic status that is powered by wind energy and utilizes algae and duckweed to treat rearing water. Approximately 5% of Ireland comprises bogs that support natural ecosystems where there is a pressing need to develop alternative innovation to that of burning peat in order to reduce Ireland's carbon emissions. This constitutes the first study to evaluate water quality from this new IMTA where intake and terminal holding tank samples were evaluated from May to August 2019. Physicochemical parameters (temperature, pH, nitrogen, phosphorus, oxygen, suspended solids, hardness and alkalinity), and ecotoxicological bioassays (*Pseudokirchneriella subcapitata* and *Daphnia pulex*), were used to investigate the potential effects that introducing aquaculture processes may have on peatlands. Nitrite ($p<0.001$), Nitrate ($p=0.016$), and chemical oxygen demand ($p=0.011$), were the only physicochemical parameters that differed significantly between the intake and holding tank water indicating that water quality for the most part remained unchanged. Low levels of toxicity were observed between the bioassays suggested the introduction of the processes into the bog were unlikely to cause adverse effects on the ecosystem and the organisms therein. Observations determined in this study were similar to or lower than those determined for intensive flow-through aquaculture processes that discharge to receiving water. Findings from this study support the use of peatlands as future locations for integrated aquaculture processes.

Keywords: freshwater-aquaculture, cutaway-peatlands, sustainable, water quality, integrated-multitrophic system

1. Introduction

Aquaculture is the fastest growing food producing industry globally (Fečkaninová *et al*., 2017; Liu *et al.*, 2017; O'Neill *et al.*, 2019). It affords one of the most sustainable forms of edible protein (Liu *et al.*, 2017; Yue and Wang, 2017). Aquacultures rapid expansion has resulted in response to dramatic increase in global population and an augmented demand for food (Seoane *et al.*, 2014). Between 1983 and 2003, global capture fisheries production increased from 71.1 M (million), to 92.6 M tonnes globally, whilst global aquaculture production increased from 6.2 M to 70.2 M tonnes (Ottinger *et al.*, 2016). In 2014, aquaculture production reached 73.8 M tonnes (Huynh *et al.*, 2017), and now accounts for ~50% of fishery products produced for human consumption (Liu *et al*., 2017). Fredricks *et al*. (2015), suggests that by 2030 aquaculture will provide an estimated 62% of fish for human consumption. However, limitations in space that would allow for expansion of existing facilities, challenges with the development of new sites due to licencing, the lack of availability of freshwater, and the ever growing concerns associated with pollution are thought to be major hurdles in the further expansion of traditional aquaculture systems (Badiola *et al.*, 2012). Concerns about the environmental impacts of the rapid expansion of intensive aquaculture systems has also led to increased research interest in integrated multitrophic aquaculture systems or IMTA (Granada *et al*., 2016)

The Irish aquaculture industry was valued at €208.4 M in 2017, producing just over 47,000 tonnes of fish (Bord Iascaigh Mhara, 2018). The industry provides valuable employment and assists in preserving rural communities (Department of Agriculture Food and the Marine, 2015a). Food Wise 2025 is a national strategy put forward by the government of Ireland to assist in the growth and development of the Irish agri-food industry, which includes seafood (Department of Agriculture Food and the Marine, 2015b). Food Wise 2025 has projected that the Agri-Food industry, or more specifically exports, have the potential to grow to €19 billion per annum by 2025 (Department of Agriculture Food and the Marine, 2015b). This growth will, in part, be achieved by expansion in the seafood sectors (Department of Agriculture Food and the Marine, 2015b). As part of the Food Wise 2025 strategy and in order to assist in meeting this goal, it has been predicted that the Irish aquaculture industry has the potential to increase export production to 81,700 tonnes by 2023 (Department of Agriculture Food and the Marine, 2015b). However, issues associated with 1) the Irish aquaculture licensing process, and 2) the potential environmental impacts caused by aquaculture and its discharge which will have an effect on the environmental EU directives adopted by Ireland (Department of Agriculture Food and the Marine, 2015b), have hampered the industry reaching this goal.

There has been increasing interest in enhancing sustainable freshwater aquaculture processes through innovation (Tahar *et al.*, 2018a, 2018b; Rowan, 2019). However, enhancement must not be at the expense of the commitments set out by the Water Framework Directive (WFD), which aims to provide a structure for water protection to assist EU member states to achieve good water status in all waters and ensure this status does not depreciate (Voulvoulis *et al.,* 2017; WFD Ireland, 2018). As part of the 2015 National Strategic Plan for Sustainable Aquaculture Development, Bord Iascaigh Mhara (BIM), the state agency for developing Ireland's seafood industry, undertook a feasibility study to assess the novel use of Irish bogs for aquaculture diversification (Department of Agriculture Food and the Marine, 2015a). Peatlands (bogs) are wetland environments characterised by the build-up of organic matter (peat), derived from dead plant material that is slowly decomposing under wet conditions. Bord na Móna, a state company that was originally developed to establish Irish peat resources for economic benefit owns or controls approximately 7% or about 80,000 hectares of Ireland's bogs. Cutaway-bog sites that were taken out of production when there is no economic supply of peat remaining have been converted to wind energy, forestry, biodiversity, amenity, and waste management. Recently, Bord Na Mona, in conjunction with BIM, has further expanded use of these cutaway bogs to develop Ireland's first integrated recirculation aquaculture process for fish production using organic principles that comprises a combined split pond and duckweed culture lagoons (Bord na Móna, 2019). The trial fish farm was established at a peatland site of a wind farm at Mount Lucas, Co Offaly which is situated in the centre of Ireland. The trial farm, is based on sustainable multitrophic culture system principles, holding European perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiis*), common duckweed (*Lemna minor*) and gibbous duckweed (*Lemna gibba*).

