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Using seasonal variability of water quality parameters to assess the risk of aquatic pollution from rainbow trout fish farms in Greece

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Abstract

Sustainable development of fresh water (FW) aquaculture requires minimal environmental impact, for example by reducing the waste discharged on the surrounding waters. To assess the water quality status and impact of flow-through trout farming in the Louros river (NW Greece), a seasonal evaluation of the trout production in the river was performed. Seasonal samples of river water entering and discharged (water inlet and outlet) were obtained to monitor parameters, such as temperature, pH, ammonium, phosphate, dissolved oxygen (DO), biochemical oxygen demand (BOD_5), specific conductivity(SpC), total dissolved solids (TDS), total nitrogen (TN), and total phosphorus (TP) of flow-through trout farms alongside the river were analysed. The monitored parameters in the water outlets indicated minimal environmental impact of the fish farms. Mean pH ranged from 7.57 to 8.03, TDS ranged from 151.43 to 242.56 mg/L, DO ranged from 6.28 to 9.16 mgO_2/L, BOD ranged from 0 to 2 mg O_2/L. As for the nutrients, mean values were below each limit set for freshwater systems. NH_4-N ranged from 0 to 0.28 mg/L, and PO_4-P ranged from 0.15 to 0.42 mg/L. Based on the comparison of water quality parameters in the outlets and on the Environmental Impacts and Environmental Quality Standards (EQS), it can be concluded that trout fish farms had no significant environmental impact on the river stream water quality during any of the tested seasons.

Keywords: *Freshwater aquaculture, Water quality, Environmental impact, Rainbow trout, Greece*.

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Sustainable development of fresh water (FW) aquaculture requires minimal environmental impact, for example by reducing the waste discharged on the surrounding waters. To assess the water quality status and impact of flow-through trout farming in the Louros river (NW Greece), a seasonal evaluation of the trout production in the river was performed. Seasonal samples of river water entering and discharged (water inlet and outlet) were obtained to monitor parameters, such as temperature, pH, ammonium, phosphate, dissolved oxygen (DO), biochemical oxygen demand (BOD_5), specific conductivity(SpC), total dissolved solids (TDS), total nitrogen (TN), and total phosphorus (TP) of flow-through trout farms alongside the river were analysed. The monitored parameters in the water outlets indicated minimal environmental impact of the fish farms. Mean pH ranged from 7.57 to 8.03, TDS ranged from 151.43 to 242.56 mg/L, DO ranged from 6.28 to 9.16 mgO_2/L, BOD ranged from 0 to 2 mg O_2/L. As for the nutrients, mean values were below each limit set for freshwater systems. NH_4-N ranged from 0 to 0.28 mg/L, and PO_4-P ranged from 0.15 to 0.42 mg/L. Based on the comparison of water quality parameters in the outlets and on the Environmental Impacts and Environmental Quality Standards (EQS), it can be concluded that trout fish farms had no significant environmental impact on the river stream water quality during any of the tested seasons.

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Introduction

Aquaculture production and the land-based systems

The aquaculture industry is highly developed in Greece, and in the last 20 years, especially, it has been highly ranked among European production standards with marine aquaculture contributing 125,772 tonnes per annum (2018) to the world aquaculture market. Freshwater systems situated mostly in land-based facilities adjacent to rivers usually focus on trout and limited quantities of carp in raceways (FAO, 2020). More than 78 farms of freshwater aquaculture are present on the mainland. Seventy-two organizations are working with lagoon aquaculture, mostly working as cooperatives, which occupies a space of 40,000 ha with a production of around 600 tones. The number of farms has been relatively stable and did not expand during the current period of economic problems in Greece (FGM, 2017).

Environmental Impacts and Environmental Quality Standards (EQS)

Several parameters can contribute to the environmental impact of aquaculture including energy consunmption, production of equipment and feed, as well several other operational features of a fish farm (Konstantinidis et al. 2020). The potential aquatic pollution of aquaculture can be divided into two categories depending on the water system and the release of its sewage (freshwater or marine systems). The impact of water quality mainly includes changes in the surface and column of the water ecosystems and changes in the sediment ecosystems (Beveridge 2004; Mavraganis et al. 2020; Mpeza et al. 2013; Telfer and Beveridge 2001). In freshwater aquaculture, the ecosystem impact can be monitored by assessing the water quality parameters to test the surface water for changes in nutrient levels (Black 2001; Bunting 2013; Pillay 2004). Information concerning the freshwater aquaculture impact has been addressed in several studies at both general (summary of impacts globally) and individual (important or local cases) levels. The farming process also causes altered oxygen levels in used water because nutrients leading to plant and organism growth use oxygen; eventually, the system develops hypoxia or in extreme cases, anoxia. For this reason, both dissolved oxygen (DO) and the biochemical oxygen demand (BOD) are important parameters for assessing water quality (Allan and Castillo 2007; Black 2001; Enderlein et al. 1996; Henriksson et al. 2015). An example of oxygen interaction is found in freshwater wetlands in which the hydric soil remains wet enough to create oxygen-poor/anaerobic conditions. Generally, DO is needed by fish and other aquatic organisms for their respiration and thereby is an important parameter both for the fish growing on the farm and for the organisms off of the farms.

Low oxygen indicates pollution stress and/or pollution phenomena (EPA 2000; White 2013). Other important parameters in addition to total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), BOD on the water quality are the total carbon (TC), calcium carbonate ($CaCO_3$), and carbon dioxide (CO_2). These parameters have been reported in various books and papers regarding water quality and environmental impact of fish farming in freshwater (Cromey et al. 2018; DFO 2015; Enderlein et al. 1996; Henriksson et al. 2015; Turner et al. 2007).

DO is utilized by the fish biomass to accommodate the metabolism and is excreted as CO_2, transferred to the plants with the used water out of the farm, and eventually consumed by the plants as part of the photosynthesis process. BOD is a traditional measure of the oxygen-consuming strength of various organic wastes; it is a useful water-quality management tool for comparison of aquaculture effluents with various other agricultural and manufacturing process waste. While aquaculture pond and flow-through effluents have BODs slightly higher than their source waters, their BOD levels are far below the degrading strength of many raw agricultural municipal and industrial process waste materials and closer to the post-treatment levels of municipal sewage. The notable exceptions for aquaculture by-products are the concentrated waste sludge from return activated sludge (RAS) and unused aquaculture feed, which has extreme degrading potential. Raw fish manure also has a high degrading potential similar to that of livestock manures (Yeo et al. 2004). Several bodies have assessed and regulated the pollution levels of the aquaculture as both single and combined processes. The common policy for the Water Framework Directive (Directive 2000/60/EC) was established by the European Union (2000) in which the approach of using a monitoring regime for the freshwater quality was recommended. Other noted directives are the 2008/105/EC, 91/676/EEC, and 91/271/EEC. Local policies and assessments have also been established so that the impact of freshwater quality can be accurately addressed. Those bodies consist of the ministries and research institutes in each country.

Fish farms effluents and modelling

As with nearly all forms of aquaculture and agriculture, fish farming sites generate considerable amounts of waste, including nutrients, waste feed and faeces, and by-products, such as chemical residues. The impact of aquaculture on the surrounding environment and ecosystem is a function of several parameters, including unconsumed feed, faeces, and both organic and inorganic elements, such as nitrogen (N) molecules (NH_X, NOx, DO_N), and phosphorus (P/PO_X) (Karakassis et al. 2005; Klaoudatos 2001; Wallace 1993). Decomposition of these nitrogenous compounds is particularly important in intensive RAS because of ammonia (NH_3), nitrite, and to some extent, nitrate toxicity. Ammonia appears to have a direct effect on the growth of aquatic animals. Unionized ammonia is toxic to fish at low concentrations with 96-h LC_{50}s varying widely by species from as low as 0.08 mg/L NH_3-N for pink salmon to 2.2 mg/L NH_3-N of common carp. According to the studies on the environmental impact of aquaculture, nutrient and chemical release constitute the major impact in aquatic ecosystems (Black 2001). High nutrient release alters the water quality of the aquatic ecosystems mainly causing eutrophication and hypoxia (Black 2001). The levels of NH_3 and phosphates (PO_4) are the core measurement parameters for estimating the impact of high nutrient release as they are the main products of fish metabolism; the chemical cycles of nitrogen and P produce substances toxic to aquatic life (Black 2001). N and P discharge from fish farms can be determined by monitoring and calculating the discharge based either on records of fish production and feed used or by using feed conservation rates (FCR), such as FCR combined with chemical analyses of feed and fish (OSPAR 2000). Guideline 2 describes the approach to quantify N and P discharge from aquaculture to surface waters (OSPAR 2000) and the model for flow-through systems (FTS) is given as $L= 0.01*(ICi- PCf)$ in which L is the quantity of P or N in a water body (tonnes/year), I is the feed used (tonnes/year), Ci is the phosphorus or nitrogen content in the feed (%), P is the production (tonnes/year), and Cf is the P or N content in the resulting organisms (%). Another quantitate bioenergetic method used for the quantification of TN and TP is described in Osti et al. (2018), in which mass balance models accurately estimated the nutrient loads, which were determined by the product of the water flow values (L/sec) and the TP and TN concentrations (μg /L) in affluent (LA) and effluent (LE) of the Nile tilapia production system in fishponds.

