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Emer A. O'Neill, Neil J. Rowan

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# Microalgae as a natural ecological bioindicator for the simple real-time monitoring of aquaculture wastewater quality including provision for assessing impact of extremes in climate variance – A comparative case study from the Republic of Ireland.

Emer A. O'Neill[1,2]*, Neil J. Rowan[1,2]

[1]Bioscience Research Institute, Athlone Institute of Technology, Dublin Road, Athlone, Co. Westmeath, Ireland.

[2]Empower Eco Sustainability Hub, Boora, Co. Offaly, Ireland.

*Corresponding author: e.oneill@research.ait.ie

**Abstract**

Aquaculture is one of the fastest growing food producing industries globally, providing ~50% of fish for human consumption. However, the rapid growth of aquaculture presents a range of challenges including balancing environmental impact that can be influenced by variations in climatic conditions. Monitoring of physicochemical parameters is traditionally used to evaluate aquaculture output quality; however, this approach does not indicate the cumulative ecotoxicological effects on receiving waters. Specifically, this case study investigated the relationship between measuring traditional physicochemical parameters and the health of the alga *Pseudokirchneriella subcapitata* in order to evaluate the potential ecotoxicological effects of freshwater aquaculture on the receiving aquatic ecosystem in the Irish midlands. This constituted the first 2-year longitudinal study conducted in 2018 and 2019 that reports on the efficacy of using algae as a natural bioindicator to monitor and assess freshwater aquaculture wastewater from a traditional flow-through fish farm producing Eurasian Perch (*Perca fluviatilis*); monitoring was compared over a same six-month period in the same location each year. Findings demonstrated significant differences between the two monitoring periods when using *P. subcapitata* for assessing the quality of aquaculture intake (P=0.030) and output (P=0.039). No stimulatory effects were observed during 2019 unlike >50% rates experienced the previous year. These observations coincided with changes in climatic conditions whereby the 2018 period experienced extended levels of drought; whereas non-drought conditions were observed during 2019. Findings suggest that reliance upon traditional monitoring techniques may not provide sufficient robustness or versatility to address emerging issues, such as extremes in climate variance, which may influence the future intensive sustainability of freshwater aquaculture. This research supports the complementary use of *P. subcapitata* as a rapid and simple early-warning bioindicator for measuring aquaculture output quality on receiving aquatic ecosystems.

**Keywords**

Algae*;* bioindicator; ecotoxicology; climate change; freshwater aquaculture; sustainability.

## 1. Introduction

The depletion of wild capture fishery practices has resulted in the rapid development of aquaculture (Han et al., 2019) making it the fastest growing food producing industry worldwide (Ottinger et al., 2016; O'Neill et al., 2019, 2020). According to the FAO (2018), aquaculture now accounts for ~50% of fish produced for human consumption; this figure is expected to rise to ~62% by 2030 (Fredricks et al., 2015; Liu et al., 2017). The dramatic increase in aquaculture production is attributed to over exploitation of wild fisheries that are now at their maximum sustainable yields, along with increased consumer demand for fish (Tahar et al., 2018a, 2018b). Farmed fish is rich in protein and is also a more efficient protein utilisation and feed conversion source than other animals destined for protein production (Tschirner and Kloas, 2017). However, despite its numerous advantages, the rapid increase in aquaculture production has resulted in the emergence of several issues within the industry that include limitations in water and space, increased incidences of disease and increased environmental concerns (Ngo et al., 2016; Troell et al., 2017; Han et al., 2019; O'Neill et al., 2019). Stenevik and Sundby (2007) have also indicated that variations in climatic conditions have demonstrated substantial effects on increases as well as decreases in stocking densities; therefore, the success of fish stock assessment depends to a large extent on the ability to predict impacts climate change has on the dynamics of aquatic ecosystems. These treats have hindered the sustainable development and expansion of the industry (Han et al., 2019).

The ecological importance of algae have received consideration in studies focusing on natural approaches to wastewater remediation in freshwater aquaculture (Naughton et al., 2020) including potential influence of climate variance on process performance (O'Neill et al., 2019). Previous researchers have also noted the potential of algal communities to exhibit many attributes as biological indicators of spatial and temporal environmental change (Omar, 2010); additionally, microalgae have been reported as potentially useful monitoring quality of water bodies (Zahgloul, 2020; Parus and Karbowska, 2020; Kadam et al., 2020; Tsanenko et al. 2021). Parus and Karbowska (2020) recently reported on the possibility of using the algae *Ulva* and *Cystoseira* as natural indicators of environmental cleanliness given that these species where shown to accumulate metals. Parmar and Rawtani (2016) described several potential advantages for use of bioindicators, namely (1) biological impacts can be determined; (2) potential synergetic and antagonistic impacts of various combined pollutants on ecosystems can be exhibited; (3) early stage diagnosis of putative harmful effects of toxins on human and animal health can be monitored; and (4) can be considered as a potentially viable economic alternative to use of conventional sophisticated methods.

According to Rindi (2014), terrestrial algae (green algae and diatoms) are more directly affected by climate change and can therefore respond in a more immediate way. This is attributed in part to the fact that algae have short generations, fast turnovers and respond quickly to changes in environmental conditions. Sarmaja-Korjonen *et al.* (2006) demonstrated that algae appeared to be comparatively good indicators of environmental conditions by representing productivity disparities during changing climatic conditions.

Hallegraeff (2010) has also indicated that changes in algal communities can putatively provide a sensitive early warning for climate-driven uncertainties in aquatic ecosystems. There has been increased interest in alternative uses for microalgae within aquaculture to assist with sustainability, in addition to enabling ecotoxicological assessment and water quality control (Han et al., 2019; O’Neill et al., 2019). According to Han et al. (2019), microalgae can also be utilised in aquaculture for wastewater assimilation, oxygen production and partial feed replacement. The microalgae *Pseudokirchneriella subcapitata* (*P. subcapitata*) has previously been suggested as a potential early warning indicator for altering issues associated with in aquaculture processing due to environmental variances, including climate change (O’Neill et al., 2019).

Fish farm wastewater is traditionally high in nutrient rich products (Ngo et al., 2016; Sikder et al., 2016). Nitrogen, phosphorus and organic matter are characteristic of this nutrient rich waste which is normally as a result of metabolic waste products and left over food (Jegatheesan et al., 2011; O’Neill et al., 2019). If this is released into a water body untreated, water pollution will develop leading to issues that may include eutrophication in that aquatic system (Martinez-Porchas et al., 2014). Eutrophication occurs when a water body is put under pressure with large levels of organic matter and nutrient waste that is taken in and biologically processed which in turn leads to algal blooms (Jegatheesan et al., 2011; Martinez-Porchas et al., 2014; Sikder et al., 2016). Algal blooms in turn can lead to decreases in light and oxygen production, which can suffocate aquatic life (Jegatheesan et al., 2011; Chislock et al., 2013; O’Neill et al., 2019). Organic matter and nutrient waste is typically as a result of the application of artificial feed supplementation which is necessary in order to increase and maintain yields to meet the increased demands (Kolarevic et al., 2014; Feucht and Zander, 2015; O’Neill et al., 2019).

Water quality is typically assessed to determine the potential effects it may have in its receiving system; this is traditionally conducted by means of physicochemical analysis (da Silva et al., 2017). The use of these parameters alone will only provide a limited window in time of the water quality for a system (O'Neill et al., 2019, O'Neill et al 2020). Inclusion of bioassays to assess the potential effects on aquatic ecosystems and the organisms therein will provide a broader scope on the quality of water. Microalgae are primary producers and are keystones in aquatic food chains. They represent an imperative group of highly sensitive photosynthetic organisms frequently used to assess aquatic systems (Rodgher et al., 2012). *Pseudokirchneriella subcapitata* (*P. subcapitata*) is unicellular green algae most commonly used and recommended for ecotoxicological assessment due to its being inexpensive, and both highly reliable and reproducible (ISO, 2012).

The hypothesis of this study is that algal traditionally used in ecotoxicology bioassays can be further utilised for the real-time sustainable enhancement of aquaculture as it provides a potential means of monitoring the influence of adverse environmental effects caused by extreme weather events attributed to variances in climate. Thus, the aim of this research is to determine the robustness of *P. subcapitata* as a putative early warning bio-indicator for monitoring impact of climate variance using an Irish freshwater aquaculture farm as a case study.