Bogs primarily make up Ireland's peatlands (National Parks & Wildlife Service, 2015). Irish bogs can be divided into two main categories; blanket bogs and raised bogs where the latter is the focus of this study. Raised bogs are best described as raised bodies of peat situated in former lakes or shallow hollows in the landscape (Irish Peatland Conservation Council, 2019). They were formed thousands of years ago, after the last ice age when much of Ireland was covered in depthless lakes as a result of the melting ice (Ward *et al*., 2019). Over the centuries, poor drainage and the build-up of decaying plants have resulted in the raised bogs we know today. They are only fed by precipitation (rainfall), and as a consequence are generally poor in nutrients (National Parks & Wildlife Service, 2015; Ward *et al*., 2019). Irish raised bogs are normally found in the midlands where moderate levels of rainfall are experienced, and are very deep, *i.e.* up to 13 m in parts (National Parks & Wildlife Service, 2015; Ward *et al*., 2019). Thus, fish farms from cutaway bogs differ from traditional aquaculture practices that use water from rivers and lakes where latter system must consider environmental impact of their expansion and possible threats arising from potential pollutants from agricultural runoff, industry and waste-water treatments plants (Tahar et al., 2017; Tahar et al., 2018c; Tiedeken et al., 2018).

Irish bogs are considered to be some of the finest examples of their type in the world (Flynn *et al.*, 2016; Irish Peatland Conservation Council, 2019). Raised bogs are important reservoirs of biodiversity (Flynn *et al.*, 2016), and are home to a range plant and animal species, such as bog cotton, frogs and moths, which are highly adapted to the bogs inhospitable conditions of low-nutrient soil and waterlogging (Ward *et al.,* 2019). As a result, Ireland has a responsibility to conserve and protect them.

Bogs are important carbon stores which help to control greenhouse gases (Flynn *et al*., 2016; Irish Peatland Conservation Council, 2019). However, human activities and inputs have caused degradation. The remaining intact bogs are small remnants of once larger bog systems. It is thought that there has been as much as a 99% loss in bog habitats (Flynn *et al.*, 2016). For example, Hammond (1981), reported that 16.2% of the island of Irelands landscape was covered in peatlands. Today, on the other hand, it is down to only 5% (Ward *et al*., 2019).

Bord na Móna was formed in 1946 by the Irish Government from the Turf Development Board after the introduction of the Turf Development Act (Bord na Móna, 2019; Ganly, 2017). The now semi-state owned company was originally established to aid in the development of a large scale peat industry for the state's economic gain (Bord na Móna, 2019; Ganly, 2017; Ward *et al.,* 2019). Peat has been used for centuries in Ireland as a fuel source for both domestic (heating homes), and later commercial use (generate electricity via power plants), as it is a country with very little oil, gas or coal (Toner, 2018). However, peat is considered to be very damaging to the environment, emitting more carbon dioxide than coal and natural gas (Toner, 2018). In 2016, peat generated approximately 8% of Ireland's total electricity which was the equivalent to 20% of the sectors annual carbon emissions (Toner, 2018).

The urgent threat of climate change, an ever complicated and increasingly problematic challenge, in addition to some of the bogs now being listed as important habitats under the European Unions (EU), Birds and Habitats Directives due to their scarcity, have resulted in dramatic changes in the peat industry (Bord na Móna, 2019; Irish Peatland Conservation Council, 2019; O'Neill *et al*., 2019; Toner, 2018; Ward *et al*., 2019). Bord na Móna are now moving away from peat harvesting (Bord na Móna, 2019; Lee, 2018). In 2018, 17 of their 62 active harvesting sites were closed and all works will cease on the remaining 42 sites by 2025 (Lee, 2018). Because of the need to decarbonise energy sources to in response to climate change, Bord na Móna has diversified its portfolio of works to include renewable energy (Bord na Móna, 2019; Lee, 2018), with more than 60% of electricity generated now coming from renewable resources *e.g.* wind turbines (Bord na Móna, 2019).

Aquaculture discharge typically contains nutrient rich waste which requires treatment prior to release (Jegatheesan *et al*., 2011; Martinez-Porchas *et al*., 2014; Ngo *et al*., 2016; O'Neill *et al.*, 2019; Sikder *et al.*, 2016). If discharged untreated, this waste can lead to water pollution in the environment (Jegatheesan *et al.*, 2011; O'Neill *et al.*, 2019; Sikder *et al.*, 2016). Water quality is generally assessed by measuring physicochemical parameters (da Silva *et al.*, 2017), such as temperature, pH, phosphorus, nitrogen, oxygen, suspended solids, hardness and alkalinity. Temperature and dissolved oxygen affects the growth and survival of fish and are therefore a critical environmental factors (Ferreira *et al.*, 2011). Ammonium (NH_4^+) and nitrite (NO_2^-) are toxic to aquatic life and require treatment by way of conversion to nitrate (NO_3^-) before being released from fish farms (Celik *et al.*, 2001; Durborow *et al.*, 1997; Pollice *et al.*, 2002; Zhang *et al.*, 2011). Orthophosphate (PO_4^{3-}), is a reactive form of phosphorus and is one of the main causes of algal blooms and hypoxic conditions that blooms ensue (Barcellos *et al.*, 2019; Brogan *et al.*, 2001; O'Neill *et al.*, 2019). The biochemical oxygen demand (BOD), is the amount of oxygen bacteria use to break down organic matter, whilst the chemical oxygen demand (COD), measures the level of stress the organic matter puts on the receiving water system (Lee and Nikraz, 2015). Suspended solids usually consist of organic matter and eutrophic conditions can occur when levels become elevated (Bilotta and Brazier, 2008; O'Neill *et al.,* 2019). Analysis of these parameters alone, although important, will only indicate potential adverse effects at a given time and not the effects on the ecosystem or organisms therein (da Silva et al., 2017; O'Neill et al., 2019; Stephens and Farris, 2004a). Therefore, ecotoxicological bioassays are used in conjunction with the physicochemical parameters (da Silva *et al.*, 2017). This may be very important as ultimately the WFD is not concerned with exact emission levels as such, but rather what can be emitted such that harmful effects can be avoided and good or excellent status can be both achieved and maintained (WFD Ireland, 2001).