Seasonal variation of Water Quality parameters

Frequently used indicators of land-based aquaculture pollution include seasonal and spatial changes in the P, N, and microbial load, of the aquatic ecosystems upstream and downstream of land-based fish farms (Ackerman and Weisberg 2003). These parameters may vary seasonally and longitudinally according to aquaculture management, flow rates, and other spatial and seasonal factors (Tahar et al. 2018). For example, some farms may use effluent treatments of varying efficacy prior to discharge while others may use different feeding regimes or use different volumes of water and discharge more or less dilute waste (Bergheim and Brinker 2003; Moraes et al. 2016; Rosenthal 1994). In Miao *et al.* (2020), 5-year monitoring results showed that N, P, and N:P ratio levels showed no obvious long-term changes in high-altitude oligotrophic waters in rainbow trout cage aquaculture. In Pejman et al. (2009), water quality parameters were tested for variations during the four seasons, and the natural and inorganic parameter and organic nutrients were the most significant parameters contributing to water quality variations during all seasons. The results from Tahar et al. (2018) in a seasonal evaluation of the water quality parameters in Irish rainbow trout farming showed that except for NH_4-N, no significant differences were observed among the average results obtained for each season for all of the assessed parameters. The analysis of the results obtained for NH_4-N revealed that mean values obtained for the spring season were significantly lower ($P < 0.01$) than the results obtained for any other season.

Greece, research gap, and aim of the present work

In Greece, no reports have been published for assessing the seasonal variations of the water quality parameters and their impact on the local river streams from land-based aquaculture; this lack of reporting can be considered a research gap.

The aim of the present work was to employ aquaculture pollution assessment tools to assess the risk of aquatic pollution by flow-through trout farming in Louros River, NW Greece.

Materials and Methods

Description of the trout farms.

The trout farms examined in this research were seven, all were growing rainbow trout (*Oncorhynchus mykiss*) in flow-through cement ponds, and located by the River Louros in North West Greece. Four out of the seven trout farms were monitored on a seasonal basis for the environmental quality standard (EQS) analysis. Aquaculture production data of all seven trout farms were used for bio-energetic modelling analysis.
The locations of the farms on the river can be seen in Figure 1, which is a map of the four farms along with the direction and extension of the Louros River. The studied farms produce 20.57 tons of fish on average annually. The farms were all located adjacent to the river and operated by challenging the required quantity of river water through a concrete channel. The individual production of fish for the all of the farms can be seen in Table 1. Farm1 was located approximately at the beginning in the main springs of the river. Farm2 was 5 km downstream of the river. Farm3 was 12 km downstream of the river, and Farm4 was 18 km downstream of the river. The project aimed to investigate water quality parameter variability during a whole year. To obtain more precise results and calculate production parameters, such as yearly TN and TP, the water flow of the outlet stations per farm was recorded. The average water flow rate was 105.7 m^3/sec and ranged from 40 to 240 m^3/sec for all of the farms combined depending on the farm size and season.

Sampling, *Lab process*, *and Analysis approach*

The samples were collected during the four seasons starting in Spring (March) to Winter (February). The sampling process and lab analyses were performed using the methodology mentioned in Tahar *et al.* (2018) and Osti *et al.* (2018). The water quality (WQ) parameters were selected based on the studies described above and local government regulations. Those parameters were the Temperature (T in ºC), pH, DO (in mg O_2/L), BOD (in mg O_2/L), specific conductivity (SpC in µS/cm), total dissolved solids (TDS in mg/L), ammonium as N (NH_4-N in mg/L), orthophosphate as phosphorus (PO_4-P in mg/L) and chloride (Cl_2 in mg/L) Temperature, pH, DO, SpC and TDS were measured in situ (HACH HQ40D). The water samples were transferred in a portable refrigerator to the laboratory.

Once the data input were finalized, data were statistically tested for outcome and dispersion by assuming quantities of the inlet and outlet parameters would not show a significant difference, and the parameters' quality standards would be less than the measured ones. Particular focus was given to the nutrients' parameters, which for this project were NH_4-N and PO_4-P. Furthermore, TN and TP were also measured and estimated by calculating the required factors obtained from the bioenergetic models mentioned in Osti *et al.* (2017) and Mavraganis *et al.* (2017). The approach was to compare the Louros quantity of TN and TP expressed in kg per produced ton of rainbow trout along with other farms that were similar in quantity. To calculate the annual TN and TP, the production and the water flow rate were used according to Moraes *et al.* (2016).

The sampling occurred at two stations per site for four farms, one before the farm located on the river (inlet station) while the second site was located after the farm in the river (outlet station). Thus, 24 measurements per site (12*2) per annum were taken for at total of 96 measurements. The sampling methodology and processing of the samples was similar to relevant studies, such as Tahar *et al*. (2018). The water quality parameters data were used to calculate the differential concentrations (Di) by deducting the outlet values (Oi) from the inlet values (Ii) for each parameter (Di = Oi − Ii) for a given sampling effort. To investigate the impact or their potential in water stream, the limits of the water quality parameters and their quality standards were considered and compared with Louros values. The actual data analyses were obtained by initially testing the normality of the groups using Shapiro testing in RStudio Desktop 1.3.1093. When the data were normally distributed, the Student's t-test (one-sample T-tests) was used to check the null hypothesis, which stated that no significant differences in water parameters existed between water inflow and outflow. For the groups that were not normally distributed, the Shapiro's and Mann–Whitney U-tests were used to check the null hypothesis. To compare and analyse data, boxplots were obtained by using RStudio Desktop 1.3.1093 and scatterplots using Microsoft Office Excel 365.

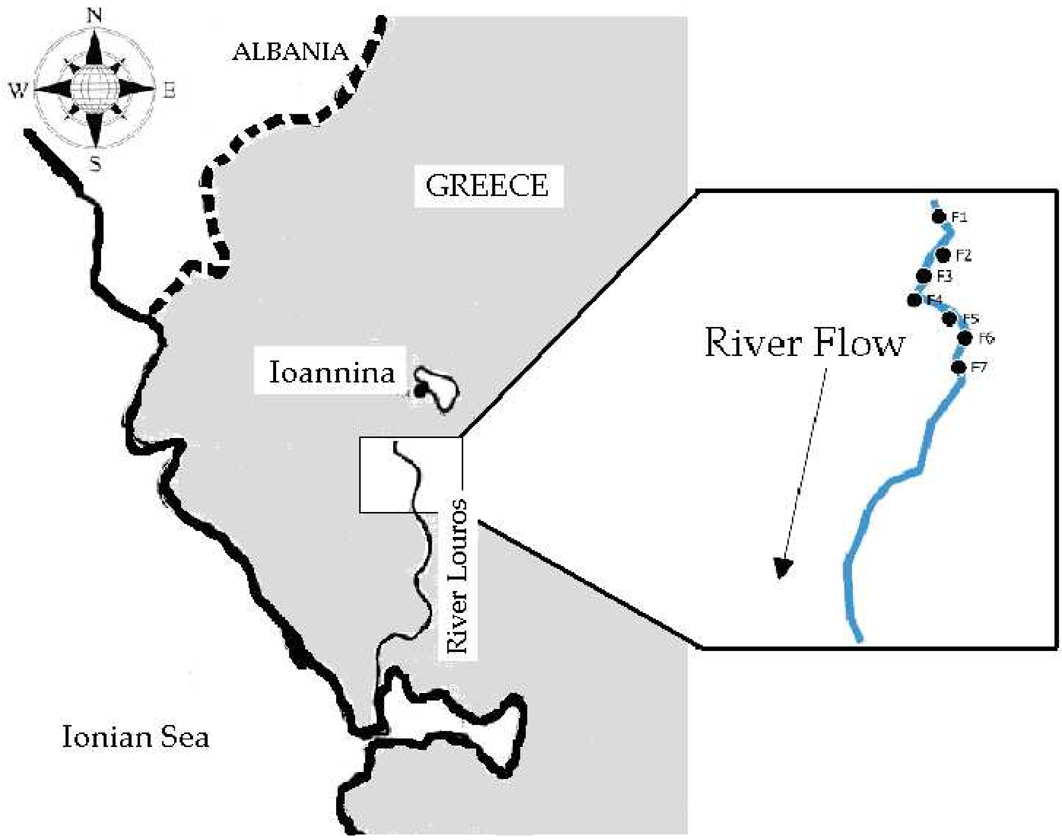


Figure 1. The Louros River in Epirus in North West Greece, the location of the seven tested farms and the water flow direction towards the South. Black arrow indicates the direction of water flow.