## 2. Materials & Methods

### 2.1. *Sampling*

Intake and output water samples were collected from a freshwater fish farm located in Boyle, Co. Sligo (Figure 1). The farm cultures European perch (*Perca fluviatilis*) and consists of three culture ponds that use a flow through system, a hatchery and nursery that use a recirculating aquaculture system (RAS) and a constructed wetland that is used for culture water treatment. Grab samples were collected in 5 L octagonal carboy HDPE bottles (Lennox) and transported directly to the lab via car approximately 70 km away. Samples were collected directly from the intake and output sources once a month from March 2019 to August 2019 as this was the time period analysed during the previous study at same freshwater aquaculture farm reported in O'Neill et al. 2019. Samples from the settlement pond were also collected for analysis in order to determine the treatment efficacy of the constructed wetland, which was was not fully operational until June 2019 reflecting period of maintenance. Wastewater collection occurred on the same day, at approximately the same time, during each month of monitoring where sampling points are displayed in Figure 2. Triplicate samples were analysed from the same 5 L grab sample.

### *2.2. Physicochemical analysis*

The Statutory Instrument (S.I.) 77/2019, S.I. 272/2009, and the Irish Environmental Protection Agency's (EPA) water quality parameters (Environmental Protection Agency, 2001; Irish Stationery Office, 2009; Irish Stationery Office, 2019) were followed to measure water quality parameters. Discharge licensing in Ireland is currently based on an individual basis. Grab samples collected represented 30 min of the 24 h period; composite sampling was not possible. To compensate for the latter, results complied in this study were also compared to previous research studies conducted on a range of aquaculture facilities (Table 7 in the supplementary data).

Physicochemical parameters – temperature, pH, $NH_4^+$, $NO_2^-$, $NO_3^-$, $PO_4^{3-}$, DO, BOD, COD, suspended solids, hardness and alkalinity were analysed within 24 h of collection to remove the need for preservation. Spectroquant® kits (Sigma Aldrich) were used as per the manufacturer's instructions to assess $NH_4^+$, $NO_2^-$, $NO_3^-$, $PO_4^{3-}$ and COD levels. Temperature and pH were analysed using the VWR pHenomenal$^{TM}$ MU 6100 L meter and VWR 111662-1157 pH probe. DO and $BOD_{5day}$ were assessed using the Jenway 9500 $DO_2$ meter and probe. The suspended solids were analysed by filtration using a Buchner flask and funnel. Alkalinity was assessed via titration using phenolphthalein indicator, methyl orange indicator and hydrochloric acid. Hardness was analysed via titration using pH 10 buffer, erichrome black and EDTA. A summary of all physicochemical methods employed in this study, including each standard method number, are shown in Table 1.

### *2.3. Ecotoxicity analysis*

The unicellular freshwater green algae *P. subcapitata* was used to determine the quality of the water. A culture 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 culture medium at 23$^{o}$C ± 2°C exposed to continuous illumination (lux 6,000-10,000). Additionally, starter cultures of *Asterionella formosa* (CCAP 1005/9) and *Monoraphidium contortum* (CCAP 245/2) were obtained from The Culture Collection of Algae and Protozoa (SAMS Limited, Scottish Marine Institute, Oban, Argyll, Scotland). *P. subcapitata* was compared with *A. formosa* and *M. contortum* to ensure that *P. subcapitata* was representative of Irish aquatic algae (Table 2). Algae were sub-cultured every three days to ensure the growth rate remained in the exponential phase. Analysis 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 output samples for 72 h at 23$^{o}$C ± 2$^{o}$C exposed to continuous illumination under static conditions. The percent of algal growth rate inhibition ($E_rC_{50}$) was calculated by comparing samples to a negative control containing just the Jarworski's culture medium. The $E_rC_{50}$ is the concentration at which the has been a 50% reduction in the growth rate relative to the control within 72h (ISO, 2012). Equations 1, 2 and 3 were taken directly from the ISO (8692:2012) guidelines (ISO, 2012) and calculations were conducted as follows;

*Equation 1*

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

where

n = the number of cells counted using a haemocytometer

*Equation 2*

$$\text{Average specific growth rate } (\mu) = \frac{lnX_n - lnX_0}{T_n - T_0}$$

where

ln = natural log of

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

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

$T_n$ = Duration of test

$T_0$ = Time zero

*Equation 3*

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

where

Cμ = Average specific growth rate for control

Tμ = Average specific growth rate for treatment

### 2.4. Statistical analysis

Statistical analyses were conducted using MINITAB 18 and GRAPHPAD PRISM 8. The generated data were grouped and subjected to normality testing (Anderson-Darling) to ensure all samples were normally distributed. Unpaired *t*-tests and ANOVA were used to identify any significant differences in the variables. $P < 0.05$ indicated a statistically significant difference. Pearson's correlation (*r*) was used to assess if any correlations between the algae and/or the physicochemical parameters existed.

## 3. Results

### 3.1. Physicochemical analysis

Results determined for the physicochemical parameters investigated in this study on Irish freshwater fish farm intake, output and settlement pond water samples are displayed in Figure

4. Increases in $NH_4^+$, $NO_2^-$, $PO_4^{3-}$, BOD and temperature, along with decreases in DO, COD, pH and alkalinity occurred when comparing the intake and output water from the fish farm. Fluctuations from month-to-month in $NO_3^-$ and hardness were also observed. With the exception of $NO_2^-$ ($P = 0.011$) and pH ($P = 0.025$), no statistically significant (one-way ANOVA) differences were indicated. With the exception of $NH_4^+$ and $NO_2^-$ levels in May and June, decreases were observed in the physicochemical parameters between the settlement pond and output water. With the exception of suspended solids ($P = 0.044$), no statistically significant (one-way ANOVA) differences were observed; one-way ANOVA was conducted across all three sampling points and no statistically significant differences were observed. Two-way ANOVA was conducted in order to take the sampling month into consideration; with the exception of BOD ($P = 0.083$) and suspended solids ($P = 0.150$), a statistically significant difference was observed in all parameters.

### *3.2. Algal Bioassay Analysis*

The percentage growth rate inhibition observed in the intake, output and settlement pond water are displayed in figure 5. With the exception of samples for May and July, a decrease in growth rate inhibition between the intake and output samples was demonstrated. A decrease was also observed in all samples between the settlement pond and output water. No statistically significant (one-way ANOVA) differences were observed for either set of samples. One-way ANOVA was conducted across the three sampling points and no statistically significant differences were indicated. Two-way ANOVA was conducted to determine whether any statistically significant differences were observed when the sampling month was taken into consideration. A statistically significant difference ($P = 0.001$) was indicated when the sampling month was included.

### *3.3. Comparative study*

Table 3 summarises an average of all results obtained during a previous study conducted at a similar time of year on the same fish farm during times of extreme weather conditions (heat wave and drought) by O'Neill et al. (2019) and those determined in this study which were conducted under normal weather conditions for the Republic of Ireland. With the exception of the dissolved oxygen, pH, alkalinity and hardness, all physicochemical concentrations decreased during the similar time periods of 2018 and 2019 in both the intake and output water samples. The pH, alkalinity and hardness remained similar whilst dissolved oxygen levels increased. For the *P. subcapitata*, considerable decreases in inhibition toxicity were observed in the intake water and no stimulation was observed in the output water from this study compared to 2018. Both of which demonstrated a statistically significant (*t*-tests) difference ($P$ = 0.030 for the intake water and $P$ = 0.039 for the output water).