Primary producers (*e.g.* algae), and primary consumers (*e.g.* micro-crustaceans), are key components in aquatic food chains (Aruoja, 2011). The microalgae *Pseudokirchneriella subcapitata* (*P. subcapitata*), is commonly used for ecotoxicological assessment of primary producers in multi-trophic testing due to its high sensitivity, reproducibility and growth rate (Suzuki *et al.,* 2018). As a result, *P subcapitata* is one of the recommended algae set out by the ISO (6892:2012), guidelines (International Organisation for Standardisation, 2012a). *Daphnia* are freshwater micro-crustaceans are commonly used for ecotoxicological assessment of primary consumers in multi-trophic testing. Similarly with *P. subcapitata,* they also are chosen due to their reproducibility and ease of culture (International Organisation for Standardisation, 2012b).

The aim of this research was to determine whether the introduction of aquaculture production into peatlands had any potential adverse effects on the very important and scarce natural habitat of the peatland. This in turn may assist the Irish aquaculture industry in reaching the goal of increasing production to the predicted levels set out by Food Wise 2025 as well as assist in limiting job losses that could face Bord na Móna employees once peat harvesting ceases.

2. Methods

2.1. Fish Farm

The multitrophic aquaculture system consists of four split (pill) ponds connected with duckweed area serves as a treatment system (Figure 1A). Fish are kept at a density that does not exceed the organic farming standard (< 20 $kg.m^{-3}$ for perch), using screens at the D-ends of each split pond (Figure 1B). The space between two D-end fish culture areas is also used to treat waste with free living algae and bacteria 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. The farm is designed to hold a maximum of 32,000 Kg of fish. Holdings reached just over 11,000 Kg in August (Table 1).

2.2. Sampling:

Water samples were collected from the trial fish farm located in Mount Lucas, Co Offaly in the Republic of Ireland. The farm, which cultures perch (*Perca fluviatilis*), and rainbow trout (*Oncorhynchus mykiss*), utilises a multi trophic approach, which reuses up to 100% of its water and a large algae and duckweed bed used to assist in treating waste water (Figure 1). The bog is undergoing restoration and is in the process of returning to its natural state before peat extraction begun. The farm is adjacent to a large natural wetland.

Grab samples were collected in 5 L octagonal carboy HDPE bottles (Lennox), and transported directly for laboratory analysis. Samples were obtained directly from the intake and holding tank sources of the farm every month from May to August 2019. The intake samples consisted of water taken directly from the bog. The terminal holding take samples contained water that had been used to culture fish in the split ponds and had then passed through the algae and duckweed channels for treatment. This water continues on after the holding tanks and returns to the culture ponds to be reused, thus is recirculated. As discharge only occurs during times of high rainfall, resulting in overflow, the samples were taken directly from the holding tank at the discharge point. Discharge did not occur during the sampling period. Collection occurred on the same day (Wednesday), and at approximately the same time (9:00 a.m.). Sampling points are shown in Figure 1. All samples were tested in triplicate. Due to issues in collected individual and composite samples, triplicate samples were taken from the 5L grab sample and analysed separately.

2.3. Physicochemical analysis:

Water parameters, including; temperature, pH, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, DO, BOD, COD, suspended solids, hardness and alkalinity, were analysed in the laboratory within 24 hours of collection to eliminate the need for preservation. Spectroquant® photometric kits (Sigma-Aldrich), were used to assess the NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and COD. Analysis was performed as per the manufacturer's instructions. Temperature and pH were analysed using a VWR pHenomenalTM MU 6100L meter and VWR 111 662-1157 pH probe. DO and BOD_{5day} were analysed using a Jenway 9500 Dissolved O_2 meter and probe. Suspended solids were analysed via filtration using 7 cm 0.45 µm filter paper. Hardness was assessed via titration using pH 10 buffer, Erichrome black and EDTA. Alkalinity was analysed by titration using phenolphthalein indicator, methyl orange indicator and hydrochloric acid. See Table 2 for a summary of all standard methods and their corresponding method numbers used in this research.

2.4. Ecotoxicity testing:

The freshwater unicellular green algae *P. subcapitata* (also known as *Raphidocelis subcapitata*), and the freshwater crustacean *Daphnia pulex* (*D. pulex*), were used in the ecotoxicity test.

A starter culture of the *P. subcapitata* was obtained from The Culture Collection of Algae and Protozoa (CCAP 278/4; SAMS Limited, Scottish Marine Institute, Oban, Argyll, Scotland, U.K.), and grown in standard Jarworski's medium under controlled conditions of 23^{o}C ± 2^{o}C exposed to continuous illumination (lux 6,000 – 10,000). Sub-culturing was conducted every two to three days to ensure the growth rate remained in the exponential phase. Ecotoxicity testing was conducted as per the Water quality – Fresh water algal growth inhibition test with unicellular green algae ISO (8692:2012), guidelines. The *P. subcapitata* was exposed to the intake and holding tank samples for 72 hours under static conditions at 23^{o}C ± 2^{o}C exposed to the continuous illumination. Inhibition of the algae growth rate, in percent, was calculated by comparing the samples to a negative control containing just the Jarworski's medium. Calculations were conducted as follows;

Equation1:

$$\textbf{Algal cells mL}^{-1} = \frac{n}{0.02}\ x\ 10^3$$

Where n = number of cells counted using a haemocytometer

Equation 2:

$$\textbf{Average specific growth rate } (\mu) \textbf{, } = \frac{\ln X_n - \ln X_o}{T_n - T_o}$$

Where ln = natural log of

X_n = Algae cells mL^{-1} at 72 hours

X_0 = Algae cells mL^{-1} at 0 hours

T_n = Duration of test

T_0 = Time zero

Equation 3:

$$\textbf{Percent inhibition in growth rate} = \frac{C\mu - T\mu}{C\mu}\ x\ 100$$

Where Cμ = Average specific growth rate for control

Tμ = Average specific growth rate for treatment

An in-house stock of the freshwater crustacean *D. pulex*, was maintained in aerated spring water. Sub-culturing was conducted twice a week to ensure the culture conditions of the crustacean remained at optimum levels. Ecotoxicity testing was conducted as per the Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute toxicity test ISO (6341:2012), guidelines. *D. pulex* were used over the more common *Daphnia magna* as the former species are more commonly found in Irish water systems. The *D. pulex* was exposed to the intake and holding tank samples for 24 hours under static conditions at 20^{o}C ± 2^{o}C exposed to a photoperiod of 16 hr bright and 8 hr dark. Immobilisation, in percent, was calculated by comparing the samples to a negative control containing just the aerated spring water. *D. pulex* was deemed immobile when no movement was observed after 15 seconds under gentle agitation. Calculations were conducted as follows;

$$\textbf{Percent immobilisation} = \left(1 - \frac{T}{C}\right) x\ 100$$

Where T = Total number of mobile *D. pulex* in the treatment

C = Total number of mobile *D. pulex* in the control

2.5. Statistical analysis:

All statistical analyses were conducted using GRAPHPAD PRISM 8 and MINITAB 18. The data generated were grouped and subject to normality tests (Anderson-Darling). T-tests and one-way ANOVA with Tukey were used to identify significant differences in the variables.