Results and Discussion

Nutrient loading and modelling

Daily TN and TP mean values were 45.11 and 7.44 mg/l as can be seen in Table 1. Farm 3 presented the highest concentrations of TN and TP for annual biomass of 30 tons while Farm 7 had the same biomass quantity, but lower nutrient quantity due to higher water flow rate. In Table 2, the annual loading of N and P for average trout farm production was 20.57 ton/year, and the TN and TP concentrations were 1.23 and 0.2 ton/year, respectively. Based on these tables, it was estimated for the River Louros that 15 tons biomass yielded 10.27 mg/l and 1.69 mg/l per day TN and TP, respectively, 20 tons produce 34.4 mg/l and 5.68 mg/l, and finally for 30 tones produce 86.3 mg/l and 14.24 mg/l, respectively. TN and TP were calculated to be 60 and 9.7 kg per ton of produced fish, respectively, for an average of 20.57 tons annually. Comparison with three FTS land-based trout farms of the same mean production showed that TN in Louros was higher and TP was lower but were within the limits of standard deviations (Figure 2). The relationship between mean TN and TP values (Figure 3) was calculated to be: TN = 6.06*TP (approximately) and is dependent on the local water flow of the river. As Figure 3 shows, the linear regressions ($r^2 = 1$) are in the form of $y = ax$, which is natural because as the TN and TP levels grow, production also grows.

Table 1. *TN and TP produced annually in tonnes and the biomass of each farm. The biomass, TN and TP per year are also presented in this table.*

Farms	Biomass t/y	L_N t/y	L_P t/y
Farm1	20	1.2	0.19
Farm2	9	0.54	0.089
Farm3	30	1.8	0.29
Farm4	20	1.2	0.198
Farm5	15	0.9	0.14
Farm6	20	1.2	0.198
Farm7	30	1.8	0.297
Average	**20.57**	**1.23**	**0.20**

Table 2. *Table of the total nitrogen and total phosphorus (TN and TP, respectively) production in mg/L per day of each farm and their average values.*

Farm	TN mg/l/d	TP mg/l/d
Farm1	32.87	5.42
Farm2	29.58	4.88
Farm3	123.28	20.34
Farm4	46.96	7.74
Farm5	10.27	1.69
Farm6	23.48	3.87
Farm7	49.31	8.137
Average	**45.11**	**7.44**

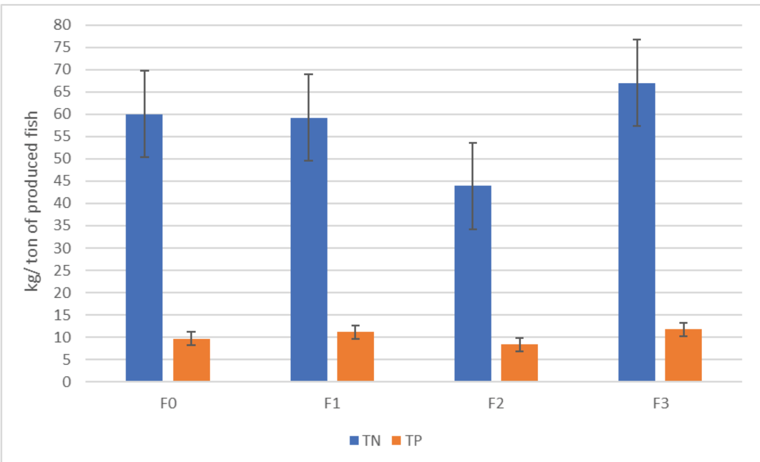


Figure 2. Bar chart of the total nitrogen and total phosphorus (TN and TP, respectively) produced per kg of produced fish during the four seasons (F0 = Spring, F1 = Summer, F2 = Autumn, F3 = Winter).

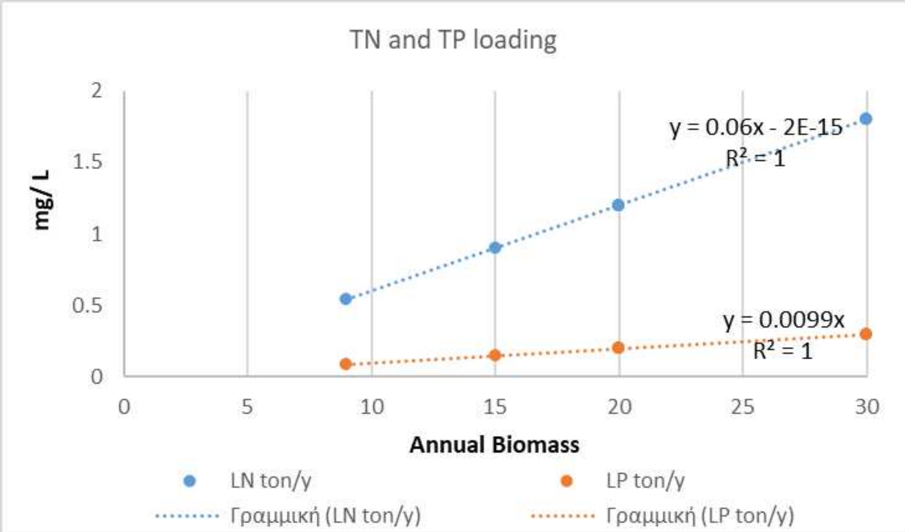


Figure 3. Scatterplot and modelling of TN (L_N) and TP (L_P) in relation to their annual biomass.

Water quality parameters of inlet/outlet relationship

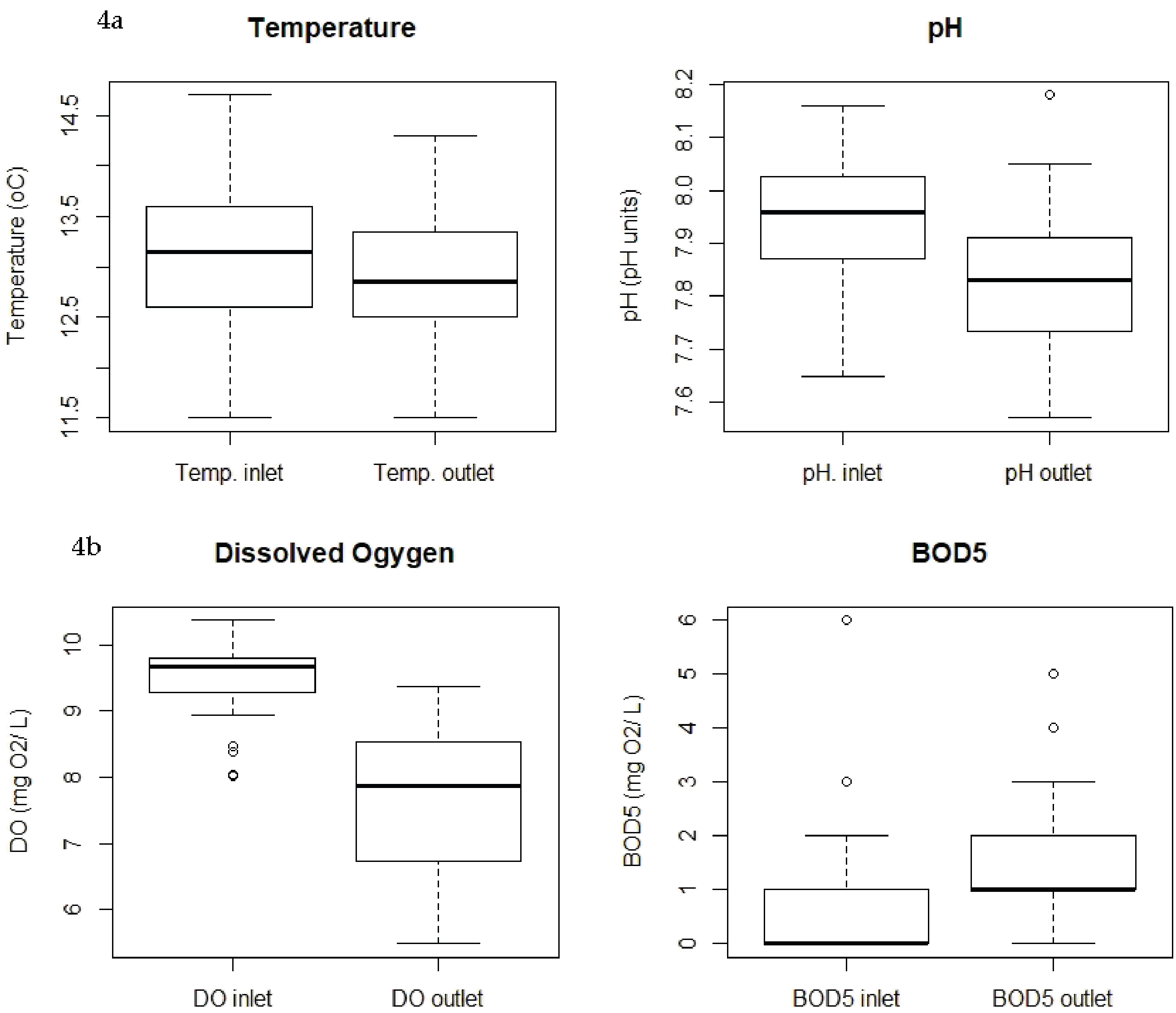
No significant differences were found between the farm inlet and outlet stations for the temperature and SpC and TDS values, showing that the tested fish farms may have not impacted the downstream river quality. Statistical analysis showed significant differences with respect to mean pH, DO, BOD, PO_4-P and NH_4-N parameters at the inlet–outlet for all seasons combined ($p < 0.05$). The temperature remained in the expected range for any season of the whole year, which was 11.6 to 14.3 ^oC (average of 13 ^oC) for all outlet stations. In Figure 4, the inlet–outlet comparison of the parameters, which showed significant differences, can be seen. The mean DO presented a 1.82 mg/L difference in the decrease and the pH presented an increased difference. Mean PO_4 showed a 0.063 mg/L difference in the increase and finally the NH_4 means a 0.066 mg/L difference in the increase.

