The use of *Pseudokirchneriella subcapitata* as a test organism for the ecotoxicological evaluation of Irish freshwater finfish aquaculture effluent and as a potential early indicator of climate change.

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Abstract

Aquaculture is one of the fastest growing food producing industries in the world. The dramatic increase in the growth of global aquaculture production has many environmental concerns. The evaluation of aquaculture effluent is frequently assessed by the measurement of physicochemical parameters but this only indicates a potential degradation caused by the effluent, not the effects on aquatic ecosystems and organisms.

Thirteen physicochemical parameters were used to assess the water quality of a freshwater finfish aquaculture effluent in Ireland, including; temperature, pH, nitrogen, phosphorus, oxygen, hardness, alkalinity and conductivity. The *Pseudokirchneriella subcapitata* algal bioassay ISO (8692:2012), was used to evaluate the potential ecotoxicological effects the freshwater aquaculture effluent on its receiving aquatic ecosystems and organisms. Influent and effluent samples were collected from a freshwater aquaculture facility every two weeks from April 2018 to October 2018.

Physicochemical analysis found that concentrations of ammonium, nitrite, orthophosphate, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand and suspended solids determined in the effluent may be cause for concern. After exposure of algae to the aquaculture effluent, stimulation of algal growth rates increased by up to >50% during the sampling period. This stimulation was observed during periods of increased temperatures which were as a result of heat wave and drought conditions experienced during the summer of 2018. Correlation studies identified a strong relationship between algal stimulation and temperature increases (-0.619), suggesting that Pseudokirchneriella subcapitata should be included in the assessment of aquaculture effluent.

Comparison of these findings to revised studies have also indicated that standard water quality parameters may not be applicable to aquaculture effluent. The results determined in this study indicate that changes in weather patterns, as a result of issues such as climate change, may have a direct impact on aquaculture effluent and its receiving aquatic ecosystem. Therefore *Pseudokirchneriella subcapitata* may have use as a potential early indicator of climate change and its effects.

1. Introduction

Aquaculture is one of the fastest growing food producing industries in the world (Fečkaninová *et al.*, 2017; Liu *et al.*, 2017), and provides one of the most sustainable forms of edible protein (Liu *et al.*, 2017; Yue and Wang, 2017). The dramatic increase in the growth of global aquaculture production indicate its importance in modern day food supply (Jegatheesan *et al.*, 2011), by providing a means to meet the growth in global demand (Seoane *et al.*, 2014). However, despite this and many other advantages (Jegatheesan *et al.*, 2011; Martinez-Porchas *et al.*, 2014), there are many environmental concerns thought to be associated with aquaculture (Martinez-Porchas *et al.*, 2014; Ngo *et al.*, 2016; Troell *et al.*, 2017), and in particular to the impacts aquaculture effluent is thought to have on the receiving aquatic ecosystem (Jegatheesan *et al.*, 2011).

Aquaculture effluent typically contains nutrient rich waste products (Jegatheesan *et al.*, 2011; Martinez-Porchas *et al.*, 2014; Ngo *et al.*, 2016; Sikder *et al.*, 2016), which if released untreated into water bodies can lead to water pollution (Jegatheesan *et al.*, 2011; Sikder *et al.*, 2016). It may cause direct negative effects such as eutrophication (Jegatheesan *et al.*, 2011; Martinez-Porchas *et al.*, 2014; Ngo *et al.*, 2016; Sikder *et al.*, 2016; Troell *et al.*, 2017), which is one of the greatest concerns (Ngo *et* *al.*, 2016). Eutrophication is a process by which a water body receives large levels of nutrients and organic matter that can be taken in and biologically processed (Martinez-Porchas *et al.*, 2014; Sikder *et al.*, 2016), which in turn can result in increased levels of algal blooms and decreased levels of oxygen which can suffocate aquatic life in the water body (Jegatheesan *et al.*, 2011; Chislock *et al.*, 2013; Ngo *et al.*, 2016).

The evaluation of aquaculture effluent is frequently assessed by the measurement of physicochemical parameters (da Silva *et al.*, 2017). However, investigation of these parameters alone only indicate the potential degradation caused by the effluent, not their effects on aquatic ecosystems and organisms (Stephens and Farris, 2004a, 2004b; da Silva *et al.*, 2017). Thus, ecotoxicological bioassays are used in conjunction with physicochemical analysis however, there are few studies that assess the toxic effects of aquaculture effluent on aquatic ecosystems and organisms (da Silva *et al.*, 2017).

Globally, aquaculture is dominated by freshwater farming and the bulk of the production is finfish (Wang *et al.*, 2015). The aim of this study was to conduct an ecotoxicological evaluation of Irish freshwater finfish aquaculture effluent using the *Pseudokirchneriella subcapitata* algal bioassay in conjunction with traditional physicochemical analysis. Correlation studies were also conducted to determine any potential relationships between the algae and the physicochemical parameters.

2. Materials & Methods

Sampling: Water samples were collected from a freshwater aquaculture farm located in Boyle, Co. Roscommon (Figure 1). The farm, which cultures perch (*Perca fluviatilis*), consisted of a recirculated aquaculture system (RAS), used for the hatchery and nursery, three culture ponds for the adult fish that uses a flow through system (FTS), a settlement pond and a constructed wetland for wastewater treatment. Samples were taken directly from the effluent source of the farm every two weeks from April 2018 to October 2018. Collection occurred on the same day (Thursdays), and at approximately the same time (10:30 a.m.). Influent samples were also collected and analysed so that any potential issues caused by works upstream of the fish farm and not as a result of works within the facility itself could be taken into consideration. Influent and effluent sampling points are shown in Figure 2.

Physicochemical analysis: Water parameters – temperature, pH, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), hardness, alkalinity and conductivity – were investigated in the laboratory within 24 hours of collection to prevent the need for preservation. Table 1 summarises the physicochemical methods employed in this study.

Toxicity testing: The freshwater unicellular green algae *Pseudokirchneriella subcapitata,* was used in the toxicity test. A starter culture of the *Pseudokirchneriella subcapitata* (*Raphidocelis 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°C ± 2°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. Toxicity testing was conducted as per the Water quality – Fresh water algal growth inhibition test with unicellular green algae ISO (8692:2012), guidelines. The *Pseudokirchneriella subcapitata* was exposed to the influent and effluent samples for 72 hours under static conditions at 23°C ± 2°C exposed to the continuous illumination. The algae growth rate inhibition and stimulation, in percent, was calculated by comparing the samples to a negative control.