3. Results

3.1. Physicochemical parameters:

The results determined for the physicochemical parameters investigated on the trial fish farm intake and holding tank samples are displayed in Figure 2. Increases in concentrations from the intake to the holding tank were observed in NO_2^-, NO_3^-, PO_4^{3-}, BOD, COD and suspended solids. Similar concentrations between the samples were observed in DO, temperature, pH, hardness and alkalinity. The only parameter to display a decrease in concentration between the intake and holding tank was NH_4^+. When the intake and holding tank samples were compared via statistical analysis, a significant difference was observed in the NO_2^- ($p = <0.001$), NO_3^- ($p = 0.016$), and COD levels ($p = 0.011$), where $p < 0.05$ indicated a statistically significant difference.

3.2. Ecotoxicological bioassays:

The *P. subcapitata* algal bioassay ISO (8692:2012), was performed on intake and holding tank samples from the trial fish farm to determine whether the rate of growth was inhibited as a result of exposure to either sample. The levels of growth rate inhibition observed in the intake and holding tank samples can be found in Figure 3A. With the exception of the July sample, growth rate inhibition decreased from the intake to the holding tank water. Statistical analysis was conducted between the intake and holding tank samples and no significant differences were observed ($p = 0.402$).

The *D. pulex* crustacean bioassay ISO (6341:2012), was performed on intake and holding tank samples from the trial fish farm to determine whether immobilisation occurred as a result of exposure to either sample. The levels of immobility observed in the intake and holding tank water can be found in Figure 3B. Increased immobilisation was observed from the intake to the holding tank samples during May and July. Immobility remained unchanged in the month of June. August displayed a decrease. Statistical analysis was conducted between the intake and holding tank samples and no significant differences were observed ($p = 0.390$).

4. Discussion

In order to determine the potential effects aquaculture processes may have on Irish peatlands, intake and terminal holding tank water samples were compared to one another. Equally important was the potential effects the quality of the bog water may have on the fish themselves. Results from this study were therefore also compared to a range of studies previously conducted on a variety of fish farms that used different techniques (*e.g.* recirculating aquaculture systems, flow through systems, *etc.*), to culture a range of different species (*e.g.* shrimp, rainbow and brown trout, perch, catfish, salmon). In order to assess the quality of the water, recommended water quality parameters set out by the Statutory Instrument (S.I.), 77/2019 (European Communities Environmental Objectives – Surface Waters- Regulations 2019), and the Irish Environmental Protection Agency's (EPA), water quality parameters were used as a guidance (Environmental Protection Agency, 2001; Irish Stationery Office, 2009). This was done so as, as far as the authors are aware, water quality parameters for aquaculture holding tank are currently unavailable in Ireland as the Irish EPA are actively investigating the regulation of freshwater aquaculture discharge (O'Neill *et al.,* 2019). Discharge licensing is currently assessed on an individual basis. It should be noted that the guidance values used (S.I. 77/2019, Freshwater Fish Directive [78/659/EEC], and/or the Irish Surface Water Regulations [1989]), are based on freshwater systems and not peatland environments. These freshwater parameters were included as the bogs freshwater receiving system is the Daingean /Philipstown River, 3.8 Km away from the farm. Emission levels set out in the fish farm's current discharge licence were not applicable here as composite sampling over 24 hours could not be conducted due to limitations. Also, the system is quite new and full operation only commenced in April.

4.1. Physicochemical analysis:

Nitrification is the process by which NH_4^+ is firstly converted to NO_2^- and then to NO_3^- via enzymatic oxidation (Subba Rao *et al.,* 2017). The presence of NH_4^+ in freshwater systems is an indication of recent pollution (Zhang *et al.,* 2011). The NH_4^+ levels detected in the terminal holding tank water were lower than that of the intake water. Levels were also below the guidance value of 1 mg NH_4^+ L^{-1}. This suggested that the fish farms and its treatment processes were reducing the levels of NH_4^+ present in the bog water. NO_2^- is also an indicator of recent pollution in freshwater systems (O'Neill *et al*., 2019). Concentrations of NO_2^- detected in both sets of samples were above the Irish EPAs guidance values of 0.03 mg L^{-1}. The levels in the holding tank water were also significantly greater ($p < 0.001$), than the intake. However, two very important points that needs to be highlighted when interpreting these results; 1) grab samples not composite samples were collected and analysed and, 2) water in the holding tank is only discharged from the farm when overflow occurs as a result of very heavy rainfall. Therefore, the concentration being released would be considerable reduced as the rain water would dilute the levels present. Results suggested that, although the treatment processes had successfully transformed NH_4^+ to NO_2^-, it had not fully converted the nitrogenous waste to NO_3^-. However, the DO levels detected should have been high enough to avoid partial nitrification. The NO_2^- levels may have been due to the water not being present in the algae and duckweed treatment bed for long enough. Also, as this is a new process, the algae and duckweed pond was not yet working to its maximum capacity (Table 1). Additional research would need to be conducted to establish the exact cause of the increase as the lack of ammonium could indicate that a low retention time may be unlikely. NO_3^- levels detected in the holding tank were also significantly greater ($p = 0.016$), than that of the intake. However, all levels were well below the guidance value of 50 mg L^{-1}. The increases in the concentrations of these compounds were due to the rise in the feed applied as a result of increased stocking densities (Table 1). The release of these nitrogen compounds may assist in the restoration of the recovering bog as plants require nitrogen for growth and development (Xu *et al.,* 2012). However, as peatlands are normally nutrient poor, additional research would need to be conducted.