Another parameter, which was reduced in the outlet was the DO and this can be combined with the increased BOD outlet and pH higher outlet values. It is natural that an increase in biochemical oxygen demand leads to a decrease in DO due to the aerobic activity while P and N are released. Yet again, this result is expected for the FTS as the oxygen is consumed by the fish for their respiration and the excreted faeces use oxygen for their decomposition. The DO values decreased to 1.82 mg O_2/L, which is very close to the differences observed in previous studies in addition to the BOD outlet values, which presented a difference of 0.625 mgO_2/L (Bonaventura et al. 1997; Tahar et al. 2018). The DO and BOD results suggest that the river has the potential to reoxygenate its water from one farm to another.

The increase of 0.065 in NH_4-N can be considered low compared to the ones found in other relevant studies (Bonaventura et al. 1997; Tahar et al. 2018). This finding was also observed for PO_4-P values with an average increase of 0.066, which is within the limits of the standard deviation of the relevant studies for trout farming over a period of a year (Bonaventura et al. 1997; Tahar et al. 2018). A common FTS outcome is an increase in NH_4-N and PO_4-P in outlet measurements, which applies to this study. Since the p-values showed a significant difference between inlet–outlet values, an impact on downstream river water quality occurred because of the potential of the P to cause eutrophication.

However, these results must be seen in combination with quality standards in addition to TN and TP to lead to a holistic conclusion (see sections above).

Finally, for the outlet results analysis, TDS and SpC values corroborate the increase in water quality parameters. The amount of solids and conductivities showed a slight increase within the boundaries of standard deviation from inlet to outlet and since the tests did not yield a significant difference, the potential for possible downstream impact was not analyzed further in this section.



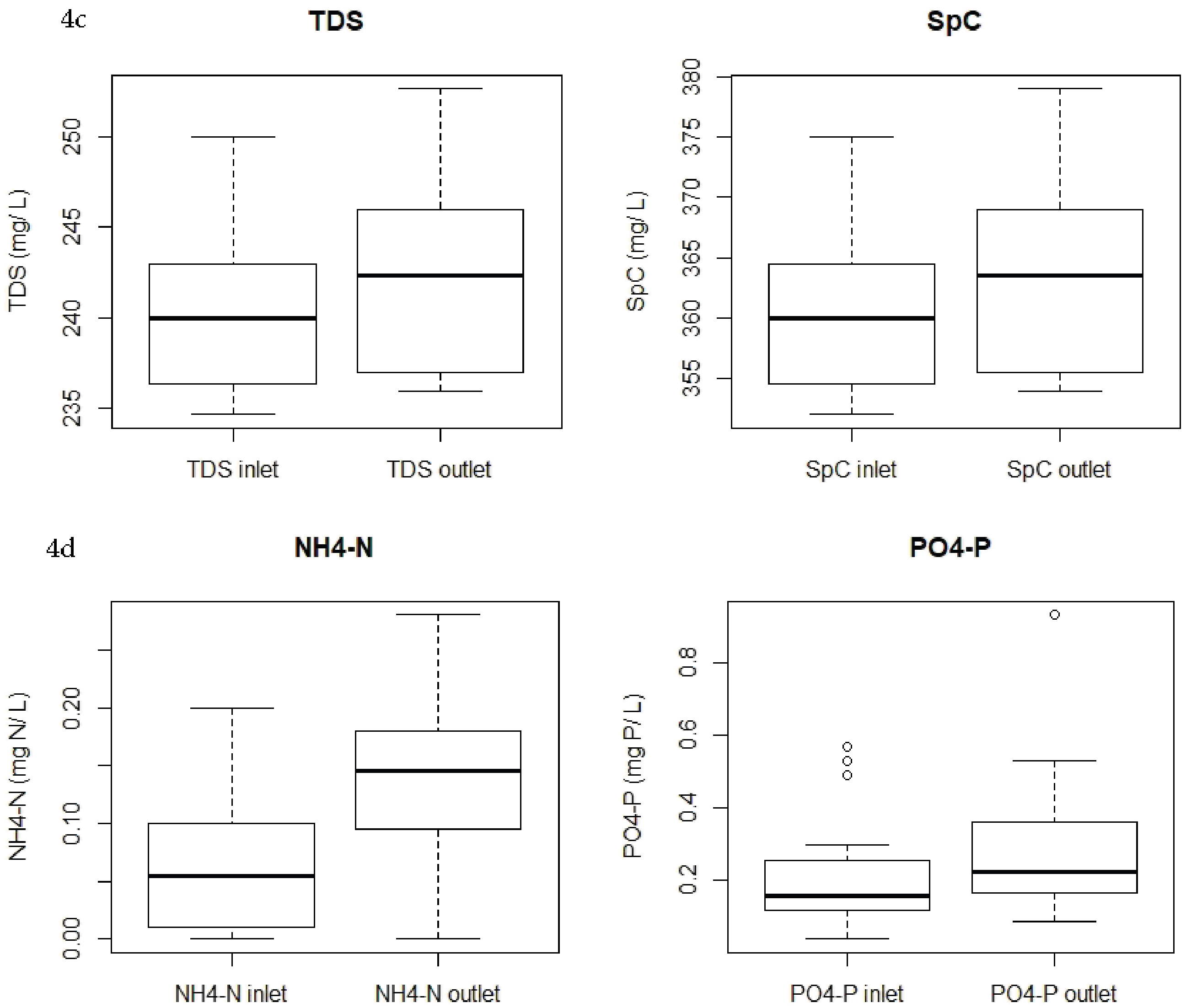


Figure 4. Boxplot representation of the inlet and outlet values for all monitored parameters throughout the whole year. a) Temperature and pH; b)DO and BOD5; c)TDS and SpC; d) NH_4-N and PO_{4-}-P

Seasonal variation and differential concentrations of the water quality parameters

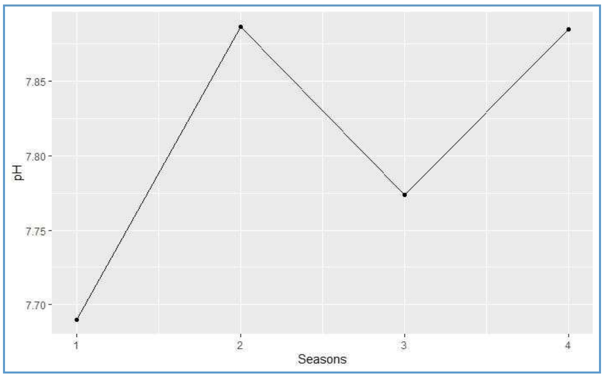
Table 3 and Figure 5 show the summary of the differential means per season for the parameters during the seasons and without distinction among the fish farms. Reductions in SpC, BOD, PO_{4-}-P and NH_4-N in outflows at all seasons were observed, while pH and DO in the outflows, increased in all seasons. These results have an expected outcome as DO outlet values decreased because of the fish growing process (mean differential= -1.82 ± 0.25) and the pH also decreased (mean differential= -0.1 ± 0.068). The BOD outlet values showed an increase (mean differential= 0.53 ± 0.21) due to the need of organisms to feed in the outlet stations. Nutrient release was higher in the outlet values, which is natural for the NH_4-N (mean differential = 0.065 ± 0.04) and PO_4-P (mean differential= 0.067 ± 0.04) values to increase after the enrichment from the culturing process. The outlet parameters did not present significant difference in their differential values (tested from Table 3) between group means as determined by one-way analysis of variance (ANOVA) of $p > 0.05$, which suggests no differences occurred for the whole year for the tested farm site discharge into the river stream.

Table 3. Table representation of the differential concentrations (Di) between inlet and outlet values of all the farms for all tested parameters. The one-way Anova F-ratio and P-values can also be seen.