Statistical analysis: All statistical analyses were conducted using GRAPHPAD PRISM 8 and MINITAB 18. The data generated were grouped and subject to normality test (Anderson-Darling). T-tests and one-way ANOVA with Tukey were used to identify significant differences in the variables. Pearson's correlation was used to assess any correlations between the algae and/or the physicochemical parameters.

Figure 1: Map of Ireland indicating the location of the fish farm (yellow) and the closest weather stations (orange).

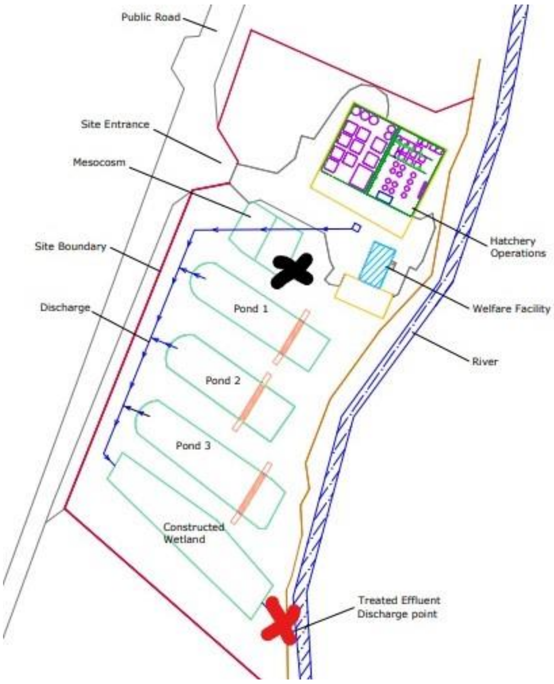

Figure 2: Schematic of the fish farm layout. Location of the collection point for the influent (black X), and effluent (red X), are indicated.

Table 1: Methods, including detection limits, used for the analyses of water quality parameters in the influent and effluent. Figure in brackets indicate the standard method number.

PARAMETER / VARIABLE	METHOD	DETECTION LIMIT (mg L^{-1})
Temperature	Thermometer (2550-B)	-
pH	Membrane Electrode (2310-B)	-
Ammonium (NH_4^+)	Photometric (4500-NH_3-F)	0.013 – 3.86 2.6 – 193.0
Nitrite (NO_2^-)	Photometric (345-1)	0.007 – 3.28
Nitrate (NO_3^-)	Photometric (4500-NO_3)	0.4 – 110.7
Phosphate (PO_4^{3-})	Photometric (4500-P-C)	0.007 – 15.3 1.5 – 92.0
Dissolved Oxygen	Membrane Electrode (4500-O G)	-
Biochemical Oxygen Demand (BOD)	Membrane Electrode (5210-B)	-
Chemical Oxygen Demand (COD)	Photometric (5220-D)	0-150 15-300
Suspended Solids	Gravimetric (2540-D)	-
Hardness	Titrimetric (2340-C)	-
Alkalinity	Titrimetric (2320-B)	-
Conductivity	Electrical Conductivity (2510-A)	-

3. Results

Physicochemical Analysis

Mean concentrations determined for the physicochemical parameters investigated on Irish freshwater aquaculture influent and effluent samples over the entire testing period are summarised in Table 2 and Figure 3 provides a monthly breakdown of the results. The average temperature in the influent was found to be 14.76°C and 15.53°C in the effluent. The mean pH was found to be 7.76 and 7.11 in the influent and effluent, respectively. When analysing the nitrogen content; ammonium (NH_4^+), levels observed in the influent was found to be 0.16 mg L^{-1} and 1.16 mg L^{-1} in the effluent. Nitrite (NO_2^-), concentrations of 0.02 mg L^{-1} and 0.32 mg L^{-1} were observed in the influent and effluent, respectively. Nitrate (NO_3^-), levels were found to be 3.62 mg L^{-1} in the influent and 5.29 mg L^{-1} in the effluent. After investigating phosphorus levels by way of orthophosphate (PO_4^{3-}), a mean concentration of 1.76 mg L^{-1} was observed in the influent and 3.78 mg L^{-1} in the effluent. Oxygen concentrations were analysed via dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD). Average DO levels were found to be 10.31 mg O_2 L^{-1} and 5.10 mg O_2 L^{-1} in the influent and effluent, respectively. BOD levels were found to be 2.27 mg O_2 L^{-1} in the influent and 3.24 mg O_2 L^{-1} in the effluent. A mean COD concentration of 45.91 mg O_2 L^{-1} was observed in the influent and a concentration of 76.44 mg O_2 L^{-1} was observed in the effluent. Suspended solids in the influent was an average of 40.17 mg L^{-1}. In the effluent, suspended solids averaged 83.67 mg L^{-1}. Alkalinity and hardness were investigated via calcium carbonate ($CaCO_3$), concentrations. For alkalinity, the average $CaCO_3$ level observed in influent was 122.55 mg L^{-1} and 128.91 mg L^{-1} in the effluent. For the hardness, $CaCO_3$ concentrations of 100.49 mg L^{-1} and 116.03 mg L^{-1} were observed in the influent and effluent, respectively. Finally, conductivity levels observed in influent was 247.18 µS cm^{-1} and 298.17 µS cm^{-1} in the effluent. When the influent and effluent were compared via statistical analysis, a significant difference was observed in the NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and DO levels.