High levels of phosphorus in freshwater systems can result in algal blooms and hypoxic conditions (O'Neill *et al.,* 2019). However, phosphorus is also an essential element for plant growth (Vance *et al.*, 2003). PO_4^{3-} levels detected in both samples were greater than the guidance value of

0.035 mg PO_4^{3-} L^{-1}. This rise was due to the increased application of feed (Table 1). Once again this concentration would be greatly decreased when diluted by rain water. Concentrations in the holding tank were greater than that of the intake however the difference was not statistically significant ($p = 0.211$), suggesting that the introduction of aquaculture processes did not appear to have altered the quality of the water. Additional research may need to be conducted in order to determine its effects on the receiving natural wetland, as well as to develop improved treatment methods if required. It should also be noted that the algae and duckweed channels used for treatment were not working to their maximum capacity (Table 1).

Similar DO levels were observed in both samples. Concentrations were above the guidance oxygen levels of 7 mg L^{-1}. The use of the and inclusion of paddle wheels in the farm allows workers to maintain full control of oxygen levels within the farm ensuring optimum levels are sustained. Alam *et al*. (2007), da Silva *et al*. (2017), and O'Neill *et al*. (2019), have also suggested that concentrations of ≥ 4 mg L^{-1} are sufficient to maintain aquatic life, therefore current DO levels were not considered to be any cause for concern. Similar BOD levels were observed in both samples. With the exception of one spike observed in June, BOD levels were below the guidance values of 6 mg L^{-1}. As the levels detected were below the guidance values, and the water quality remained unchanged, no issues were foreseen. COD levels detected in the holding tank were significantly greater ($p = 0.011$), than that of the intake. Holding tank sample levels were also just above the guidance value of 40 mg L^{-1}. This may have highlighted a potential issue. However, as previously mentioned, this level of COD would not be released as dilution would occur after the heavy levels of rainfall that would result in its release.

Suspended solids can cause increased gill irritation. Therefore, the guidance concentration of 25 mg L^{-1} set out by the Freshwater Fish Directive [78/659/EEC], was deemed a more suitable maximum allowable concentration (MAC), than the 50 mg L^{-1} indicated in the Irish Surface Water Regulations [1989]. With the exception of one spike observed in May which may have resulted after recent rainfall, suspended solids were similar in both the samples, and levels remained below the guidance concentration.

Water temperatures remained consistent in both samples, as too did the pH values. Water may not be released from facilities if temperatures above 20^{o}C are observed, and the temperature of the samples may have risen during its transportation to the laboratory. Therefore, there were no issues with the temperature. The pH values were well within the recommended value of between pH 6 and pH 9.

Both alkalinity and hardness were measured as $CaCO_3$. Results for hardness suggested that water was slightly too moderately hard. This correlates with water hardness maps of Ireland which indicated that water around Ballycon, Co Offaly was slight too moderately hard.

Some of the results observed (NO_2^-, NO_3^-, PO_4^{3-} and COD), suggested potential issues. No research conducted on the use of peatlands for aquaculture could be found in the available literature. Additionally, this research only focused on one fish farm in a peatland setting. As a results all physicochemical results were then compared to a range of studies conducted on traditional fish farms (Table 3). All results obtained were similar to or below the concentrations determined in the previous aquaculture studies investigated, including NO_2^-, NO_3^-, PO_4^{3-} and COD (Boaventura *et al.*, 1997; Camargo, 1994; Caramel *et al*., 2014; Costanzo *et al.*, 2004; da Silva *et al.*, 2017; Guilpart et al., 2012; Lalonde *et al*., 2014; Moreira *et al.*, 2010; Namin *et al*., 2013; Noroozrajabi *et al.*, 2013; O'Neill *et al.*, 2019; Pulatsü *et al.*, 2004; Stephens and Farris, 2004a; Ziemann *et al.*, 1992; Živić *et al.*, 2009). This indicated that there appeared to be no observable differences between the water quality after traditional aquaculture settings, and that of bog-land settings.

4.2. Ecotoxicological analysis:

Low levels of inhibition in the growth rate of the *P. subcapitata* were observed in both the intake and terminal holding tank water. This suggested that the quality of water in the intake samples and the holding tank water, in terms of its effect on the algae, remained unchanged and that the water within the bog was unlike to cause growth inhibition, therefore the recovery of the bog would not be affected. Most of the previous research using *P. subcapitata* in assessing water were based on polluted river systems and drainage water (Guéguen *et al*., 2004; Ivanova and Groudeva, 2006). These studies displayed higher growth rate inhibition levels than those observed in this study. Only two previous studies could be found in the available literature that focused on the use of the algae in the context of aquaculture. The research conducted by O'Neill *et al.* (2019), found considerably higher inhibition levels than those reported in this study. Miashiro *et al.* (2012), and O'Neill *et al.* (2019), also reported stimulation of growth in the algae which could results in eutrophic conditions. As no stimulation of the algae was observed in this study, issues with eutrophic would seem highly unlikely.

Low levels of immobilisation in the *D. pulex* were observed in both samples. This suggested that the water quality seemed unlikely to cause any adverse effects on any of the aquatic organisms present in the bog. However, no studies in the available research focused on the use of *D. pulex* to assess aquaculture discharge, therefore additional research may need to be conducted.

5. Conclusion

No differences were observed between the physicochemical parameters of the intake and holding tank samples, with the exception of NO_2^-, NO_3^- and COD, indicating that aquaculture processes did not adversely alter the quality of the water. The sub-lethal levels of toxicity observed in the ecotoxicological bioassays have also demonstrated that the introduction of aquaculture processes into peatlands may be unlikely to cause any adverse effects on the ecosystem and organisms within it. This suggested that the use of cutaway bog as future locations for integrated multitrophic aquaculture (IMTA) processes holds promising potential. The use of bogs may aid in achieving the aims set on in the Food Wise 2025 initiative to increase Irish aquaculture production to 81,700 by 2023, assisting in the enhancing freshwater aquaculture processes. This constitutes the first study to report on sustainable development of new fish farms in cutaway bogs that were powered by wind turbine and used algae and duckweed as natural processes for treating water adopting organic principles. IMTA may also assist in providing an economically sound alternative for sustainable land use for stakeholders once all peat harvesting processes cease. However, while results so far have indicated positivity, additional research needs to be conducted in order to fully appreciate all parameters governing large scale up of this peatland aquaculture process to ensure commercially viability that includes fine tuning hydronamics, microbial ecology and probiotic benefits of algal, bacterial and duckweed interactions.