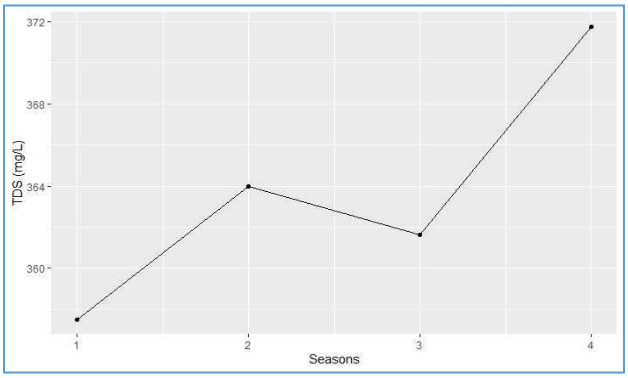
Parameter	Spring	Summer	Autumn	Winter	F-ratio	p-value
pH	−0.16	−0.11	−0.142	−0.01	0.997	0.378
DO	−2.16	−1.73	−1.825	−1.57	1.892	0.164
SpC	0	2.75	3.25	6.5	3.036	0.07
BOD	0.5	0.75	0.625	0.25	1.2	0.319
PO4P	0.08	0.11	0.023	0.05	1.91	0.161
NH4N	0.02	0.058	0.063	0.117	3.067	0.058

The seasonal mean variations for all farms per season can be seen separately in Figure 5. The data were tested for normality and then ANOVA tests were used among the four seasons groups to test the significant differences. No significant differences for any of the parameters were noted, except DO, which showed a significant difference among all groups ($p = 0.0048$). As seen in Figure 5, seasonal variations show a pattern of increasing quantities from spring to summer and decreasing scheme from summer towards winter.

a)



b)



c)

d)

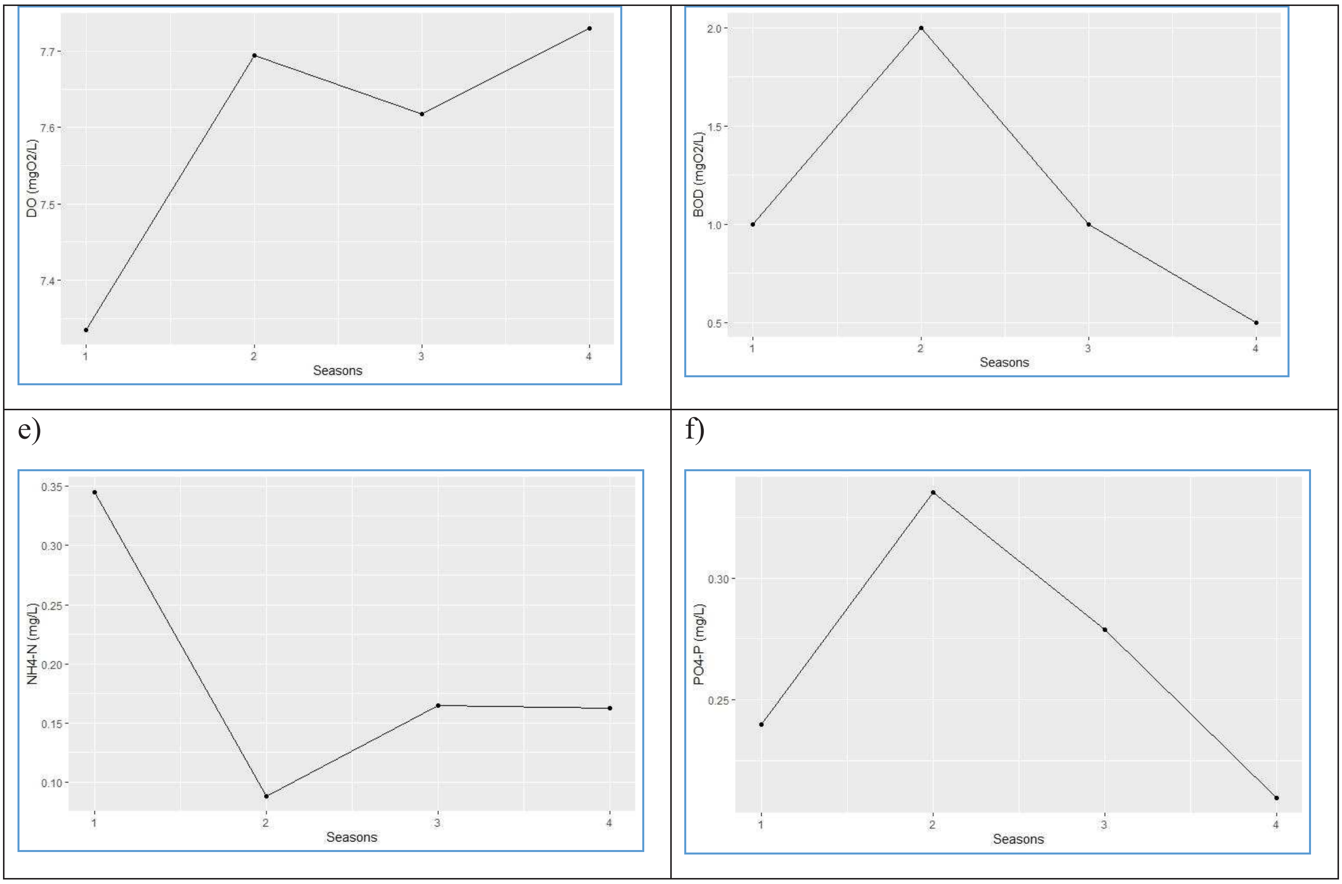


Figure 5. Line graph of the seasonal variation of the mean values grouped for each parameter pH (a), DO (b), SpC (c), BOD (d), PO_4P (e), NH_4N (f), 1= Spring, 2= Summer, 3= Autumn and 4= Winter.

Water Quality environmental impacts

Fig. 6 shows the mean and maximum outlet values of the parameters in reference to EQS standards for all seasons combined in which the means of the parameters were compared to each other for all seasons but separately for each farm. Analytically, the pH ranged from 7.57 to 8.03, which was within the optimal pH levels for both sustaining life in freshwater streams, which is 6.5 to 8.5 (Bonaventura et al. 1997; Tahar et al. 2018) and also the optimal value for fish farms. It has been noted that at a temperature of 13 ºC in conjunction with the mean pH values provides an ideal condition for fish farming.

The TDS must be below 500 mg/L according to the set limits, and the measured TDS values in Louros outlet stations ranged from 151.43 to 242.56 mg/L, which was below this limit and less than its half for the maximum value. The DO values must be higher than 3 mgO_2/L. The DO values of the outlet stations in Louros were all higher than 3 mgO_2/L, ranging from 6.28 to 9.16 mgO_2/L. The BOD5 values for quality water must be below 3 mgO_2/L. The measurements for Louros FTS farms showed a range ranged from 0 to 2 mg O_2/L, indicating the means were much lower at exactly 1 mgO_2/L.

As for the nutrients, the results showed that the mean values were below each limit set for freshwater systems. The NH_4-N means ranged from 0 to 0.28 mg/L, which is below the quality standard value of 0.5 mg/L, and the PO_4-P means ranged from 0.15 to 0.42 mg/L, which is below the standard value of 0.7 mg/L. The comparison of the Louros water quality outlet parameter values along with their limits and

EQS suggest that fish farming had no impact on the river stream water quality during any of the tested seasons.