Table 2: Physicochemical parameters investigated on Irish freshwater finfish aquaculture influent and effluent samples from April 2018 to October 2018. Results are presented as means ± S.D. *Significant differences, where $p < 0.05$, are indicated. (n = 12)

PARAMETER	INFLUENT	EFFLUENT	P VALUE
Temperature (°C)	14.76 ± 2.53	15.53 ± 2.66	0.8539
pH	7.76 ± 0.19	7.11 ± 0.18	0.7078
Ammonium (mg NH_4^+ L^{-1})	0.16 ± 0.18	1.16 ± 0.64	0.001*
Nitrite (mg NO_2^- L^{-1})	0.02 ± 0.01	0.32 ± 0.38	<0.0001*
Nitrate (mg NO_3^- L^{-1})	3.62 ± 1.60	5.29 ± 5.56	0.0057*
Orthophosphate (mg PO_4^{3-} L^{-1})	1.76 ± 0.84	3.78 ± 2.00	0.0128*
DO (mg O_2 L^{-1})	10.31 ± 0.87	5.10 ± 2.85	0.0009*
BOD (mg O_2 L^{-1})	2.27 ± 1.47	3.24 ± 1.95	0.1850
COD (mg O_2 L^{-1})	45.91 ± 40.81	76.44 ± 59.06	0.2301
Suspended Solids (mg L^{-1})	40.17 ± 79.08	83.67 ± 144.33	0.0733
Hardness (mg $CaCO_3$ L^{-1})	100.49 ± 9.22	116.03 ± 16.80	0.0922
Alkalinity (mg $CaCO_3$ L^{-1})	122.55 ± 17.71	128.91 ± 18.19	0.9153
Conductivity (µS cm^{-1})	247.18 ± 57.82	298.17 ± 57.12	0.2409

DO = Dissolved Oxygen, BOD = Biochemical Oxygen Demand, COD = Chemical Oxygen Demand

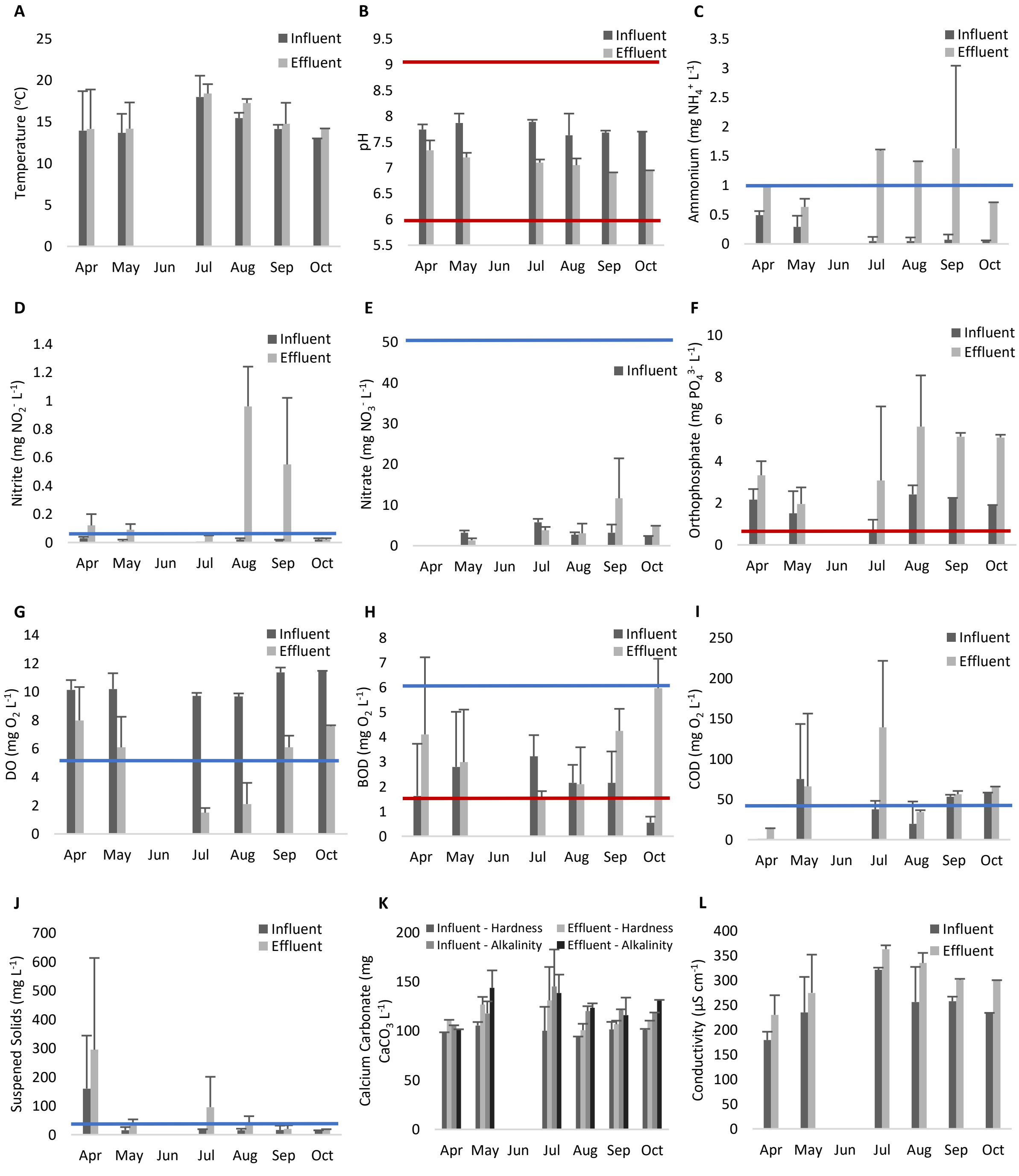

Figure 3: Bar charts displaying monthly means for the physicochemical parameters investigated on Irish freshwater finfish aquaculture influent and effluent from April 2018 to October 2018. Parameters investigated were A) temperature, B) pH, C) NH_4^+, D) NO_2^-, E) NO_3^-, F) PO_4^{3-}, G) dissolved oxygen, H) Biochemical Oxygen Demand, I) Chemical Oxygen Demand, J) suspended solids, K) hardness and alkalinity via $CaCO_3$ content, and L) conductivity. S.D. indicated, n = 12. Red lines indicate levels set out by S.I. 272 of 2009, which is the European Communities Environmental Objective (Surface Waters) Regulations of 2009. Blue lines indicated levels set out by the Irish EPAs Parameters for water quality, which are based on Freshwater Fish Directive [78/659/EEC], Salmonid Waters Regulations [1988], and Surface Water Regulations [1989]. NOTE: The dilution factor of the receiving water body has not been taken into consideration. Lines do not appear on temperature, $CaCO_3$ content and conductivity as not limits were indicated and/or the limit is well above the range of the graph.