Conflicts of Interest

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

Acknowledgements

The authors would like to thank Athlone Institute of Technology (President Seed Fund), Bord Iascaigh Mhara (co-funded by the Irish Government and European Union), also under the EcoAqua and CENAKVA projects for providing financial support for this research. The authors also acknowledge Bord Iascaigh Mhara for furnishing aerial image of AquaMona farm used in graphic abstract, and to Bord no Móna for providing access to same farm in order to conduct this research.

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Highlights

- Peatlands (bog) offer a new sustainable approach for integrated multitrophic aquaculture using organic principles
- Trial farm within cutaway bog showed favourable intake and holding tank water quality
- Use of algae and duckweed show promise as natural means of treating rearing water
- Ecotoxicological analysis revealed no differences between intake and holding tank water.
- Water quality findings were similar to previously reported traditional aquaculture processes.

Table 1: Summary of production characteristics on AQUAMÓNA fish farm from May to August 2019.

Month	Actual Biomass (Kg)	Biomass Gain (Kg)	Feed Applied (Kg)	FCR	N Applied	P Applied	Duckweed Area (m^2)	Duckweed Area (%)
May	6282	2128	2288	1.08	148	41	182	1.67
June	8410	2373	3144	1.33	206	57	662	6.07
July	10783	783	3083	3.94	207	58	2999	27.49
August	11565	1643	3146	1.91	213	59	4982	45.66

FCR = Feed Conversion Ratio, N Applied = Protein % in feed, P Applied = Phosphorus % in feed.

Table 2: Summary of methods used for the analysis of water quality in the trial fish farm intake and holding tank water samples. The standard water and wastewater analysis methods and their corresponding number are listed. Detection limits for all kits have been included.

Parameter	**Standard Method & Number**	**Detection Limit (mg L^{-1})**
Alkalinity	Titrimetric (2320-B)	-
BOD	Membrane Electrode (5210-B)	-
COD	Photometric (5220-D)	0-150 15-300
DO	Membrane Electrode (4500-O G)	-
Hardness	Titrimetric (2340-C)	-
NH_4^+	Photometric (4500-NH_3-F)	0.013-3.86 2.6-193.0
NO_2^-	Photometric (345-1)	0.007-3.28
NO_3^-	Photometric (4500-NO_3)	0.4-110.7
pH	Membrane Electrode (2310-B)	-
PO_4^{3-}	Photometric (4500-P-C)	0.007-15.3 1.5-92.0
Suspended Solids	Gravimetric (2540-D)	-
Temperature	Thermometer (2550-B)	-

Table 3: Summary of previous studies conducted on aquaculture discharge. Location, culture species and physicochemical parameters used have been listed. Many studies included additional parameters however, for the purpose of this paper, only the same physicochemical parameters investigated in this study have been included.

Reference	Culture Species	Location	Physicochemical Parameters
Boaventura *et al.* (1997)	Rainbow Trout (*Oncorhynchus mykiss*)	Portugal	DO, BOD, A, NH_4-N, PO_4-P, SS, pH, H, NO_3-N, NO_2-N, T
Camargo (1994)	Rainbow Trout (*Oncorhynchus mykiss*) & Brown Trout (*Salmo trutta*)	Spain	H, pH, DO, T, NO_3^-
Cao *et al.* (2007)	Range of Marine & Freshwater Aquaculture	China	NH_4-H, COD, BOD, SS
Caramel *et al*. (2014)	Rainbow Trout (*Oncorhynchus mykiss*)	Brazil	PO_4-P, NH_4-N, NO_2-N, NO_3-N, pH, SS, DO, T
Costanzo *et al.* (2004)	Banana Shrimp (*Penaeus merguiensis*)	Australia	T, pH, SS, NH_4^+, NO_3^-
da Silva *et al.* (2017)	Shrimp (*Litopenaeus vannamei*)	Brazil	pH, DO, SS, NO_2^-, NO_3^-, PO_4^{3-}, COD
Fadaeifard *et al.* (2011)	Rainbow Trout (*Oncorhynchus mykiss*)	Iran	DO, BOD, PO_4-P, NO_3^-, NO_2^-, H, pH, SS
Guilpart *et al.* (2012)	Rainbow Trout (*Oncorhynchus mykiss*)	France	NH_4^+, NO_3^-, PO_4^{3-}, SS
Lalonde *et al.* (2014)	Atlantic Salmon (*Salmo salar*)	Canada	SS, NO_3^-, pH, A, H, PO_4^{3-}, BOD
Miashiro *et al.* (2012)	Nile Tilapia (*Oreochromis niloticus*)	Brazil	NH_4-N, PO_4^{3-}, pH, DO, T

Moreira *et al.* (2010)	Freshwater Prawn (*Macrobrachium rosenbergii*)	Brazil	T, pH, NH_4^+, NO_2^-, NO_3^-, DO, BOD, H
Namin *et al.* (2013)	Rainbow Trout (*Oncorhynchus mykiss*)	Iran	DO, pH, SS, T
Noroozrajabi *et al.* (2013)	Rainbow Trout (*Oncorhynchus mykiss*)	Iran	T, pH, DO, NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}
O'Neill *et al.* (2019)	European Perch (*Perca fluviatilis*)	Ireland	T, pH, DO, BOD, COD, NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, A, H, SS
Pulatsü *et al.* (2004)	Rainbow Trout (*Oncorhynchus mykiss*)	Turkey	T, DO, pH, BOD, SS, NO_2^-, NO_3^-
Stephens and Farris (2004a)	Channel Catfish (*Ictalurus punctatus*)	United States of America	pH, DO, T, A, H, SS, PO_4^{3-}, NO_2^-, NO_3^-, NH_4-N
Stephens and Farris (2004b)	Channel Catfish (*Ictalurus punctatus*)	United States of America	NO_3^-, NO_2^-, SS, PO_4^{3-},
Tahar *et al.* (2018)	Rainbow Trout (*Oncorhynchus mykiss*)	Ireland	BOD, NO_2-N, PO_4-P, SS, NH_4-N, T, DO, pH
Ziemann *et al.* (1992)	Range of Marine & Freshwater Fish & Shrimp	Hawaii	PO_4^{3-}, SS, NH_4-N
Živić *et al.* (2009)	Rainbow Trout (*Oncorhynchus mykiss*)	Serbia	PO_4^{3-}, NO_2^-, NO_3^-, DO, T, pH, NH_4^+