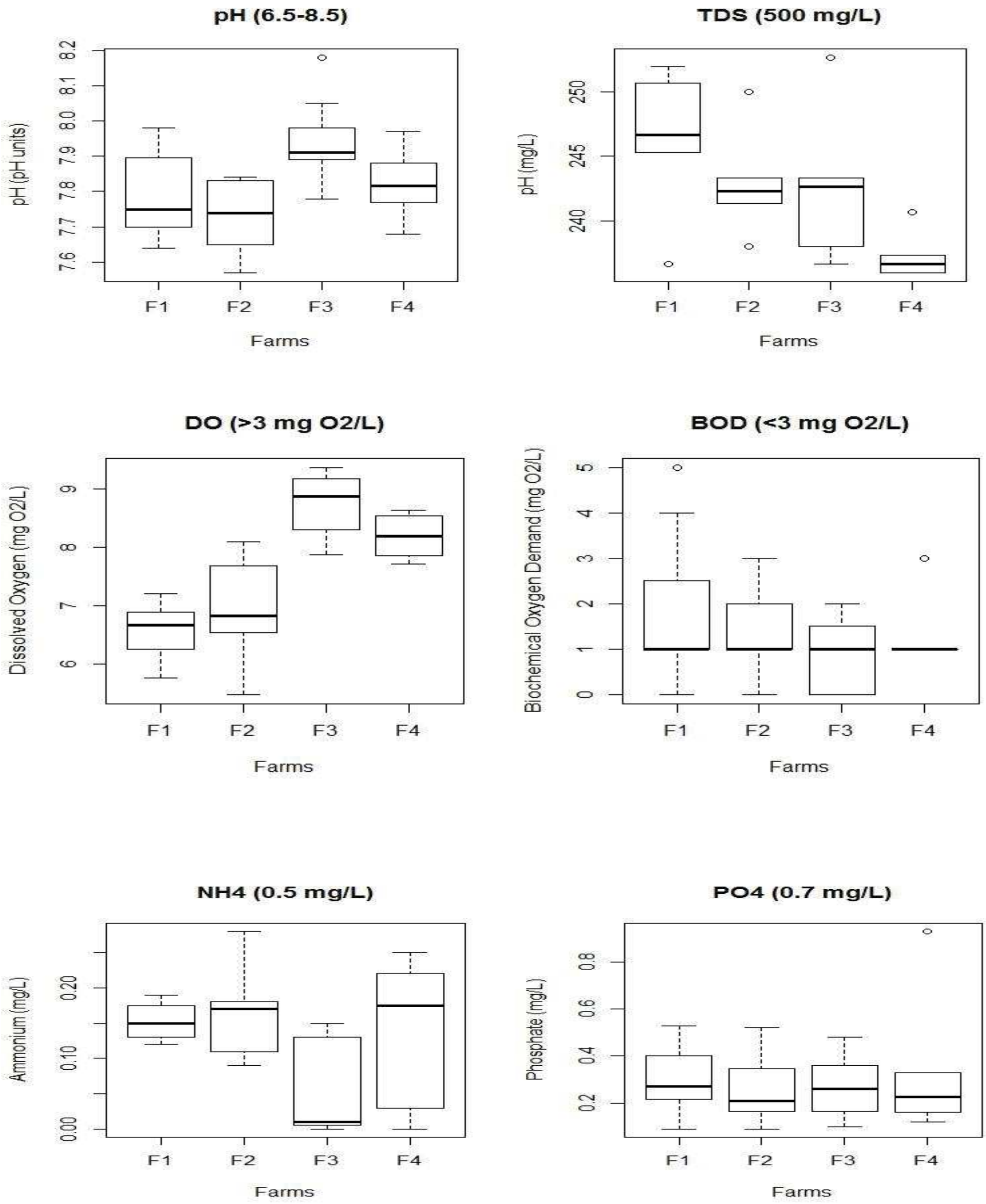


Figure 6. Boxplot of the outlet concentrations for each parameter of all farms and in brackets their limit standards (EQS). Median, Q1, Q4, and Standard deviatiosn are also presented in which F1= Spring, F2= Summer, F3= Autumn, and F4= Winter.

Conclusion

Total Nitrogen and Total Phosphorus of the Flow-Through System farms situated in the Louros River were within the production values which were found on other Flow-Through System farms with the same production rate and Total Nitrogen and Total Phosphorus amount. The farms produced nutrients, solids, and toxicity, which was natural with no indication of farming-related impacts as the outlet values of the relevant water quality parameters were not higher than any established limit or standard value. It was also noted that seasonal variation in the water quality parameters followed the natural course of variations within a year, and even though a natural alteration of the parameters was present in outlet values, their ecological differences were null. All parameters presented strong increasing trends during spring and summer and low decreasing trends in autumn and winter. In conclusion, the results indicate minimal aquaculture impact on river Louros water quality examined parameters.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure

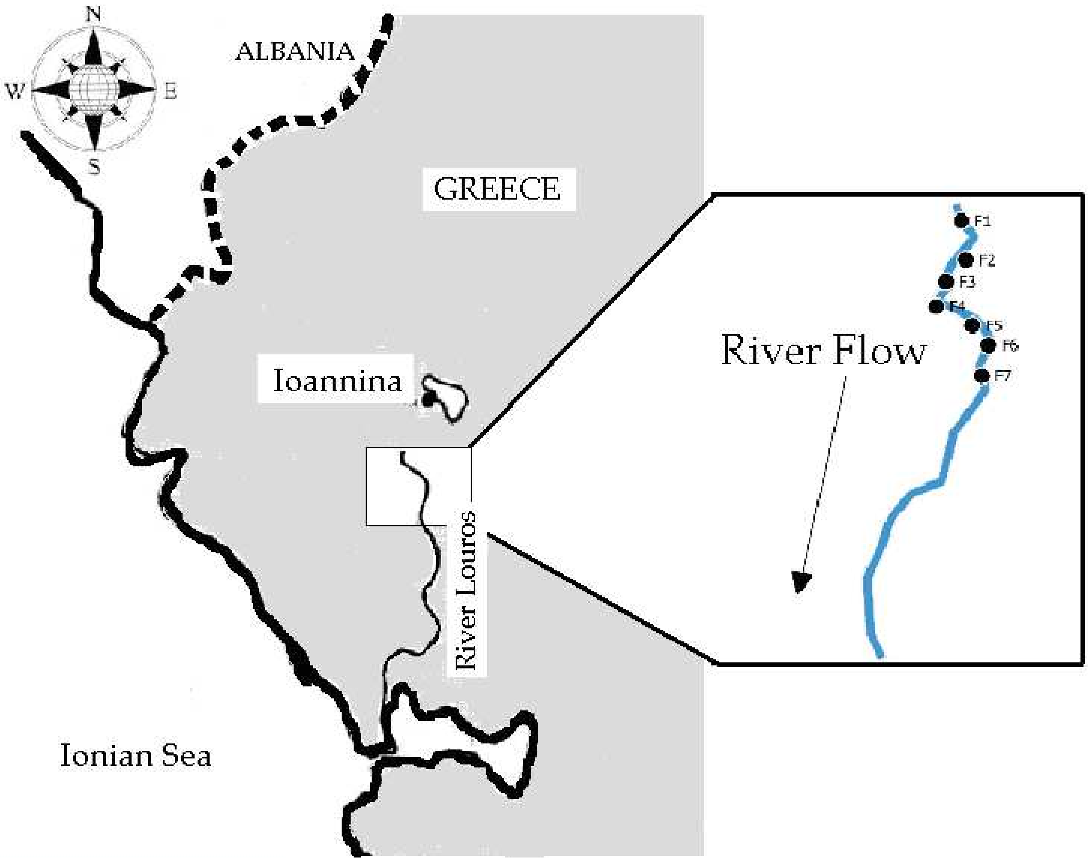


Figure 1. The Louros River in Epirus in North West Greece, the location of the seven tested farms and the water flow direction towards the South. Black arrow indicates the direction of water flow.

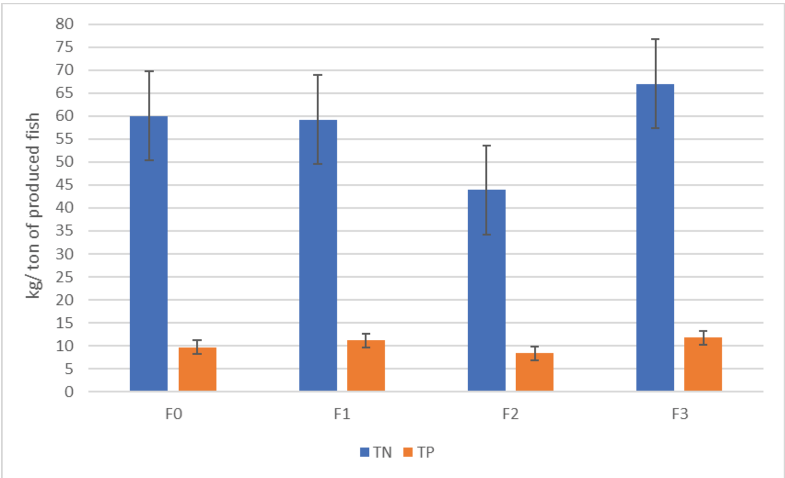


Figure 2. Bar chart of the total nitrogen and total phosphorus (TN and TP, respectively) produced per kg of produced fish during the four seasons (F0 = Spring, F1 = Summer, F2 = Autumn, F3 = Winter).