<u>Algal Bioassay</u>

The *Pseudokirchneriella subcapitata* algal bioassay was performed on influent and effluent samples from the Irish freshwater aquaculture facility every two weeks to determine whether or not growth inhibition or stimulation were observed as a result of exposure to either sample. Growth inhibition of up to 75% was observed in the influent and up to 27% in the effluent (Figure 4). Growth stimulation of more than 50% was observed in the effluent (Figure 5). No growth stimulation was observed in the influent (Figure 5). Statistical analysis was conducted between the influent and effluent and a significant difference was observed (p = <0.0001 where <0.05 indicates a significant difference).

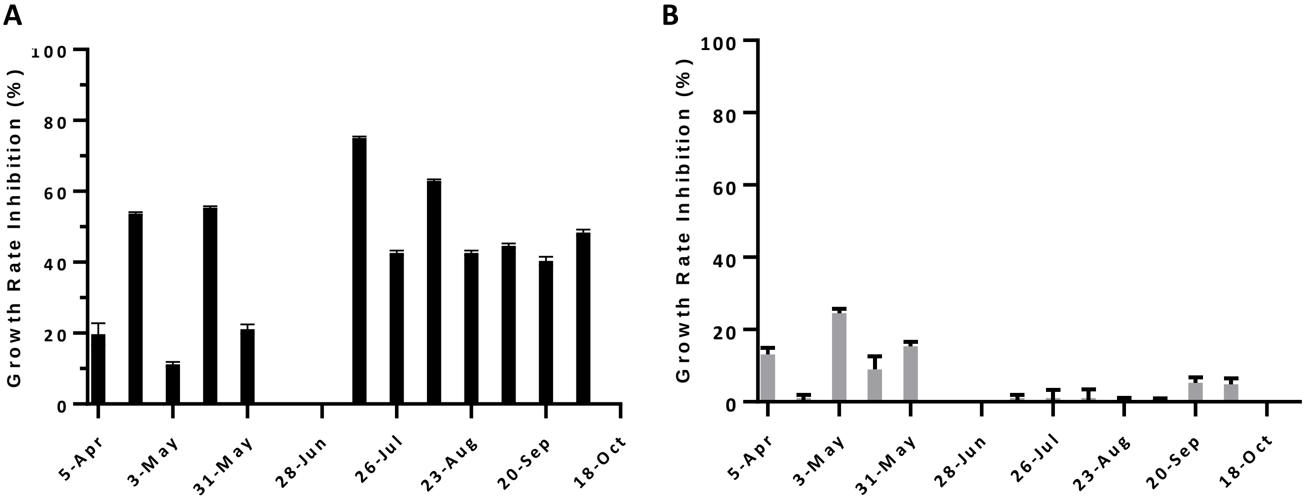

Figure 4: The percentage growth rate inhibition observed in *Pseudokirchneriella subcapitata* after exposure to the freshwater finfish aquaculture A) influent and B) effluent for 72 hours at 23°C ± 2°C under continuous illumination. Samples were collected and analysed from April 2018 to October 2018. (n = 3, SEM indicated)

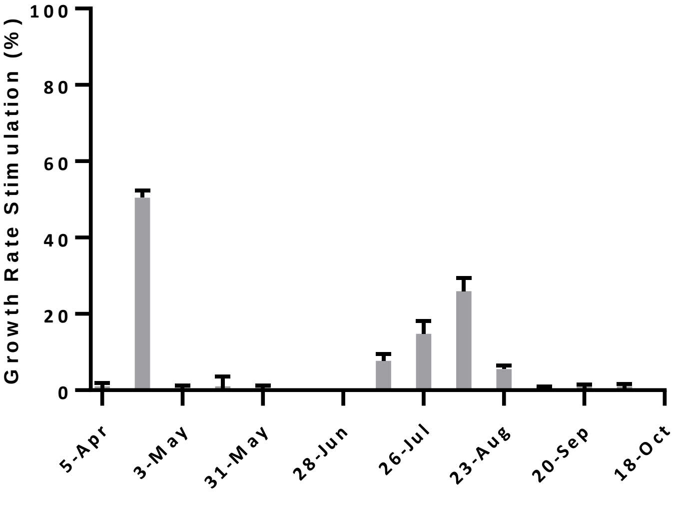

Figure 5: The percentage of *Pseudokirchneriella subcapitata* growth rate stimulation observed in the freshwater finfish aquaculture effluent. Exposure was for 72 hours at 23°C ± 2°C under continuous illumination. No stimulation was observed in the influent. Samples were collects and analysed from April 2018 to October 2018. (n = 3, SEM indicated).

Correlation Studies

The Pearson's correlation test (Table 3), demonstrated that in the effluent, the algae (*Pseudokirchneriella subcapitata*), was negatively correlated with temperature and suspended solids and positively correlated with alkalinity. Nitrite was positively correlated with orthophosphate. Temperature was negatively correlated with dissolved oxygen and positively correlated with conductivity. The parameters hardness and alkalinity were negatively correlated with orthophosphate. Dissolved oxygen was negatively correlated with conductivity and positively correlated with nitrate. Hardness was positively correlated with alkalinity and a negative correlation with nitrite. The pH had a negative correlation with conductivity.

Table 3: Correlation matrix for the algae (*Pseudokirchneriella subcapitata*), and physicochemical parameters investigated on the Irish freshwater finfish aquaculture effluent. Bold figures indicate where significant differences ($p < 0.05$), have been observed. Positive figures indicate a direct relationship, *i.e.* as the concentration in one parameter increases, so does the concentration in the other parameter under investigation. Negative figures indicate an inverse relationship, *i.e.* as the concentration in one parameter increases, it decreases in the other parameter. Breakdown of correlation figures are indicated in box. Breakdown of correlation values is based on Ratner (2009), and his work correlation coefficient values.