### 3.4. *Correlation studies for monitoring periods of freshwater aquaculture farm*

Correlation studies were conducted between all parameters investigated at the three sampling points. A positive correlation between two parameters indicates that as one parameter increases or decreases, so too does the other parameter. A negative correlation between two parameters indicates that as one parameter increases or decreases, the opposite occurs with the other parameter *i.e.*, an inverse relationship. All results for the intake, output and settlement pond water samples are displayed in Tables 3, 4, 5, and 6 respectively. In the intake samples a negative correlation was observed between *P. subcapitata* and temperature as well as pH. A negative correlation was indicated between $NH_4^+$ and $NO_2^-$. A positive correlation was identified between $NO_2^-$ and alkalinity. The $NO_3^-$ demonstrated a negative correlation with DO and a positive correlation with suspended solids. A positive correlation was observed between $PO_4^{3-}$ and alkalinity. A negative correlation was identified between DO and suspended solids. In the output samples a positive correlation between temperature and $NO_3^-$ was indicated. A negative correlation was identified between DO and $NH_4^+$ as well as $NO_2^-$. Hardness displayed a positive correlation between $PO_4^{3-}$ and alkalinity. In the settlement pond a positive correlation between *P. subcapitata* and $PO_4^{3-}$ was observed. Temperature demonstrated a positive correlation with pH, $NH_4^+$, hardness and alkalinity. The pH indicated a positive correlation with $NH_4^+$, BOD hardness and alkalinity. A positive correlation was identified between $NH_4^+$ and DO as well as BOD. A positive correlation was observed between $NO_2^-$ and $NO_3^-$. DO demonstrated positive correlations with $PO_4^{3-}$ and BOD. Finally, a positive correlation was identified between hardness and alkalinity.

### *3.5. Weather conditions influencing water quality on monitored aquaculture farm*

Due to observations determined in the previous study, conducted by same authors (O'Neill et al. 2019), as a result of dramatic weather conditions experienced during 2018 in the Republic of Ireland that coincided with occurrence of the hottest summer recorded to date by Irish Meteorological Office (Met Eireann) resulting in a nationwide hosepipe ban due to limit negative impacts of drought; weather conditions during the sampling period have been included. For continuity, mean temperature and rainfall data collected by Met Eireann at the same three weather stations surrounding the fish farm as the previous study have been included for this sample period (Met Éireann, 2019). Stations were situated at Markree Co. Sligo, Knock Co. Mayo and Mount Dillon Co. Roscommon, as shown in Figure 1. Increases in the mean rainfall (Figure 5a) and decreases in the mean temperature (Figure 5b) were observed for 2019 versus the same time period in 2018 across the three stations. Maxima temperatures had also decreased (Figure 6 – supplementary). Statistical analysis found that the relationships between the algae and the rainfall and temperature switched. A moderately strong inverse relationship ($r$ = -0.559) between the algae and temperature, and a weak inverse relationship ($r$ = -0.209) between the algae and rainfall now existed.

## 4. Discussion

### *4.1. Physicochemical evaluation*

In order to ascertain whether the processes conducted in the fish farm altered the quality of the water, the physicochemical results determined in the intake and output water were compared firstly to one another and then to the previous study conducted. It should be appreciated that the dilution factor of the receiving river on potential impact of aquaculture effluent has not been considered in this research. The presence of $NH_4^+$, $NO_2^-$ and $NO_3^-$ in the output water suggested that the nitrification process (enzymatic oxidation of $NH_4^+$ to $NO_3^-$ by way of $NO_2^-$) was occurring. Increases in these parameters between the intake and output samples suggested that, for the most part, their production was due to practices within the farm. These were most likely due to the presence of fish waste and uneaten artificial pelleted feed used in the cultural process. Increases above guidance levels (1 mg $NH_4^+$ $L^{-1}$ and 0.03 mg $NO_2^-$ $L^{-1}$) in the parameters were only observed between May and June, which suggested a potential cause for concern. However, this was most likely attributed to the constructed wetland, which was not functioning to its optimal capacity due to undergoing maintenance works: no discharge of aquaculture effluent was released during these times. Levels of monitored physicochemical parameters dropped below guidance values, once the wetland was fully functional where low levels were also observed in the intake water. It is likely that agricultural processes (cattle and sheep farming) and forestry processes (tree felling) occurring upstream of the fish farm contributed to these measured physicochemical parameters.

The $PO_4^{3-}$ levels in the output water was greater than that of the input as a result of the processes within the aquaculture farm. However, levels observed in the intake suggested that agricultural and forestry processes upstream of the farm could have also contributed to levels. Concentrations of $PO_4^{3-}$ were greater than guidance levels (0.35 mg $PO_4^{3-}$ $L^{-1}$) suggesting a potential cause for concern as excess levels can result in the promotion of algal blooms leading to potential hypoxic conditions in the water body (O’Neill et al., 2019). However, once maintenance was completed on the constructed wetland, levels detected in the output water were reduced to guidance levels indicating no foreseen issues.

A decrease in oxygen levels was observed between the intake and output water due to the aquaculture process. This decrease may also have been due to changes in seasonality. According to Alam et al. (2007), da Silva et al. (2017) and O'Neill et al. (2019) oxygen levels $\geq 4$ mg $O_2$ $L^{-1}$ are sufficient to maintain aquatic life. Although levels in the output water dropped just below the guidance value (7 mg $O_2$ $L^{-1}$ for cyprinid waters), levels remained above the critical 4 mg $O_2$ $L^{-1}$ level and as a result had indicated no cause for concern. BOD levels between the intake and output water fluctuated *i.e.*, BOD increased between the intake and output water during March, June and July, whilst decreases were observed in April, May and August. Despite fluctuations, BOD levels remained below the guidance value of 5 mg $O_2$ $L^{-1}$ for cyprinid waters suggested by the Irish EPA. Additionally, the dilution factor of the receiving water system has not been included therefore BOD levels would further decrease upon release. With the exception of March, COD levels decreased between the intake and output water. This suggested that the COD levels were not due to processes within the farm and were more likely due to works being conducted upstream. Despite this, COD levels were well below the guidance value of 40 mg $O_2$ $L^{-1}$.

Suspended solids levels were greater between the intake and output water during the months of March, April and May which were most likely due to increases in production processes in the farm. However, this trend reversed for the months of June, July and August. This was most likely due to high levels of tree felling being conducted in the forestry upstream of the farm during this time. Levels were greater than the guidance level of 25 mg $L^{-1}$ during May and June but this was most likely due to the constructed wetland maintenance work as once the wetland became fully functional again after the June maintenance, concentrations dropped well below this level. Once again, it should be noted that water did not leave the farm during this time.

Temperature between the intake and output water samples remained consistent with increases observed during the summer months, as would be expected. Fish farms must not release water that is greater than $20^{o}C$. At no point during the study did temperatures rise to this level. Aquaculture waters are recommended to have a pH of between 6 and 9 (EPA, 2001). All samples remained within this range. The intake samples were slightly more alkaline than the output samples. Output samples had greater $CaCO_3$ levels and therefore a greater buffering capacity which may account for pH levels of just about neutral (pH 7) in the output water. $CaCO_3$ levels were measured for hardness. Results suggested that the water was slightly to moderately hard. This correlates with water hardness demonstrated around Boyle, Co. Roscommon (O'Neill et al., 2019).

All parameters were then compared to the previous year's study. In 2018, Ireland experienced its hottest summer on record whereby the country experienced long periods of drought. The physicochemical parameters were greater in 2018 than that of this study (2019) for the similar time period (O'Neill et al., 2019). This was most likely due to increased flow rates as a result of increased rainfall resulting in no drought conditions being observed in 2019. As this research only focused on one type of fish farm culturing one specific species of fish (European Perch) results from this study were also compared to previous aquaculture studies. These studies were located worldwide and encompassed a range of different aquaculture systems culturing several different species of fish, as shown in Table 6. The studies reviewed demonstrated similar or higher levels than the concentrations observed in this study.

### *4.2. Algal bioassay evaluation*

Inhibition of the growth rate of the *P. subcapitata* was observed in both the intake and output water samples. The presence of growth rate inhibition suggested that algal blooms downstream of the fish farm would be unlikely. However, growth rate inhibition is still demonstration of a toxic effect. This inhibition may result in loss of biodiversity in the receiving water body (Rabalais, 2002; O’Neill et al., 2019). Exclusive of the months of May and July, the percentage of growth rate inhibition was found to decrease between the intake and output water samples. The inhibition toxicity detected throughout the study were at sub-lethal levels. Additionally, toxicity was reduced once the water had passed through the fish farm’s constructed wetland. This suggested that the farm itself was successfully improving the water quality.