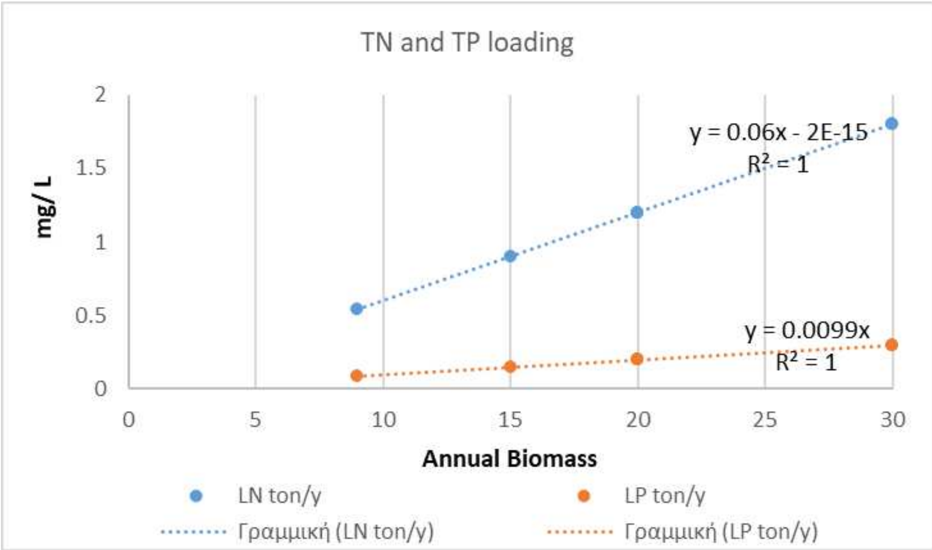
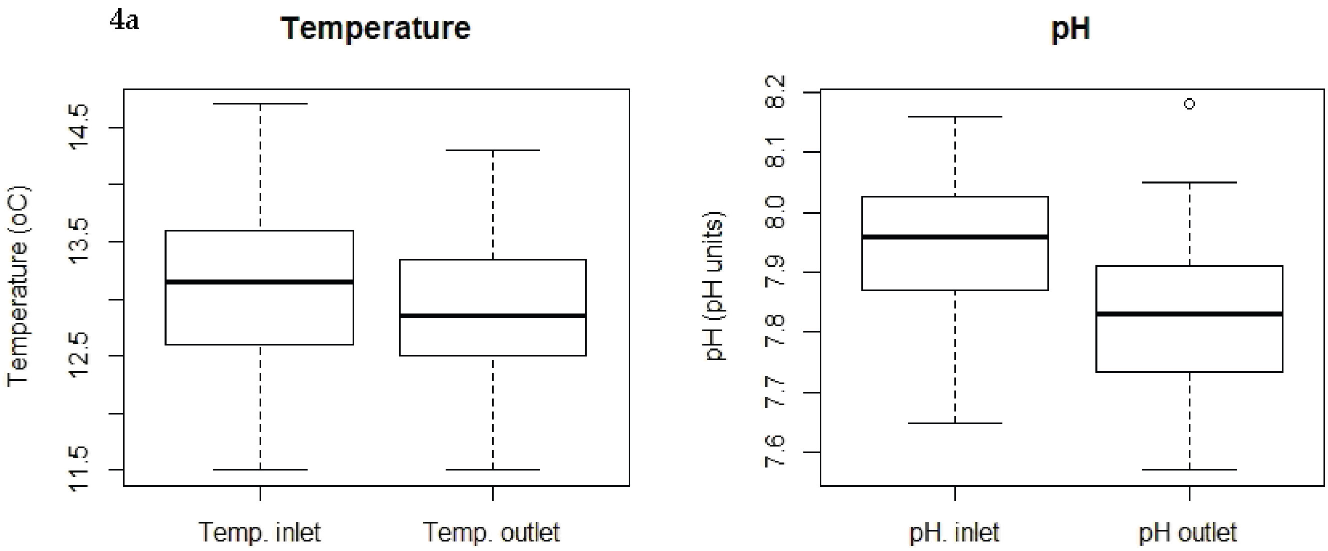
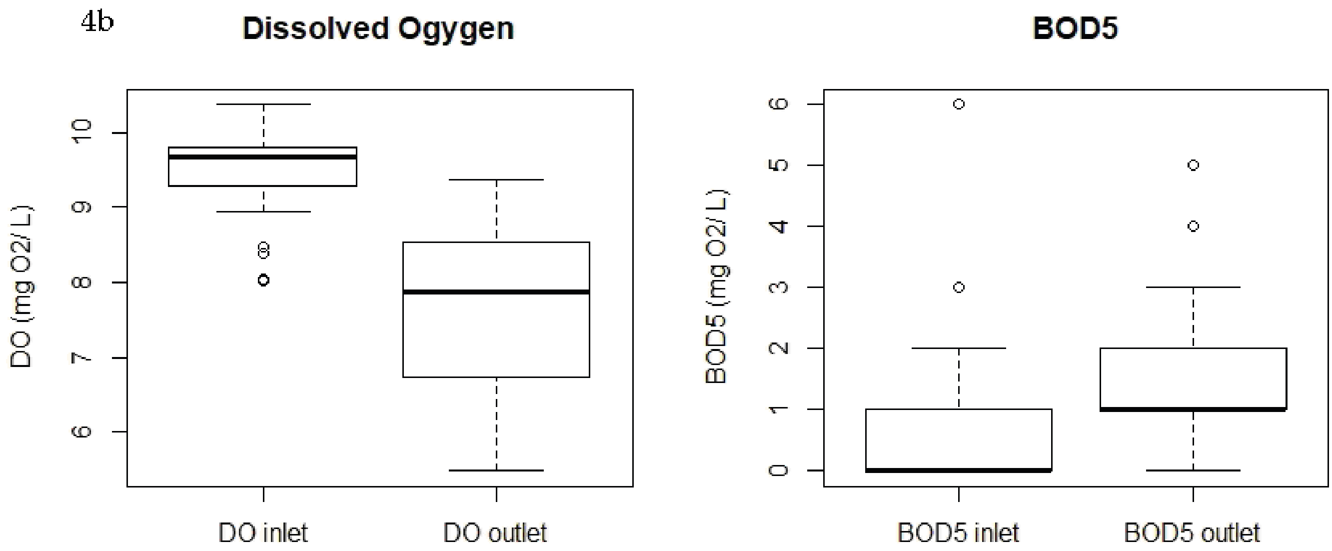


Figure 3. Scatterplot and modelling of TN (L_N) and TP (L_P) in relation to their annual biomass.

4a


Temperature
Temperature (oC)
11.5
12.5
13.5
14.5
Temp. inlet
Temp. outlet
pH
pH (pH units)
7.6
7.7
7.8
7.9
8.0
8.1
8.2
pH. inlet
pH outlet

4b


Dissolved Ogygen
DO (mg O2/ L)
6
7
8
9
10
DO inlet
DO outlet
BOD5
BOD5 (mg O2/ L)
0
1
2
3
4
5
6
BOD5 inlet
BOD5 outlet

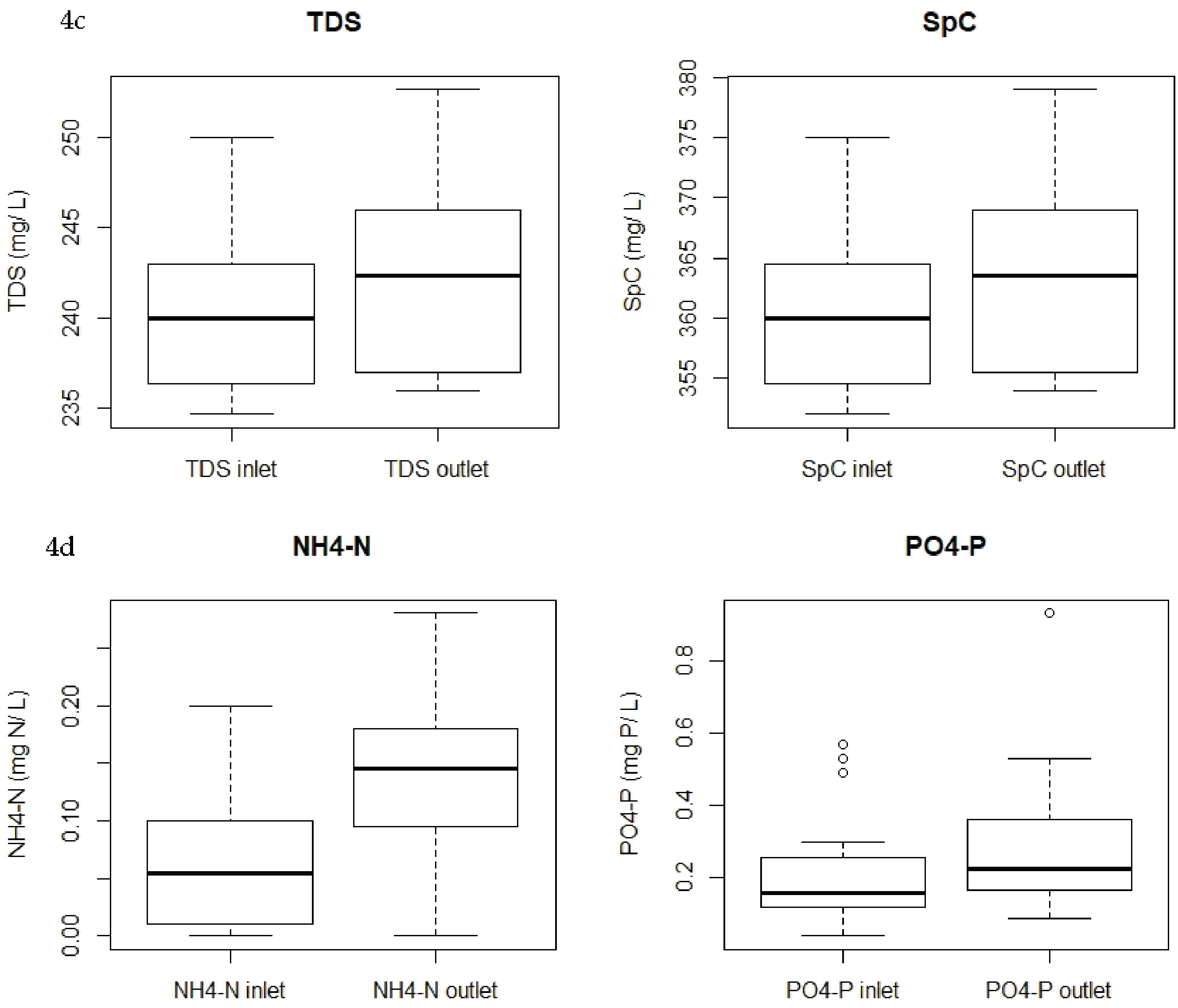


Figure 4. Boxplot representation of the inlet and outlet values for all monitored parameters throughout the whole year. a) Temperature and pH; b)DO and BOD5; c)TDS and SpC; d) NH_4-N and PO_4-P