	Algae	**pH**	**Temp**	**NH_4^+**	**NO_2^-**	**NO_3^-**	**PO_4^{3-}**	**DO**	**BOD**	**COD**	**SS**	**Hard.**	**Alk.**
Algae	1												
pH	0.112	1											
Temp	**-0.619**	-0.480	1										
NH_4^+	-0.285	-0.437	0.414	1									
NO_2^-	-0.307	-0.389	0.213	**0.554**	1								
NO_3^-	0.164	-0.468	-0.475	-0.346	-0.100	1							
PO_4^{3-}	-0.393	-0.435	0.209	0.308	**0.651**	0.320	1						
DO	0.356	0.408	**-0.846**	-0.492	-0.298	**0.578**	-0.028	1					
BOD	-0.197	-0.307	0.008	-0.065	0.048	0.251	0.310	0.422	1				
COD	0.270	0.310	-0.216	-0.083	-0.430	-0.003	-0.088	0.004	-0.495	1			
SS	**-0.727**	0.228	0.244	-0.064	-0.191	-0.121	-0.139	0.069	0.399	-0.345	1		
Hard.	0.343	0.393	-0.021	-0.095	**-0.580**	-0.335	**-0.885**	-0.156	-0.199	0.085	0.115	1	
Alk.	**0.607**	0.224	0.034	-0.408	-0.493	-0.228	**-0.657**	-0.240	-0.359	0.098	-0.368	**0.701**	1
Cond.	-0.292	**-0.547**	**0.841**	0.394	0.251	-0.485	0.138	**-0.851**	-0.227	-0.083	-0.174	0.101	0.344

0 = No relationship
>0 – 0.3 = Weak relationship
0.3 – 0.5 = Moderately weak relationship
0.5 – 0.7 = Moderately strong relationship
0.7 - <1 = Strong relationship
1 = Perfect linear relationship

Temp = Temperature, DO = Dissolved Oxygen, BOD = Biochemical Oxygen Demand, COD = Chemical Oxygen Demand, SS = Suspended Solids, Hard. = Hardness, Alk. = Alkalinity, Cond. = Conductivity.

Weather Conditions

Ireland experienced one of its hottest summers on record in 2018 (Met Eireann, 2018b). Drought conditions and a national hose pipe ban was put in place for most of the country up to the end of August 2018 (Irish Water, 2018). As a result of these unusual weather conditions mean rainfall and temperature data collected at three Met Eireann weather stations surrounding and closest to the fish farm (Figure 1), were observed. These stations were located in; Markree, Knock and Mount Dillon. Decreases in the average monthly rainfall and increases in temperature were observed across the weather stations (Figure 6). Statistical analysis suggested that moderately strong inverse relationship existed between the algae and the rainfall (r = -0.562), and a weak relationship between the algae and the temperature (r = 0.276). Figure 7 compares the average temperatures observed across the stations and the average maximum temperatures experienced. When the maximum temperatures were taken into account, a moderately strong relationship between the algae and temperature was observed (r = 0.505).

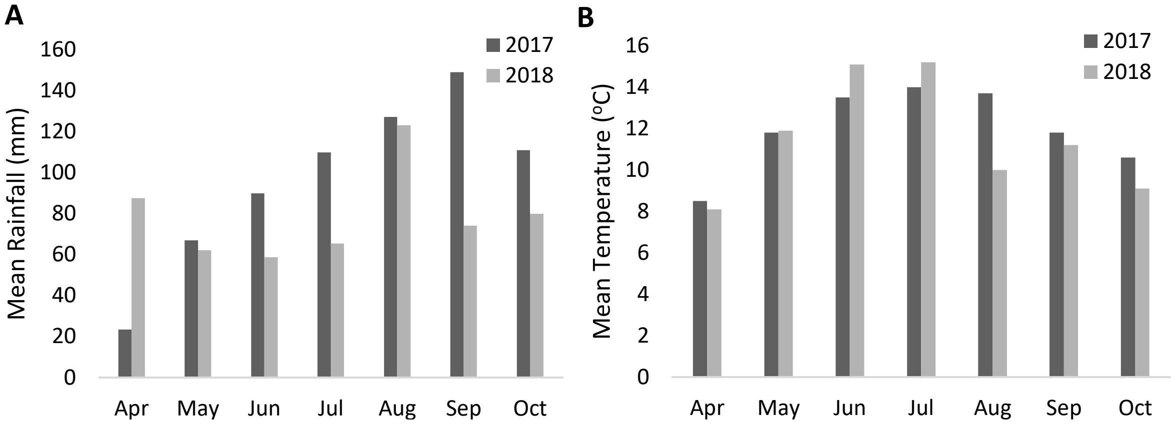

Figure 6: Mean rainfall in mm (A), and temperature in ºC (B), collected from three Met Eireann weather stations located at; 1) Markree, Co. Sligo, 2) Knock, Co. Mayo, and 3) Mount Dillon, Co Roscommon. These stations were selected as they were located to the north-west, south-west and south-east of the fish farm investigated in this study. Data from April to October in 2017(dark grey), and 2018 (light grey), were examined.

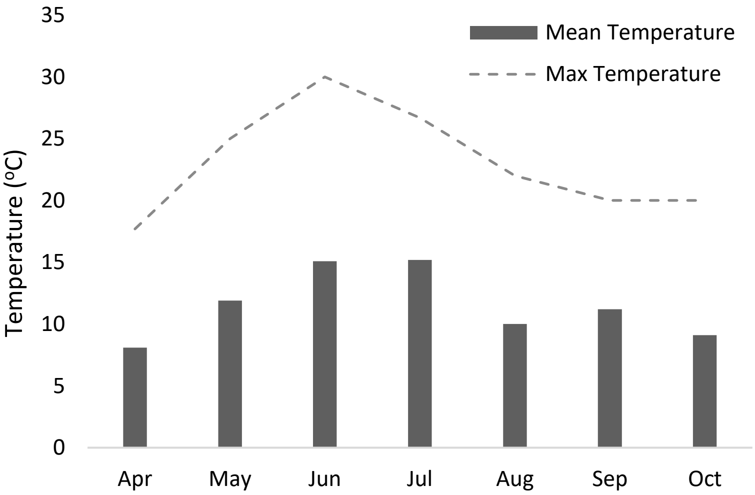

Figure 7: Mean temperatures (Bar Chart), and maximum temperatures (Line Chart), observed at three Met Eireann weather stations located closest to the fish farm, from April 2018 to October 2018. Data was based on all temperature readings collected at the three weather stations by Met Eireann (Met Eireann, 2018a).