When results were compared to the previous study of 2018, a statistically significant difference was observed in both the intake (P = 0.030) and the output (P = 0.039) water samples. Unlike the previous study, no growth rate stimulation was observed in the output water. Equally, considerably lower levels (sub-lethal) of toxicity were observed in the intake water, e.g. levels of up to 75% growth rate inhibition were observed during the drought conditions of 2018 (O’Neill et al., 2019). This reduction was most likely due to the reduced temperatures and resulting increased flow rates.

Results were than compared to previous studies that utilised *P. subcapitata* to assess fish farm output water. Miashiro et al. (2012) demonstrated similar results in a Brazilian study (with a traditionally much warmer climate than Ireland) to the previous study conducted on the fish farm by O’Neill et al. (2019) during the heat wave and drought conditions, where by similar levels of growth rate stimulation were observed. The current study however, conformed to most of the available research on the effects of fish farm output water on *P. subcapitata*. Guéguen et al. (2004), Ivanova and Groudeva (2006) and Ma et al. (2006) all observed similar growth inhibition levels to those demonstrated. These studies were also conducted in countries (Poland and Bulgaria) with similar temperate weather conditions to those normally experienced in Ireland.

### *4.3. Constructed wetland evaluation*

The previous study conducted by O’Neill et al. (2019) indicated that there may have been issues with the constructed wetland due to increased concentration of nitrogenous and phosphorus waste in the output water samples. However, it was unclear whether this issue may have been due to the extreme weather conditions experienced during 2018 in Ireland. As a result, samples were included at the exit point of the settlement pond to ascertain the efficacy of the wetland. This was the point at which the wastewater entered the constructed wetland. Evaluation of the settlement pond demonstrated that, when fully functional after the June maintenance, the constructed wetland was effective in the removal of waste products from the water. This efficacy may also be due in part to the re-introduction of duckweed (*Lemna minor*). The previous study found spikes in nitrogenous waste concentrations when the duckweed was removed from the farm. Duckweed has the ability to use $NO_3^-$ as a nutrient source (O’Neill et al., 2019) and research is ongoing in this area.

### *4.4. Climate change*

According to the Intergovernmental Panel on Climate Change (2020), the momentum of climate change had greatly increased in 2019. Climate change is the most troubling scientific issues of our time (Bulkeley and Newell, 2015). The Bulletin of the Atomic Scientists (2020) have now moved the hypothetical Doomsday Clock to 100 seconds to midnight which is the closest it’s ever been to the “point of no return” represented by midnight. Originally introduced in 1947 due to the threat of nuclear weapons, climate change is now considered an equal threat to that (Weisberger, 2020). This research has further indicated that climate change has a direct impact on fish farming, as suggested by the lack of algal growth stimulation or high levels of growth inhibition due normal weather conditions reported in this study. Algal growth and temperature still demonstrate a strong correlationship ($r$ = -0.830) in the intake samples. This research has further demonstrated the ability of *P. subcapitata* to be utilised as an early warning indicator for climate change ambiguity in freshwater aquaculture.

## 5. Conclusion

- The findings of this timely study responds to the main tenets of the recent intergovermental report on global climate change (IPCC, 2021) that seeks urgent viable and resilient technological solutions to help future proof for a climate-smart environmentally friendly agri-food sector, including fisheries. Moreover, this ‘code red for humanity’ IPCC report on climate change clearly highlights that human or anthropogenic activity has contributed greatly to greenhouse gas levels in the atmosphere where there is a pressing need to reduce carbon dioxide and methane emissions, and to stall rising global temperatures that leads to extreme weather events.
- Regarding the latter, there is pressing need for countries to use innovative approaches to support and to develop sustainable food systems delivering benefits for the sector, for society, and for the environment. The findings of this present study will support and enable viable and resilient primary producers to provide food that are safe, nutritious and appealing; thus, using eco-technologies and talent to inform innovative, competitive and resilient agri-food sector regionally, and internationally (Rowan and Pogue, 2021).
- Specifically, this study revealed that the freshwater microalga *P. subcapitata* can be used for the real-time prediction of potential adverse environmental issues associated with freshwater aquaculture wastewater, which can be seen as complementary to relying upon using traditional physicochemical parametric measurements.
- As this research focused exclusively on one type of fish farm in the Republic of Ireland, use of this algal bioindicator technique should be also applied to evaluate different types of aquaculture farms including pond-based, flow-through, and recirculation in order to ensure harmonised results across a range of culture systems and fish species.
- Inclusion of additional ecotoxicological bioassays *such as* a full test battery,

encompassing different trophic levels (*e.g.*, *Daphnia magna* – primary consumer, *Vibrio fischeri* – decomposer) (Garvey et al., 2013) within the aquatic ecosystems should also be considered for future studies in order to develop a better understanding of the potential environmental effects' aquaculture processes could have on water bodies.

- There is merit in conducting molecular profiling of naturally occurring microalgae in order to incorporate these as a cocktail of native species representative of local natural aquatic ecosystems, which will support and inform biodiversity, conservation management along with enhanced bioindicator performance. For example, Kadam et al. (2020) identified 33 Taxa belonging to 27 genera of microalgae when they considered development of a putative 'Algal Genus Pollution Index' for potentially assessing water bodies in the Doon valley, India.
- The constructed wetland servicing this aquaculture farm needs to be increased in size in order to be effective in treating volume of the wastewater effluent where efficacy of treatment can be also influenced by extreme weather events that influence flow rates.
  - The lack of growth rate stimulation and decrease in growth rate inhibition when compared to the previous study (O'Neill et al., 2019) supports future use of *P. subcapitata* as an early warning indicator to potential issues in fish farms associated with climate change where unpredictable and more erratic weather conditions may become more frequent. It is appreciated that there a dearth in evidence-based literature on the use of microalgae as a bioindicator for monitoring impact of climate change and its potential effects in aquaculture.
- While this present research has demonstrated interested findings, there is a need to pursue catchment based-studies that incorporates an extended number of locations and inter-laboratory evaluations for to improve technological rigor and stakeholder

acceptance including policy-makers.

- Increasing $NH_3$ levels in the monitored fish ponds can be potentially toxic to fish that require further investigation.
- There are emerging opportunities for use of natural microalgae in the development of predictive environmental risk models that will help inform the quality status of water catchments, along with evaluating commensurate efficacy of intervention strategies, such as municipal wastewater treatment plants (Tahar et al., 2017).

## Declaration of Competing Interest

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

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**Table 1:** Summary of the methods used to assess the physicochemical parameters investigated on the Irish freshwater aquaculture intake, output and settlement pond water samples. The method employed, detection limit for all kits used and standard water and wastewater analysis methods numbers have been included.

| Physicochemical parameter | Method | Detection limit (mg $L^{-1}$) | Standard method number |
|---|---|---|---|
| **Alkalinity** | Titrimetric | - | 2320-B |
| **BOD** | Membrane electrode | - | 5210-B |
| **COD** | Photometric | 0-150<br>15-300 | 5220-D |
| **DO** | Membrane electrode | - | 4500-O G |
| **Hardness** | Titrimetric | - | 2340-C |
| **$NH_4^+$** | Photometric | 0.013-3.86<br>2.6-193.0 | 4500-$NH_3$-F |
| **$NO_2^-$** | Photometric | 0.007-3.28 | 345-1 |
| **$NO_3^-$** | Photometric | 0.4-110.7 | 4500-$NO_3$ |
| **pH** | Membrane electrode | - | 2310-B |
| **$PO_4^{3-}$** | Photometric | 0.007-15.3<br>1.5-92.0 | 4500-P-C |
| **Suspended solids** | Gravimetric | - | 2540-D |
| **Temperature** | Thermometer | - | 2550-B |

$NH_4^+$ = ammonium, $NO_2^-$ = nitrite, $NO_3^-$ = nitrate, $PO_4^{3-}$ = orthophosphate, DO = dissolved oxygen, BOD = biochemical oxygen demand, COD = chemical oxygen demand