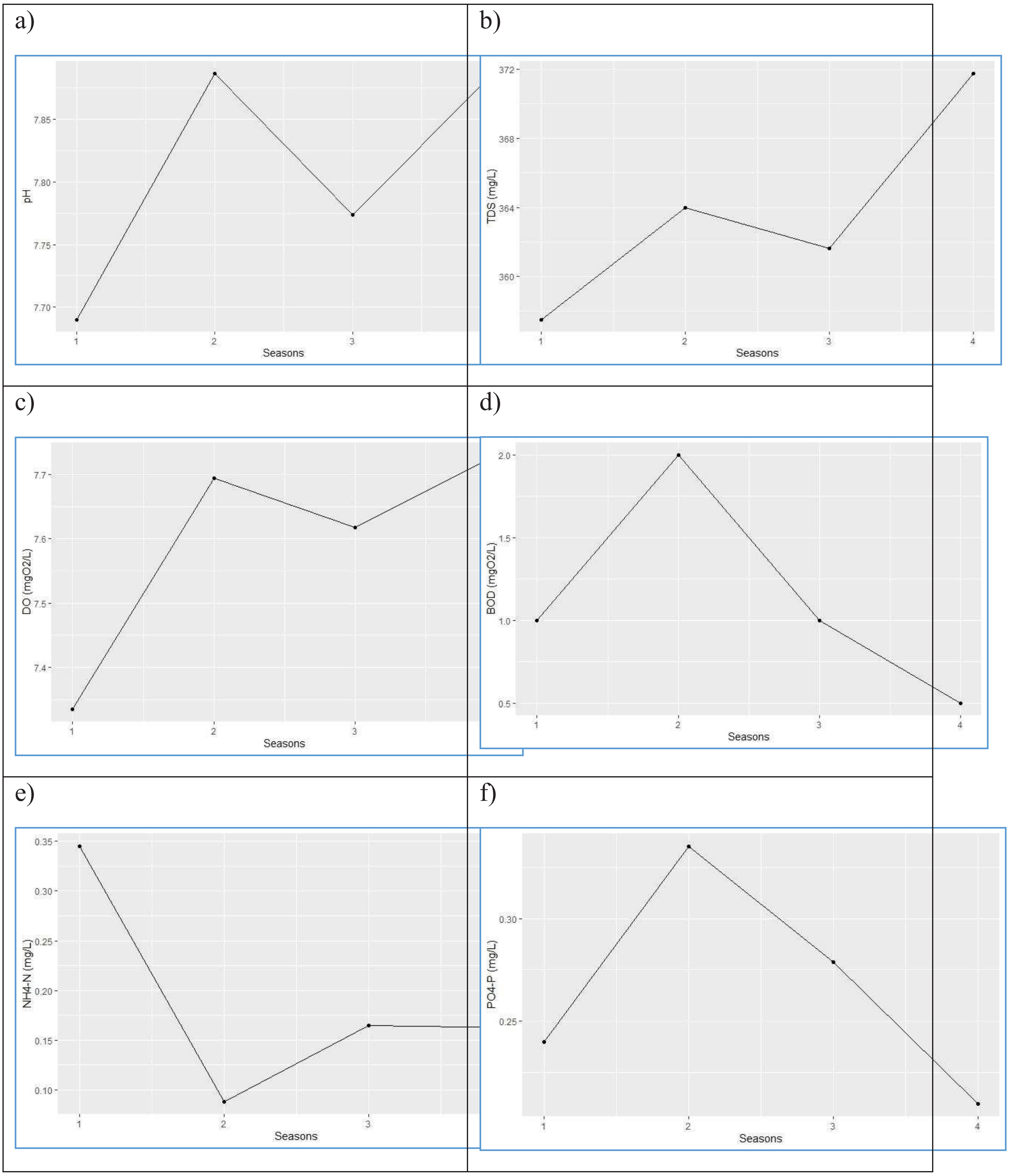


Figure 5. Line graph of the seasonal variation of the mean values grouped for each parameter pH (a), DO (b), SpC (c), BOD (d), PO_4P (e), NH_4N (f), 1= Spring, 2= Summer, 3= Autumn and 4= Winter.

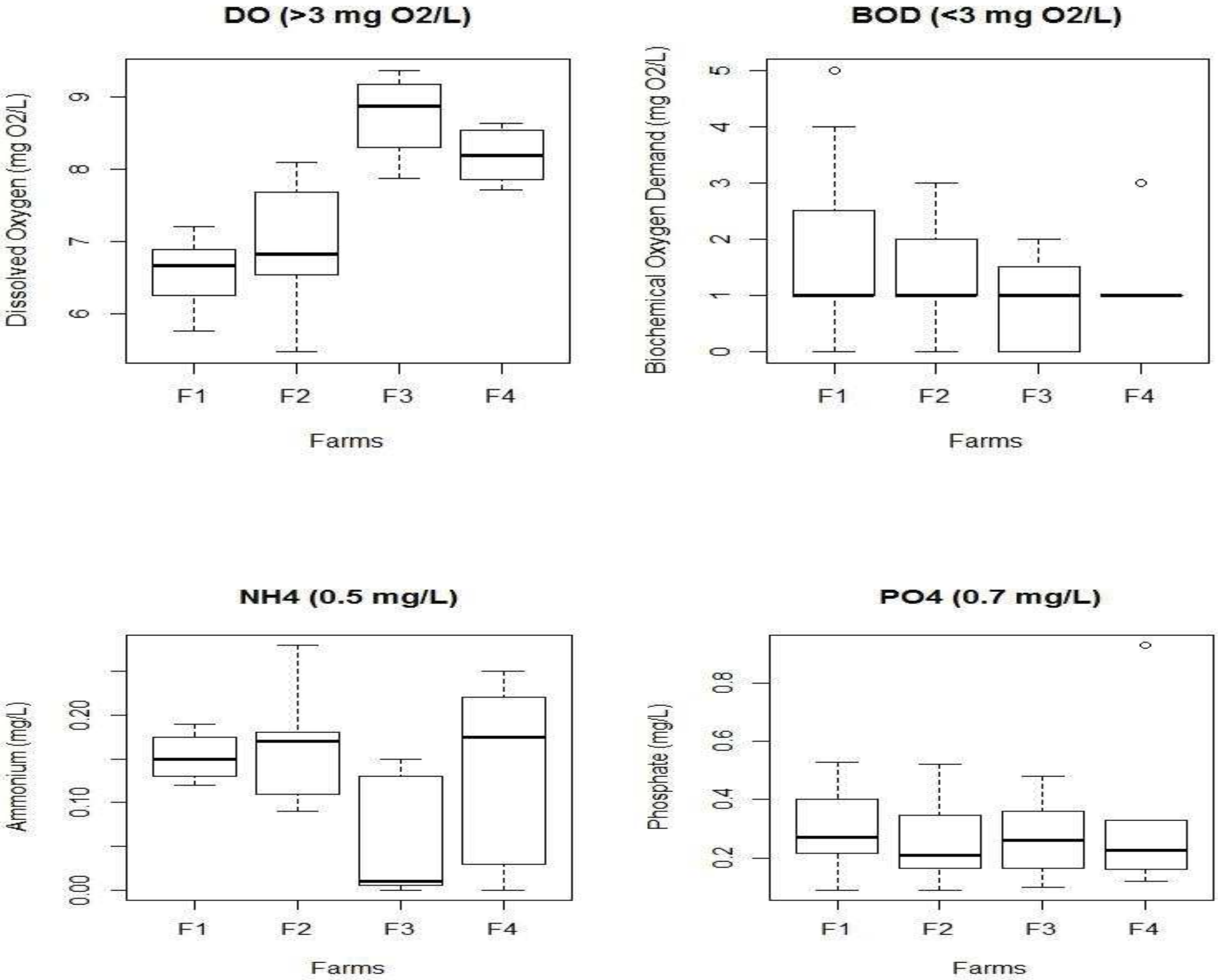


Figure 6. Boxplot of the outlet concentrations for each parameter of all farms and in brackets their limit standards (EQS). Median, Q1, Q4, and Standard deviatiosn are also presented in which F1= Spring, F2= Summer, F3= Autumn, and F4= Winter.