T = temperature, DO = dissolved oxygen, BOD = biochemical oxygen demand, COD = chemical oxygen demand, NH_4-N / NH_4^+ = ammonium, NO_2-N / NO_2^- = nitrite, NO_3-N / NO_3^- = nitrate, PO_4-P / PO_4^{3-} = orthophosphate, A = alkalinity, H = hardness, SS = suspended solids.

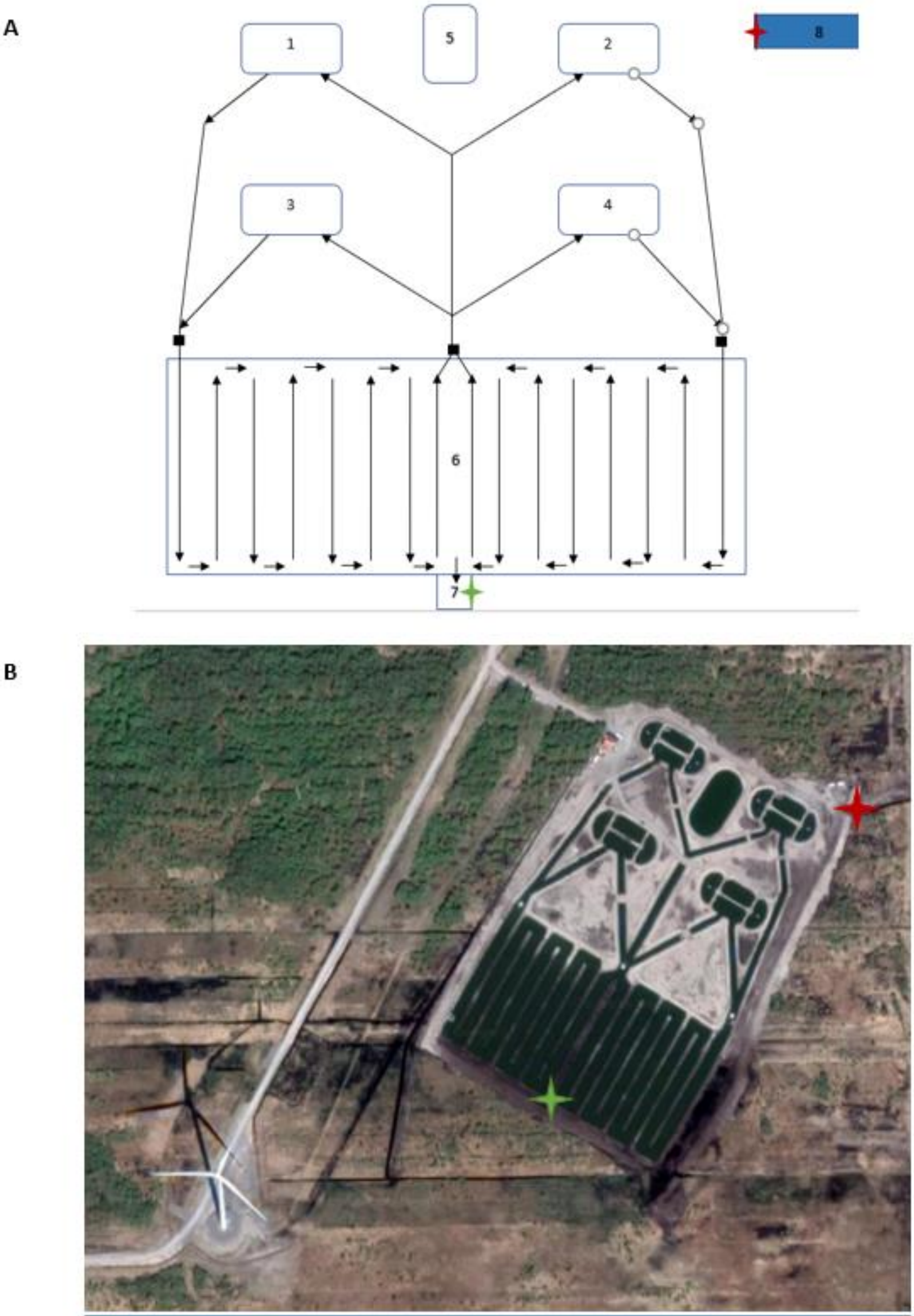

Figure 1: **A)** Schematic of trial fish farm, located in Ballycon, Co. Offaly. 1, 2, 3 & 4) Split Ponds for culturing, 5) Water Reservoir, 6) Algae & Duckweed Treatment Channels, 7) Holding Tank, 8) Bog River (intake source). Black lines indicate the direction of the flow of water. Black boxes indicate the locations of the paddle wheels. *NOTE: Schematic is not to scale.* **B)** Aerial view of the trial fish farm within the peatlands. The wind turbine used to provide all electrical needs for the farm is included. The D-ends of each split pond can be seen. The red stars indicate the location of the intake sampling point. The green stars indicate the location of the holding tank sampling point.