**Table 2:** Mean concentrations calculated for each parameter investigated in this study conducted in 2019 (intake and output water 2019) and the previous study conducted in 2018 on the same fish farm (intake and output water 2018) by O’Neill *et al.* (2019). All data is based on the average across six months. S.D. indicated

| **Parameter** | **Intake Water** | | **Output Water** | |
|---|---|---|---|---|
| | **2018** | **2019** | **2018** | **2019** |
| **$NH_4^+$ (mg $L^{-1}$)** | 0.16 ± 0.18 | 0.06 ± 0.09 | 1.16 ± 0.64 | 0.53 ± 0.53 |
| **$NO_2^-$ (mg $L^{-1}$)** | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.32 ± 0.38 | 0.10 ± 0.07 |
| **$NO_3^-$ (mg $L^{-1}$)** | 3.62 ± 1.60 | 1.81 ± 1.27 | 5.29 ± 5.56 | 1.74 ± 1.10 |
| **$PO_4^{3-}$ (mg $L^{-1}$)** | 1.76 ± 0.84 | 0.63 ± 1.14 | 3.78 ± 2.00 | 0.77 ± 0.51 |
| **DO (mg $O_2$ $L^{-1}$)** | 10.31 ± 0.87 | 10.76 ± 2.75 | 5.10 ± 2.85 | 7.66 ± 3.06 |
| **BOD (mg $O_2$ $L^{-1}$)** | 2.27 ± 1.47 | 2.68 ± 0.70 | 3.24 ± 1.95 | 2.80 ± 0.96 |
| **COD (mg $O_2$ $L^{-1}$)** | 45.91 ± 40.81 | 25.97 ± 9.98 | 76.44 ± 59.06 | 19.24 ± 11.68 |
| **Temperature ($^oC$)** | 14.76 ± 2.53 | 13.85 ± 1.35 | 15.53 ± 2.66 | 14.23 ± 1.48 |
| **pH** | 7.76 ± 0.19 | 7.70 ± 0.14 | 7.11 ± 0.18 | 7.14 ± 0.06 |
| **Suspended solids (mg $L^{-1}$)** | 40.17 ± 79.08 | 20.50 ± 8.00 | 83.67 ± 144.33 | 19.22 ± 9.23 |
| **Hardness (mg $CaCO_3$ $L^{-1}$)** | 100.49 ± 9.22 | 106.24 ± 12.18 | 116.03 ± 16.80 | 111.58 ± 22.45 |
| **Alkalinity (mg $CaCO_3$ $L^{-1}$)** | 122.55 ± 17.71 | 135.03 ± 20.49 | 128.91 ± 18.19 | 129.47 ± 17.98 |
| ***P. subcapitata* (% Growth Rate Inhibition)** | 43.14 ± 18.47 | 13.66 ± 1.44 | -2.70 ± 20.41 | 9.73 ± 2.03 |

$NH_4^+$ = ammonium, $NO_2^-$ = nitrite, $NO_3^-$ = nitrate, $PO_4^{3-}$ = orthophosphate, DO = dissolved oxygen, BOD = biochemical oxygen demand, COD = chemical oxygen demand

**Table 3:** Correlation matrix for *P. subcapitata* and all physicochemical parameters investigated on the Irish freshwater aquaculture intake water samples. Bold figures indicate where statistically significant differences were observed. Breakdown of correlation ranges are also indicated.

| | ***P. sub*** | **T** | **pH** | **$NH_4^+$** | **$NO_2^-$** | **$NO_3^-$** | **$PO_4^{3-}$** | **DO** | **BOD** | **COD** | **SS** | **H** | **A** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***P. sub*** | 1.000 | | | | | | | | ***0 = No relationship*** | | | | |
| **T** | **-0.830** | 1.000 | | | | | | | ***>0-0.3 = Weak relationship*** | | | | |
| **pH** | **-0.951** | 0.794 | 1.000 | | | | | | ***0.3-0.5 = Moderately weak relationship*** | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$NH_4^+$** | -0.183 | 0.360 | 0.451 | 1.000 | | | | | ***0.5-0.7 = Moderately strong relationship*** | | | | |
| **$NO_2^-$** | -0.260 | 0.037 | -0.020 | **-0.845** | 1.000 | | | | ***0.7-<1 = Strong relationship*** | | | | |
| **$NO_3^-$** | -0.727 | 0.687 | 0.557 | -0.173 | 0.656 | 1.000 | | | ***1 = Perfect linear relationship*** | | | | |
| **$PO_4^{3-}$** | -0.286 | 0.024 | 0.133 | -0.444 | 0.785 | 0.728 | 1.000 | | | | | | |
| **DO** | 0.726 | -0.809 | -0.553 | 0.057 | -0.468 | **-0.876** | -0.400 | 1.000 | | | | | |
| **BOD** | 0.307 | -0.627 | -0.351 | -0.582 | 0.232 | -0.465 | -0.073 | 0.625 | 1.000 | | | | |
| **COD** | -0.623 | 0.448 | 0.372 | -0.523 | 0.712 | 0.623 | 0.353 | -0.749 | 0.007 | 1.000 | | | |
| **SS** | -0.702 | 0.594 | 0.466 | -0.380 | 0.730 | **0.855** | 0.559 | **-0.915** | -0.295 | 0.924 | 1.000 | | |
| **H** | -0.061 | 0.326 | -0.138 | -0.426 | 0.588 | 0.638 | 0.471 | -0.502 | -0.264 | 0.254 | 0.438 | 1.000 | |
| **A** | -0.394 | 0.201 | 0.218 | -0.497 | **0.851** | 0.800 | **0.916** | -0.440 | 0.017 | 0.400 | 0.577 | 0.659 | 1.000 |

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

**Table 4:** Correlation matrix for *P. subcapitata* and all physicochemical parameters investigated on the Irish freshwater aquaculture output water samples. Bold figures indicate where statistically significant differences were observed. Breakdown of correlation ranges are also indicated.

| | **P. sub** | **T** | **pH** | **NH4+** | **NO2-** | **NO3-** | **PO4 3-** | **DO** | **BOD** | **COD** | **SS** | **H** | **A** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **P. sub** | 1.000 | | | | | | | | ***0 = No relationship*** | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T** | -0.537 | 1.000 | | | | | | | ***>0-0.3 = Weak relationship*** | | | | |
| **pH** | -0.332 | 0.419 | 1.000 | | | | | | ***0.3-0.5 = Moderately weak relationship*** | | | | |
| **NH4+** | -0.210 | 0.496 | -0.262 | 1.000 | | | | | ***0.5-0.7 = Moderately strong relationship*** | | | | |
| **NO2-** | 0.131 | 0.213 | -0.645 | 0.733 | 1.000 | | | | ***0.7-<1 = Strong relationship*** | | | | |
| **NO3-** | -0.627 | **0.929** | 0.234 | 0.439 | 0.161 | 1.000 | | | ***1 = Perfect linear relationship*** | | | | |
| **PO43-** | -0.488 | 0.139 | -0.500 | 0.433 | 0.656 | 0.213 | 1.000 | | | | | | |
| **DO** | 0.296 | -0.670 | 0.370 | **-0.816** | **-0.811** | -0.700 | -0.612 | 1.000 | | | | | |
| **BOD** | -0.102 | -0.518 | -0.702 | 0.128 | 0.431 | -0.405 | 0.766 | -0.112 | 1.000 | | | | |
| **COD** | 0.007 | -0.362 | -0.505 | -0.529 | -0.092 | -0.070 | 0.252 | 0.095 | 0.385 | 1.000 | | | |
| **SS** | -0.442 | -0.194 | -0.004 | 0.304 | 0.124 | -0.267 | 0.525 | 0.033 | 0.634 | -0.324 | 1.000 | | |
| **H** | -0.574 | 0.344 | -0.514 | 0.494 | 0.603 | 0.522 | **0.902** | -0.769 | 0.558 | 0.364 | 0.246 | 1.000 | |
| **A** | -0.639 | 0.479 | -0.327 | 0.494 | 0.299 | 0.736 | 0.544 | -0.701 | 0.197 | 0.296 | 0.019 | **0.839** | 1.000 |

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

**Table 5:** Correlation matrix for *P. subcapitata* and all physicochemical parameters investigated on the Irish freshwater aquaculture settlement pond water samples. Bold figures indicate where statistically significant differences were observed. Breakdown of correlation

ranges are also indicated.