4. Discussion

Physicochemical evaluation:

As far as the authors are aware, water quality parameters specific for aquaculture effluent in Irish water are not currently available and the Irish EPA are currently investigating the regulation of Irish freshwater aquaculture effluent. Recommended water quality parameters set out by the Statutory Instrument (S.I.) 272/2009 (European Communities Environmental Objectives – Surface Waters – Regulations 2009), and the Irish Environmental Protection Agency's (EPA), parameters of water quality were therefore used as guidance (Environmental Protection Agency, 2001; Irish Stationery Office, 2009a, 2009b). The parameters set out in the above-mentioned EPA document are based on the Freshwater Fish Directive [78/659/EEC] and/or Surface Water Regulations [1989].

Ammonium is highly toxic to aquatic life (Zhang *et al.*, 2011), and requires treatment before its release into its receiving water body (Celik *et al.*, 2001). When comparing both samples, the concentration of ammonium present in the influent is lower than that of the effluent. This suggested that the levels of ammonium detected are being generated within the farm itself. However, the small amount detected in the influent also suggests some form of pollution is being generated upstream of the farm. The concentrations observed in the effluent were more than five times greater than the one mg L^{-1} suggested by the EPA (Environmental Protection Agency, 2001). However, the concentrations observed in this study were similar to those determined by Boaventura *et al.* (1997), in their study on trout effluent, and Costanzo *et al*. (2004), in their study on shrimp pond effluent. Presence of ammonium is an indicator of recent pollution. As expected, a moderately strong correlation was observed between the ammonium and nitrate levels ($r = 0.554$), *i.e.* as ammonium levels increased so did nitrite levels as the ammonium was converted to nitrite.

As expected, nitrite levels detected were very low. These low levels are mainly due to the fact that, although highly toxic to aquatic life (Pollice *et al.*, 2002), nitrite is highly unstable and only remains in this form during for a short period of time during the transformation of ammonium to nitrate (Durborowet *et al.*, 1997). When comparing the nitrite levels in both samples, levels in the effluent were very much higher than that of the influent. Similar with the ammonium, the small amount detected in the influent suggests some form of pollution is being generated upstream of the farm. The higher levels detected in the effluent are generated within the farm itself. The concentrations detected in the effluent were one log dose greater than the 0.03 mg L^{-1} for cyprinid waters, as per the EPAs suggested water quality parameters (Environmental Protection Agency, 2001). Despite this, nitrite levels determined were in agreement with levels observed by other research conducted on shrimp, trout and prawn effluents (Mcintosh and Fitzsimmons, 2003; Pulatsü *et al.*, 2004; Moreira *et al.*, 2010; Herbeck *et al.*, 2013; Caramel *et al.*, 2014). Similar with ammonium, the presence of nitrite is an indication of recent pollution. As previously mentioned, a relationship was observed between the nitrite and ammonium. There was also a moderately strong relationship observed between the nitrite and the orthophosphate suggesting that increases in nitrite levels coincided in increases in orthophosphate levels.

Nitrate levels observed in the influent indicated that nitrate may have been entering the river upstream of the fish farm. It was also observed that the nitrate in the influent was at a greater concentration than the effluent. The decrease in nitrate levels between the influent and effluent may be due to its being utilised within the farm, *e.g*. *Lemna minor* (duckweed), was present in the farm and can use nitrate as a source of nutrients (Stewart and Rhodes, 1976). A spike in the effluent at the end of September 2018 was observed and may have been due to the fact that the *Lemna minor* within the farm had been removed. Nitrate levels were well below the guidance value of 50 mg L^{-1} suggested by the EPA (Environmental Protection Agency, 2001). Similar levels of nitrate detection as those observed in this study where replicated by Camargo (1994), Boaventura *et al.* (1997), Pulatsü *et al.* (2004), Guilpart *et al.* (2012), and Lalonde *et al.* (2014), who all investigated trout aquaculture, and by Mcintosh and Fitzsimmons (2003), Biao *et al.* (2004), Costanzo *et al.* (2004), Ferreira *et al.* (2011) and Herbeck *et al.* (2013), and their studies on shrimp. A correlation was found between nitrate and dissolved oxygen levels ($r = 0.578$), suggesting that as dissolved oxygen levels increased so did nitrate levels. This was expected as oxygen is required for the aerobic conversion of nitrite (NO_2^-), to Nitrate (NO_3^-). Additionally, due to *Lemna minor*s ability to use nitrate as a nutrient source and the spikes observed in the farm after its removal suggests that the duckweed hold great potential as a potential wastewater treatment option for aquaculture and further research into this possibility needs to be conducted.

Orthophosphate, a reactive form of phosphorus (Brogan *et al.*, 2001), was detected in both the influent and effluent. The levels observed in the influent were less than that in the effluent. This suggested that phosphorus pollution is entering the river upstream of the fish farm, as well as being generated within the farm itself. Levels in both the influent and effluent may be cause for concern as orthophosphates are one of the main causes of algal blooms and the hypoxic conditions which may occur in water bodies (Brogan *et al.*, 2001; Barcellos *et al.*, 2019). Concentrations detected were just over two log doses greater than the recommended value of 0.035 mg L^{-1} set out by the S.I. 272/2009 for good water status (Irish Stationery Office, 2009b). However, they were in accordance with Stephens and Farris (2004b, 2004a), and Ziemann *et al.* (1992) and their studies on finfish farming effluent, including catfish. The farm uses a constructed wetland pond for treatment of nitrates, phosphates, *etc.*, before being released. However, it has been suggested that the wetland may need to be 0.7 to 2.7 times the size of the culture area to be effective and can be less efficient in the removal of phosphorus wastes (Jegatheesan *et al.*, 2011; Sharrer *et al.*, 2016). As previously indicated, a correlation between phosphate and nitrite was observed. A strong and moderately strong relationship was observed between hardness ($r = -0.885$), and alkalinity ($r = -0.657$), respectively. This suggested that as increases in orthophosphate was observed, decreases in both hardness and alkalinity occurred.