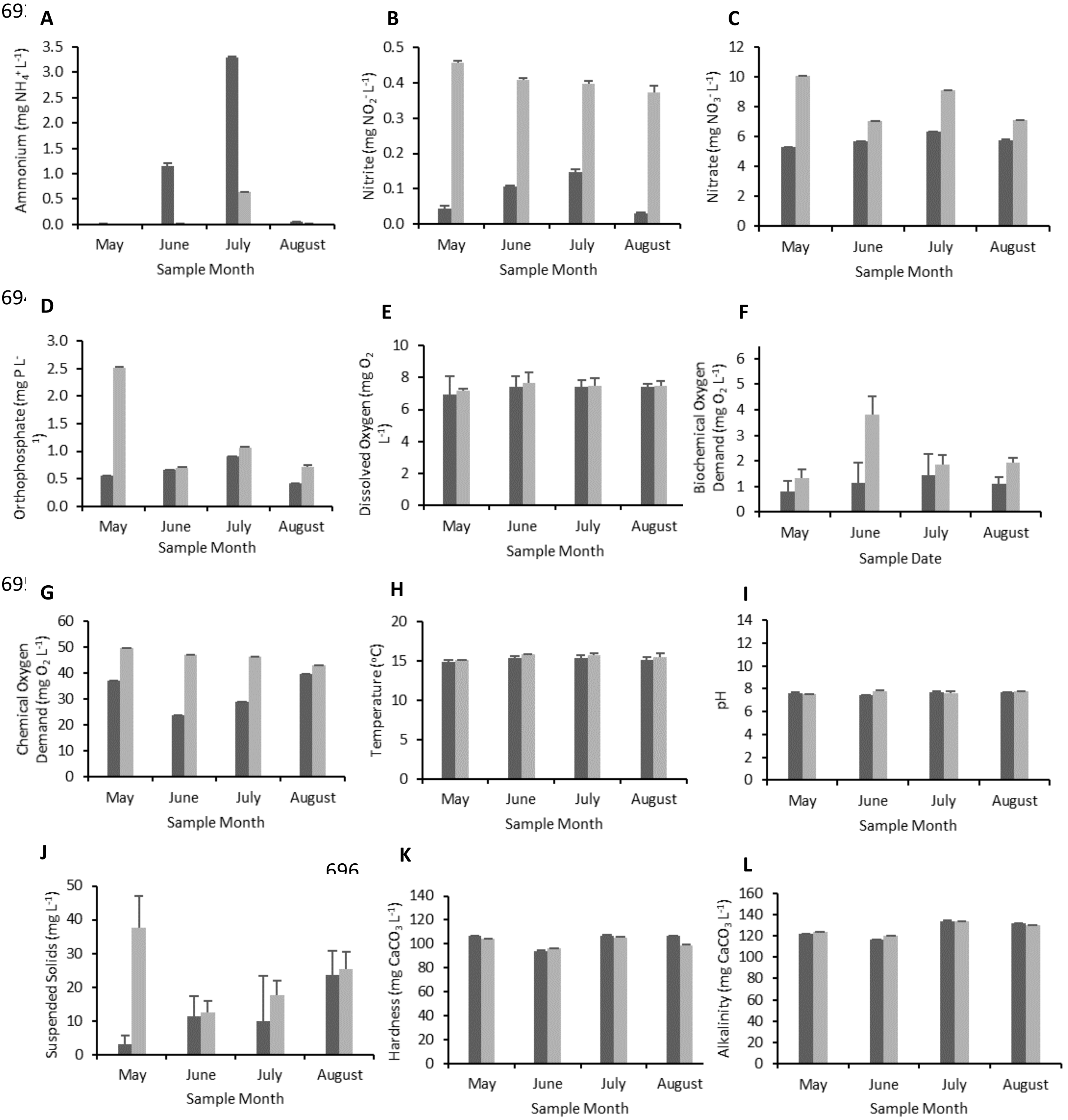

Figure 2: Bar charts indicating the physicochemical results determined for the trial fish farm intake samples (dark grey), and holding tank samples (light grey), from May 2019 to August 2019. Parameters investigated were A) NH_4^+, B) NO_2^-, C) NO_3^-, D) PO_4^{3-}, E) dissolved oxygen, F) Biochemical Oxygen Demand, G) Chemical Oxygen Demand, H) temperature, I) pH, J) suspended solids, K) hardness and L) alkalinity. S.D. indicated, n = 3.

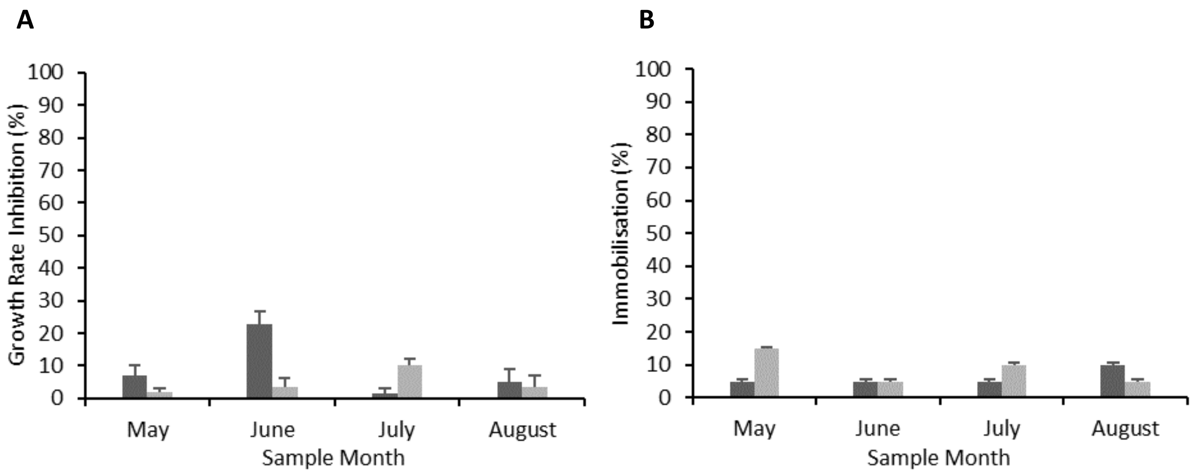

Figure 3: Bar charts displaying the results determined from the A) *Pseudokirchneriella subcapitata* bioassay and B) *Daphnia pulex* bioassay investigated on the trial fish farm intake samples (dark grey), and holding tanks samples (light grey), from May 2019 to August 2019. S.D. indicated, n = 3.