| | ***P. sub*** | **T** | **pH** | **$NH_4^+$** | **$NO_2^-$** | **$NO_3^-$** | **$PO_4^{3-}$** | **DO** | **BOD** | **COD** | **SS** | **H** | **A** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***P. sub*** | 1.000 | | | | | | | | ***0 = No relationship*** | | | | |
| **T** | 0.386 | 1.000 | | | | | | | ***>0-0.3 = Weak relationship*** | | | | |
| **pH** | 0.566 | **0.938** | 1.000 | | | | | | ***0.3-0.5 = Moderately weak relationship*** | | | | |
| **$NH_4^+$** | 0.714 | **0.816** | **0.958** | 1.000 | | | | | ***0.5-0.7 = Moderately strong relationship*** | | | | |
| **$NO_2^-$** | 0.133 | 0.464 | 0.229 | 0.107 | 1.000 | | | | ***0.7-<1 = Strong relationship*** | | | | |
| **$NO_3^-$** | 0.168 | 0.417 | 0.182 | 0.079 | **0.992** | 1.000 | | | ***1 = Perfect linear relationship*** | | | | |
| **$PO_4^{3-}$** | **0.976** | 0.393 | 0.580 | 0.715 | -0.004 | 0.032 | 1.000 | | | | | | |
| **DO** | 0.784 | 0.525 | 0.765 | **0.883** | -0.288 | -0.287 | **0.847** | 1.000 | | | | | |
| **BOD** | 0.679 | 0.786 | **0.945** | **0.986** | -0.034 | -0.060 | 0.714 | **0.927** | 1.000 | | | | |
| **COD** | 0.593 | 0.362 | 0.523 | 0.532 | -0.322 | -0.336 | 0.730 | 0.744 | 0.616 | 1.000 | | | |
| **SS** | -0.255 | 0.468 | 0.514 | 0.438 | -0.085 | -0.183 | -0.271 | 0.201 | 0.441 | -0.077 | 1.000 | | |
| **H** | 0.251 | **0.898** | **0.843** | 0.677 | 0.205 | 0.147 | 0.341 | 0.512 | 0.709 | 0.609 | 0.412 | 1.000 | |
| **A** | 0.356 | **0.942** | **0.920** | 0.781 | 0.279 | 0.212 | 0.407 | 0.571 | 0.785 | 0.582 | 0.488 | **0.974** | 1.000 |

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

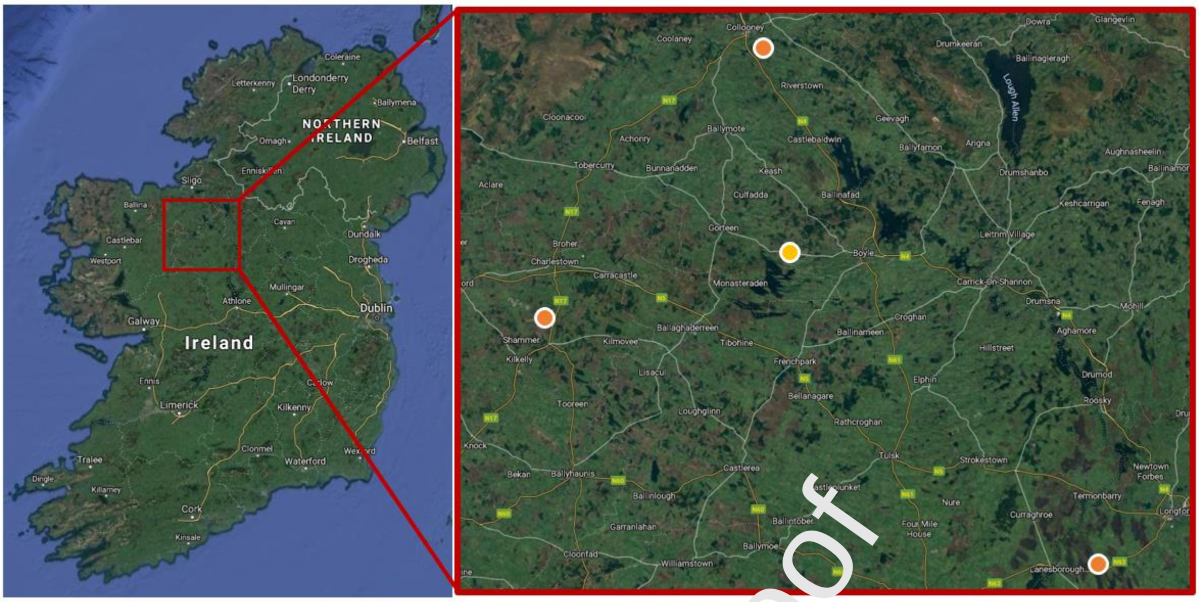


**Figure 1:** Map of Ireland indication the approximate location of the freshwater fish farm (53$^{o}$58’16” N, 08$^{o}$24’44” W) indicated with yellow, and the three closest Met Eireann weather stations (Markree – 54$^{o}$10’30” N, 08$^{o}$27’20” W; Mount Dillon – 53$^{o}$43’37” N, 07$^{o}$58’51” W; Knock – 53$^{o}$54’22” N, 08$^{o}$49’4” W) surrounding the farm, indicated by orange.

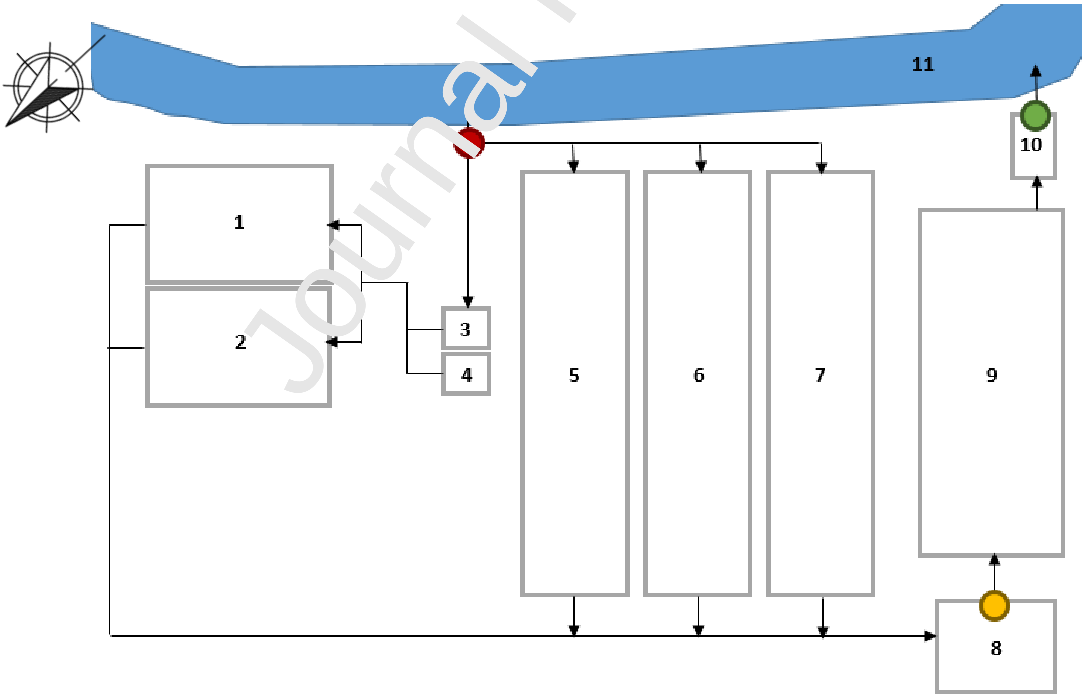
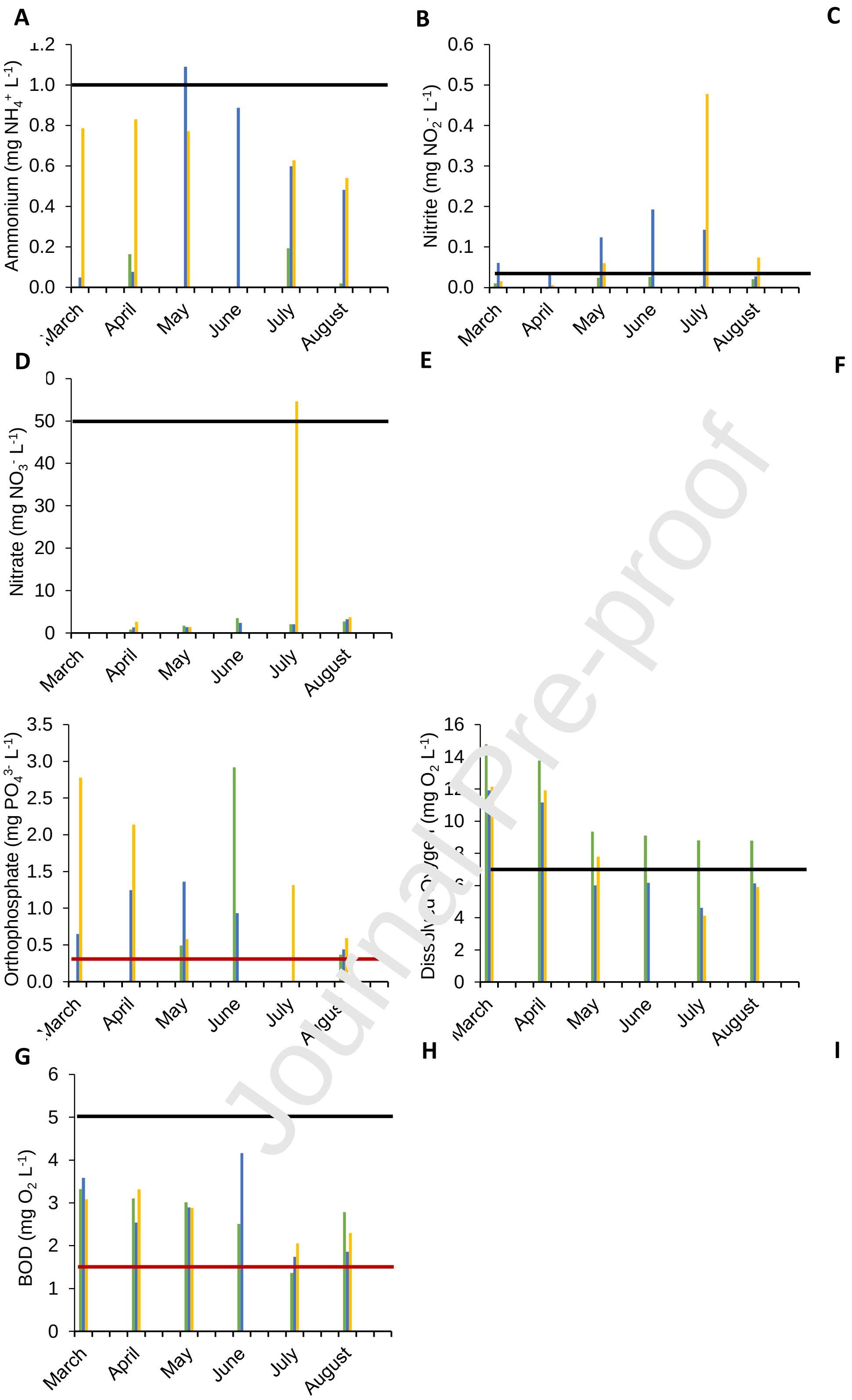