The recommended dissolved oxygen concentration present in salmonid waters should be ≥ 9 mg L^{-1} and cyprinid waters (*e.g.* perch) should be ≥ 7 mg O_2 L^{-1} (Environmental Protection Agency, 2001). There are no issues with the DO levels present in the influent however there may be cause for concern with levels observed in the effluent as they were well below the recommended concentration of ≥ 7 mg O_2 L^{-1}. Levels below this concentration were only observed during the heat wave and drought conditions and the unusual weather conditions may have played a role. Conditions began to improve once weather conditions had returned normal. Alam *et al*. (2007), and da Silva *et al*. (2017), have suggested that oxygen concentrations of ≥ 4 mg L^{-1} are sufficient for maintenance of aquatic life. Dissolved oxygen levels in other studies, which included shrimp, catfish, prawn and trout farming, were similar to the concentrations observed in this study (Camargo, 1994; Mcintosh and Fitzsimmons, 2003; Biao *et al.*, 2004; Stephens and Farris, 2004b, 2004a; Moreira *et al.*, 2010; Namin *et al.*, 2013; da Silva *et al.*, 2017). Relationships were observed between the dissolved oxygen and the temperature and conductivity results. A strong negative correlation between the temperature and dissolved oxygen levels was indicated ($r = -0.846$), indicating that as temperatures increased, dissolved oxygen levels decreased. A strong negative correlation was also observed between dissolved oxygen concentrations and conductivity measurement ($r = -0.851$), suggesting as dissolved oxygen levels decreased, conductivity increased.

BOD is the amount of oxygen used by bacteria in breaking down organic matter in the water (Lee and Nikraz, 2015). S.I. 272/ 2009 recommend a mean BOD concentration of 1.3 mg L^{-1} for high water status and 1.5 mg L^{-1} for good water status. However, the EPA has suggested ≤ 3 mg L^{-1} and ≤ 5 mg L^{-1} for salmonid and cyprinid waters, respectively (Environmental Protection Agency, 2001). The current BOD levels detected in the influent suggested no issues. The concentration of BOD detected in the effluent may be cause for concern. Although the level was below that suggested by the EPA for cyprinid water, it was greater than that suggested in the S.I. 272 of 2009. This research was then compared to results determined by other researchers. Although not many studies included BOD, those that were revised demonstrated higher levels than the concentrations detected in this study

(Boaventura *et al.*, 1997; Mcintosh and Fitzsimmons, 2003; Ansah *et al.*, 2012; Miashiro, Lombardi and Mercante, 2012).

COD measures the stress a quantity of organic matter puts on a receiving water body (Lee and Nikraz, 2015). COD was detected in both the influent and effluent. The levels observed in both sets of samples may be cause for concern, especially the effluent. The mean concentration was almost double the suggested 40 mg L^{-1} set out by the Irish EPA (Environmental Protection Agency, 2001). Very few studies included COD as a parameter in their investigations. However, research conducted by da Silva *et al.* (2017), reported some COD levels similar to those determined in this research.

Suspended solids often consist of organic matter and elevated levels can be an indicator of eutrophic conditions (Bilotta and Brazier, 2008). Two concentrations of suspended solids have been suggested by the Irish EPA (Environmental Protection Agency, 2001). Fifty mg L^{-1} as per the Surface Water Regulations [1989], and 25 mg L^{-1} as per the Freshwater Fish Directive [78/659/EEC], and Salmonid Waters Regulations [1988]. Suspended solids can increase gill irritation and blanket the benthos (Bilotta and Brazier, 2008), therefore the lower concentration of 25 mg L^{-1} was taken as the maximum allowable concentration (MAC), in this study of an Irish freshwater finfish farm. The average levels detected in both the influent and effluent may be cause for concern as they were above the 25 mg L^{-1}. However, suspended solid concentrations in a range of studies (shrimp, prawn, salmonid, catfish, brown trout and rainbow trout), were similar to those established in this study (Ziemann *et al.*, 1992; Camargo, 1994; Boaventura *et al.*, 1997; Mcintosh and Fitzsimmons, 2003; Costanzo *et al.*, 2004; Pulatsü *et al.*, 2004; Guilpart *et al.*, 2012; Caramel *et al.*, 2014; Lalonde *et al.*, 2014). As previously mentioned, a strong relationship was indicated between the stimulation of algae and increases in suspended solid concentrations.

With growing concerns associated with climate change and global warming, increases in temperatures may become more frequent. Temperature is a critical environmental factor for aquaculture due to its effect on growth, metabolism, survival, immune responses and oxygen consumption (Ferreira *et al.*, 2011). Fluctuations in temperature were observed in both sets of samples. These rises in temperatures were only observed during the elevated temperatures experienced in Ireland in 2018. The results for the pH indicated that the influent was slightly more alkaline than the effluent, which held a pH of just above neutral (pH 7). The slight difference in pH levels in the samples may have been due to the alkalinity levels observed in the samples. The effluent had a higher level of $CaCO_3$ and thus a better buffering capacity. The recommended pH levels should be between 6 and 9. Levels in both the influent and effluent are well within this level and therefore are present no issues. The mean temperature and pH results observed were similar to the those recorded in the revised studies that focused on freshwater finfish, *i.e.* catfish, brown trout and rainbow trout (Boaventura *et al.*, 1997; Pulatsü *et al.*, 2004; Stephens and Farris, 2004a; Živić *et al.*, 2009; Namin *et al.*, 2013; Noroozrajabi *et al.*, 2013; Caramel *et al.*, 2014). A strong relationship was indicated between increases in temperature and increases in algal growth stimulation, increases in conductivity ($r = 0.841$), as well as decreases in dissolved oxygen levels.

Calcium carbonate ($CaCO_3$) improves conditions for benthic animals and microbial activity, increases CO_2, phosphorus and other nutrient availability, improves survival and production, and enhances phytoplankton growth (Ferreira *et al.*, 2011). The alkalinity is the buffering capacity of the water body and is related to important factors in aquaculture (Ferreira *et al.*, 2011). It has been measured as $CaCO_3$. Results suggested that the effluent has a better buffering capacity. This is further confirmed by the pH results. Alkalinity results from shrimp and catfish studies demonstrated similar results (Mcintosh and Fitzsimmons, 2003; Ferreira *et al.*, 2011) . Water hardness is the amount of dissolved calcium and/or magnesium present in the water. The $CaCO_3$ levels were measured for this study. Results suggested that the water is slight to moderately hard. This correlates with water hardness maps of Ireland which demonstrated water around Boyle, Co. Roscommon was slightly too moderately hard. There are no guidance values for alkalinity or hardness as the alkalinity and buffering capacity of water bodies vary throughout the country, as does the water hardness. Similar hardness results were observed in revised studies on catfish and Atlantic salmon (Stephens and Farris, 2004a, 2004b; Lalonde *et al.*, 2014). A strong correlation was observed between harndess and alkalinity (r = 0.701), demonstrating that as hardness levels increased, so too did the alkalinity of the water.