**Figure 2:** Schematic of the Irish freshwater fish farm layout indicating the locations of the collection points for the intake (red), output (green) and settlement pond (yellow) water samples. 1) hatchery, 2) nursery, 3-4) mesocosms, 5-7) culture ponds, 8) settlement pond, 9) constructed wetland, 10) holding tank, 11) river. Black arrows indicate flow of water. NOTE: Schematic is not to scale.


A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ammonium (mg $NH_4^+$ $L^{-1}$)
March
April
May
June
July
August
B
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Nitrite (mg $NO_2^-$ $L^{-1}$)
March
April
May
June
July
August
C
D
0
50
40
30
20
10
0
Nitrate (mg $NO_3^-$ $L^{-1}$)
March
April
May
June
July
August
E
F
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Orthophosphate (mg $PO_4^{3-}$ $L^{-1}$)
March
April
May
June
July
August
16
14
12
10
8
6
4
2
0
Dissolved Oxygen (mg $O_2$ $L^{-1}$)
March
April
May
June
July
August
G
H
I
6
5
4
3
2
1
0
BOD (mg $O_2$ $L^{-1}$)
March
April
May
June
July
August

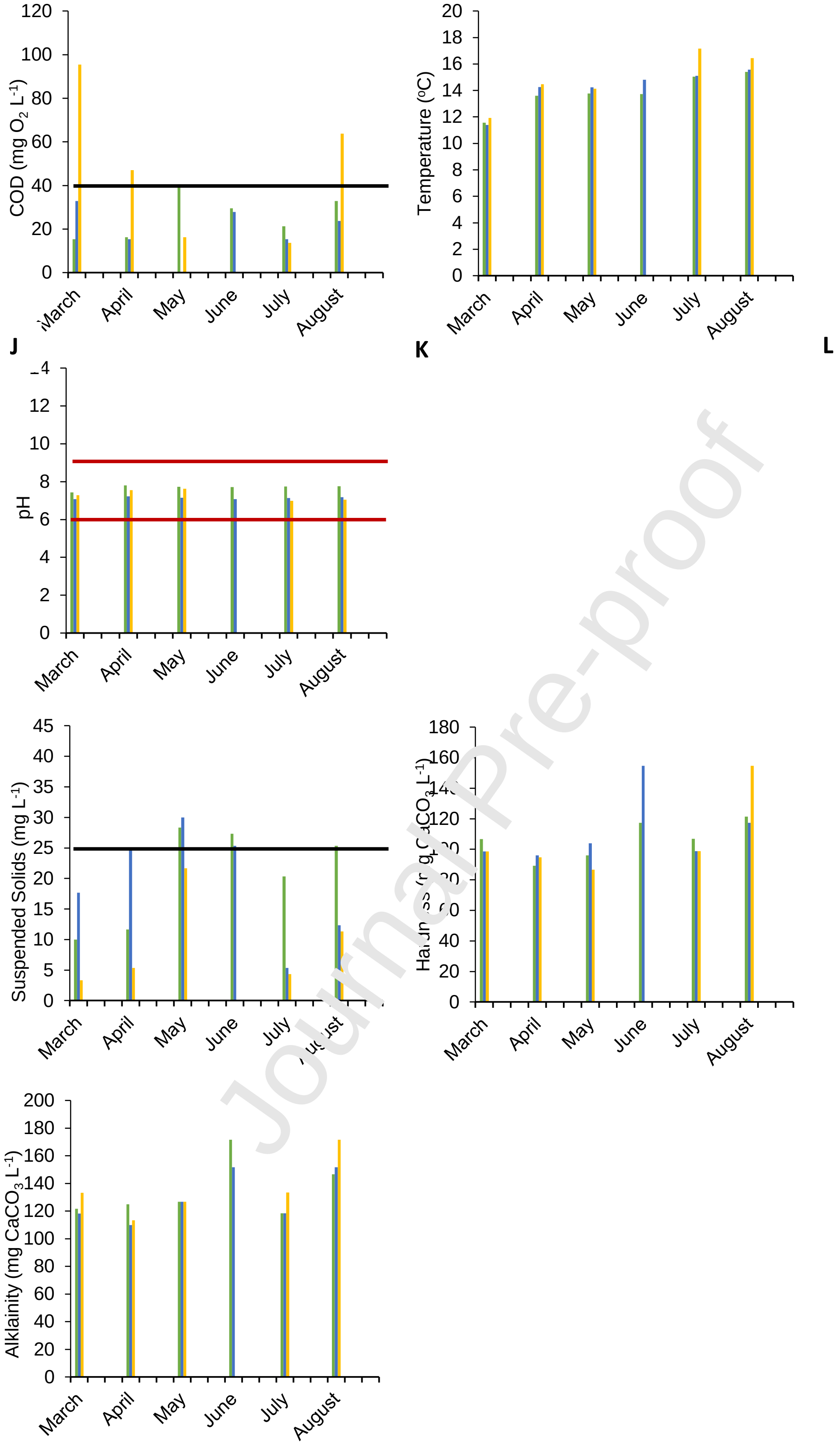


**Figure 3:** Breakdown for the physicochemical parameters investigated on Irish freshwater aquaculture intake (green), output (blue) and settlement pond (yellow) water samples from March 2019 to August 2019. Parameters investigated were A) $NH_4^+$, B) $NO_2^-$, C) $NO_3^-$, D) $PO_4^{3-}$, E) DO, F) BOD, G) COD, H) temperature, I) pH, J) suspended solids, K) hardness and

L) alkalinity. Red lines indicate levels set out by S.I. 272 of 2009 and 77 of 2019. Black lines indicate levels set out by the Irish EPAs parameters for water quality. NOTE: Dilution factor of the receiving water body has not been included. Lines do not appear on temperature and CaCO3 as no limits were indicated. S.D. indicated, *n* = 9.

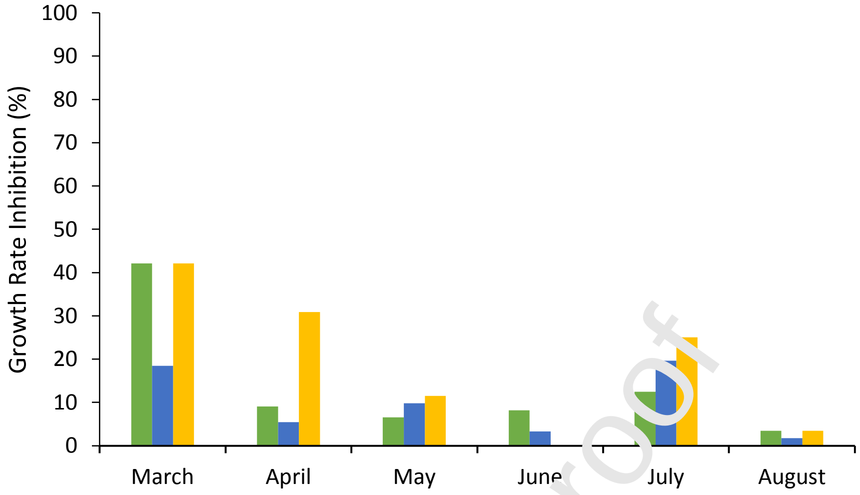


**Figure 4:** Percentage growth rate inhibition observed in *P. subcapitata* proceeding exposure to Irish freshwater aquaculture intake (green), output (blue) and settlement pond (yellow) water samples for 72 h at 23°C ± 2°C under continuous illumination from March 2019 to august 2019. S.D. indicated, *n* = 9.

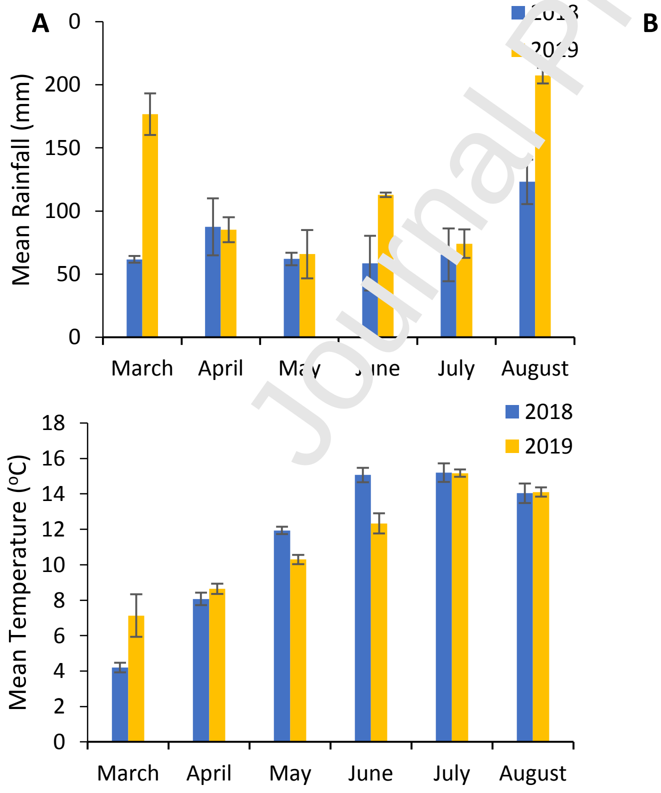
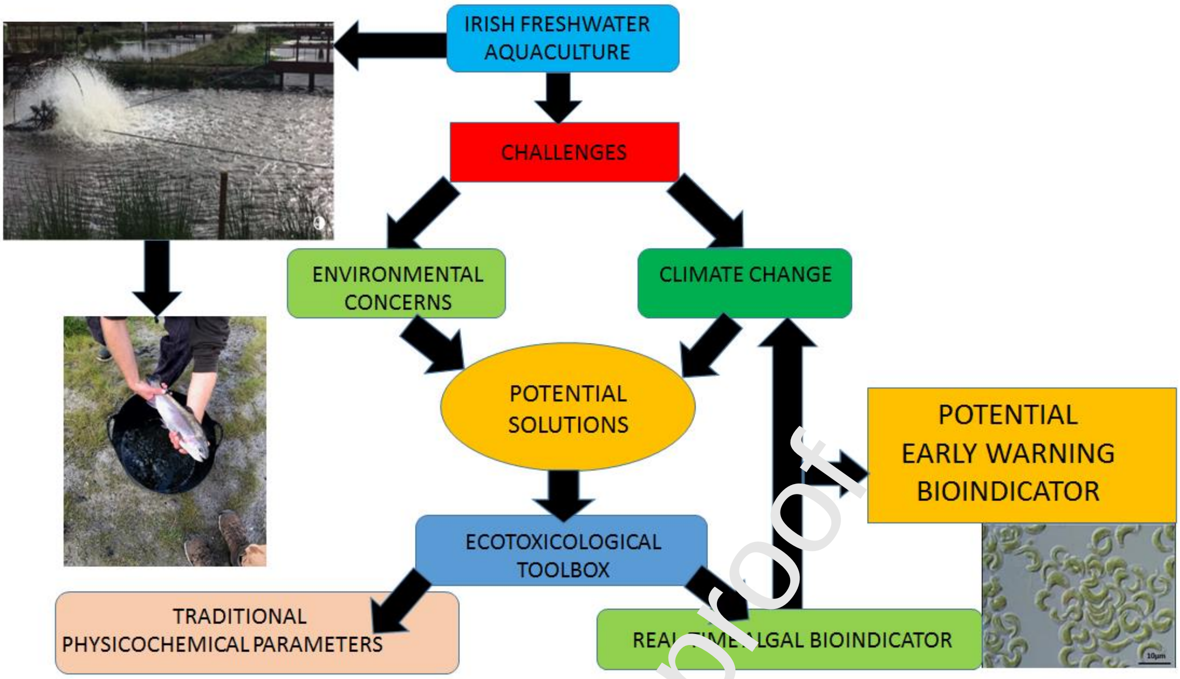


**Figure 5:** Average A) rainfall and B) temperature recorded for 2018 (blue) and 2019 (yellow) at three Met Eireann weather stations surrounding the freshwater fish farm during the sampling period of March 2019 to August 2019. Stations were located at 1) Markree, Co. Sligo, 2) Knock, Co. Mayo and 3) Mount Dillon, Co. Roscommon. Stations were located north-west, south-west and south-east of the fish farm, respectively.

**CRediT Author Statement**

Emer A. O’Neill, Neil J. Rowan: Conceptualization
Emer A. O’Neill: Data Curation
Emer A. O’Neill: Formal Analysis
Neil J. Rowan: Funding Acquisition
Emer A. O’Neill: Investigation
Emer A. O’Neill: Methodology
Emer A. O’Neill: Project Administration
Emer A. O’Neill, Neil J. Rowan: Resources
Emer A. O’Neill: Software
Neil J. Rowan: Supervision
Emer A. O’Neill: Validation
Emer A. O’Neill: Visualization
Emer A. O’Neill: Roles/Writing – Original Draft
Emer A. O’Neill, Neil J. Rowan: Writing – Review & Editing

## Graphical abstract



**Highlights**

- Traditional monitored of aquaculture outputs using physicochemical parameters has limited efficacy
- Complementary use of algae supports environmental monitoring of aquaculture
- Duckweed supports and improves efficacy of aquaculture wastewater treatment
- Algae is a potentially rapid and sensitive bioindicator of aquaculture water quality
- Algae is a potential early warning tool for assessing impacts of climate change in aquaculture