Ecotoxicological bioassay evaluation:

Growth stimulation was observed in the effluent. Stimulation occurred in mid-April and then again from July to September. This coincided with the elevated temperatures and drought conditions experienced in Ireland in the summer of 2018. Ireland's mean summer maxima temperature is between 18°C and 20°C (Walsh, 2012). In 2018, temperatures exceeded 30°C (Met Eireann, 2018b). This resulted in low rainfall levels, *e.g.* the three weather stations (Markree, Knock and Mount Dillion), measured an average total rainfall of only 61.9 mm for the months of May, June and July 2018 compared to 88.9 mm for the same three months in 2017 (Met Eireann, 2018a), leading to a national hose pipe ban and water restrictions (Irish Water, 2018). The ability of the effluent to cause growth stimulation suggested that the possibility of algal blooms (resulting in eutrophication), downstream of the fish farm are more likely to occur. This may result in loss of biodiversity, habitat and submerged aquatic vegetation, disruption of the ecosystems functionality, deficiencies in oxygen and modifications in food webs (Rabalais, 2002). The correlation studies demonstrated a moderate to moderately strong negative/inverse relationship between algal growth inhibition and increases in temperature (r = -0.619), *i.e.* algal growth stimulation increased as temperatures increased. No research readily available to the authors indicated the potential for *Pseudokirchneriella subcapitata* growth stimulation as an indicator of eutrophication suggesting that the alga is being underutilised.

A higher level of growth inhibition was observed in the influent compared to the effluent. This suggested that the influent would seem unlikely to cause issues such as algal blooms. However, the high level of growth inhibition in the influent also indicated toxicity and may result in losses to the biodiversity of the receiving water body (Rabalais, 2002). This toxicity may result in the loss of primary producers (*e.g.* algae), in the aquatic ecosystem. This may subsequently cause indirect adverse effects on the aquatic food chain, *e.g*. micro-crustaceans feed on algae and fish in turn, feed on the micro-crustaceans. Loss of the algae removes the food source for the micro-crustaceans, resulting in their potential loss. This in turn, could result in the removal of a valuable food source for the fish. It should be noted that this toxic effect does not occur within the fish farm itself and suggests potential issues upstream of the farm.

Most of the available research involving *Pseudokirchneriella subcapitata* focused on inhibition of growth (Guéguen *et al.*, 2004; Ivanova and Groudeva, 2006; Ma *et al.*, 2006). One study involving *Pseudokirchneriella subcapitata* and aquaculture effluent was published by Miashiro *et al*. (2012), demonstrated similar results to this study, *i.e.* growth stimulation instead of inhibition was observed. Miashiro *et al*. (2012), suggested that the stimulated algal growth may have been due to the high concentration of nutrients that were observed. High levels of nutrients, were also observed in this study. The lack of available research suggested an under use of *Pseudokirchneriella subcapitata* as an early indicator of potential issues in aquaculture. Correlation studies found a strong negative relationship between the algae and concentration of suspended solids (r = -0.727), suggesting that as the concentration of suspended solids increased, the stimulation of algal growth also increased. Further research will need to be conducted in order to establish the exact cause for the relationship.

Climate Change:

"There's one issue that will define the contours of this century more dramatically than any other, and that is the urgent threat of a changing climate. Climate change is no longer some far-off problem; it is happening here, it is happening now" (Obama, 2015). "We have a single mission: to protect and hand on the planet to the nest generation. The time is past when humankind thought it could selfishly draw on exhaustible resources. We know now the world is not a commodity" (Hollande, 2015). Climate change is any noteworthy change in the average weather over a period of time such as rainfall or temperature measurement (Environmental Protection Agency, 2019), and is considered to be one of the most troubling, challenging and unrelenting scientific issues of our time (Bulkeley and Newell, 2015). Climate change, including global warming, is a complicated and increasingly problematic challenge causing changes to rainfall and hydrology, *e.g.* extensive summer droughts caused by changes in rainfall (Paerl and Scott, 2010; Paerl *et al.*, 2016).

The results observed in this study have demonstrated that climate change may have a direct impact on aquaculture, as suggested by the moderately strong relationship observed between the increases in temperature and the stimulation of algal growth rates, which could lead to increased instances of eutrophication. *Pseudokirchneriella subcapitata* has thus demonstrated its ability to be utilised as a potential early warning indicator of climate change. Additional studies focusing more specifically of climate change and aquaculture will need to be conducted.

5. Conclusion

Evaluation of aquaculture effluent should include ecotoxicological bioassays in order to determine any potential effects the effluent may have on the receiving aquatic ecosystem. Inclusion of the Pseudokirchneriella subcapitata Algal Bioassay in this study has demonstrated the potential eutrophication implications as a result of releasing untreated effluent from fish farms. Additional bioassays that focus on different trophic levels should also be considered in order to develop a broader picture of the potential effect's aquaculture effluent poses on its receiving ecosystems.

Water quality parameters specific for aquaculture effluent has not yet been established in Ireland. The EPA has begun the process of regulating aquaculture effluent. Results observed in this study have demonstrated that water quality parameters suggested by S.I. 272/2009 and the EPA may not be applicable to aquaculture effluent as these results were similarly displayed in other aquaculture studies. However, the dilution factor of the receiving aquatic ecosystem is important and therefore also needs to be taken into consideration when these water quality parameters are to be determined.

Results have also indicated that influent water quality is also as important when assessing aquaculture effluent as it may indicate potential environmental issues as a result of works upstream and not that which is occurring within the farm.

Although this study focused on the ecotoxicological effects freshwater aquaculture effluent may induce on its receiving aquatic ecosystem, the research suggested that the changes in temperatures that were observed during the heat wave and drought conditions experienced in the summer months of 2018 had a direct relationship with the increased levels of algal growth stimulation detected. With irregular weather patterns becoming more frequent, especially rises in mean temperatures, due to global warming, further research into the effects of climate change on aquatic ecosystems, aquaculture effluent and the effects of effluents on its receiving ecosystem will need to be conducted. The research conducted in this study has suggested a potential toolbox that may provide an early warning system for adverse effects as a result of climate change.

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