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RESEARCH PAPER

Evaluation of Fish Farm Effluent Contamination Using Bio-Indicators Based on Macroinvertebrate Communities

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Abstract

The progress of the aquaculture industry in Iran as an environmental and economical maintainable process needs an efficient and low-cost tool for regular checking of adjacent environments. Biological monitoring by macroinvertebrate is operative for assessment of water quality. Sampling operation was carried out to investigate the influence of aquaculture wastewater on macroinvertebrate communities at 4 stations over one year (spring, summer, autum and winter) in the Chehel Chai River. 2040 (19% spring, 18% summer, 25% autumn and 38% winter) macroinvertebrate specimens belonging to 6 orders and 14 families were recognized. The most abundance among the stations belonged to the Diptera and Chironomidae. Two groups of macroinvertebrate assemblages (sensitive to tolerance) and three groups of stations (upstream, outfall and down1 and down2) were identified with Heat map analysis. The study was showed that rainbow trout farm most often significantly increase in the influence of fine particulate organic material (FPOM %), NO_3 and PO_4. According to HFBI results, organic water pollution at different stations was classified as good (upstream), appropriate, relatively poor and poor (outfall). The combined results of the biomarkers were showed that the outfall station had more organic contamination than its predecessor station (relatively poor water quality) which required more efficient management based on the self-purification capacity of the Chehel Chai River.

Introduction

Growing aquaculture activities to meet the growing global demands for fish consumption have increased the risks of adverse environmental effects, such as water pollution and loss of biodiversity (Fao 2012). Turcios and Papenbrock (2014) stated that most aquaculture methods can affect the natural aquatic system. In general, aquaculture practices release contaminations into the culture ponds/tanks that probably include fish metabolic and food waste due to over-feeding. These issues may affect the system by increasing dissolved nutrient concentrations that in turn influence the health index bacteria at later stage (Dauda et al., 2019). The impact of farms discharge depends on the structure of farms, the diversity of cultured fish, the identity of produced effluents, and the water features at the discharge site (Amirkolaie, 2011). Currently, there is an extensive range of ecological rules in Asian and European nations regarding standards of aquaculture control, effluent disposal as well as improved freshwater quality to support fish life. Iran's regulatory laws deal with specific aspects of production performs, licensing supplies for aquaculture facilities and control of insecticides and veterinary drugs in aquaculture yields (Wells et al. 2002). Nevertheless, the possible influence of freshwater fishery effluent in Iran has not been well recognized. This study presents physical, and chemical parameters of water body collected as part of a Chehel Chai River by monitoring plan in the northern part of Iran that evaluates the ecological impact of fish waste on water quality and macroinvertebrate communities.

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Physicochemical changes caused by residual nutrients and wastewater effluents result in increasing suspended solids and nutrients that further shows several environmental impacts on the surrounding waters such as a decrease in dissolved oxygen, increases BODs, and settling of suspended solids (Aazami et al. 2019). So far, biological changes have been less considered and may rely on the management and location of fish farms alongside the river and the environmental features of the river. Macroinvertebrate are more susceptible to deposition of sediments due to inactivity or less activity than fish and are more at risk. In addition to the major direct role of macroinvertebrate in the food chain, they have a significant role in the nutrient cycle such as phosphorus and nitrogen (Camargo 2019). Therefore, macroinvertebrate communities are one of the qualitative evaluation criteria that have a direct relationship with the quality of their habitat.

Barbosa et al. (2001) considered macroinvertebrate diversity as water quality indices to assess the health of some Brazilian aquatic ecosystems. In this study, due to the abundance of aquatic groups (such as Plecoptera, Gripopterygidae, Coleoptera, Psephenidae, Trichoptera, Hydrobiosidae; Chironomidae, Diptera), Low Electrical Conductivity and dominance of Oligotrophy Conditions Soa Francisco, Doce, and Parana Rivers had low nutrient levels (Staicu et al. 1998). Quantitative and qualitative changes in macroinvertebrate communities have been used as a qualitative (biological) indicator to identify pollutants in water resources (Sharma et al. 2006).

Since there has been no scientific research on the development of rainbow trout farms in the north of Iran, especially in Golestan province, the question is how aquaculture can become a sustainable activity? Therefore, a comprehensive assessment of farm activity and its impact on river ecosystems should be undertaken. The present study was conducted to consider the effect of fish farms effluent on the macroinvertebrate communities and their dominant groups. An evaluation of water quality and its self-purification potential in the study area was also performed using a combination of biomarkers. Finally, the water quality class was considered using the Hilsenhoff Biotic Index (HFBI).

Material and Methods

Study Region

The Chehel Chai catchment area is approximately 25,000 hectares in the northern slopes of eastern Alborz. The study basin was located at the east longitude of 55°22'30" to 55°37'30" and the north latitude of 36°57'30" to 37°15'00". The hydrological flow of the Chehel Chai basin has a significant impact on the economy of the basin and out of the basin and the fertile and desirable lowlands of the Gorganroud River. Chehel Chai River originates from the southern highlands of Minoodasht City and passes around the city where alluvial sediments deposited on its margins are the most suitable source of drinking water for Minoodasht. It has both local and non-local features, and the effects of the Caspian Sea, northern slopes, altitude, and Mediterranean cyclone movement are fully recognizable and vegetation reflects the diversity and gradual changes in climate.

The most of river water in Chehel Chai basin water is used for agricultural activities and another portion is used for service and industrial use (Ravanab Consulting Engineering Company 2005). Because the study area is mountainous and its villages are predominantly in elevated areas away from the main waterways, the inhabitants of the villages are not at high risk of flooding, but the shape of the river and the effects of past floods indicate the flood situation of this basin. The economic structure of the Chehel Chai Basin is agriculture and animal husbandry.

There are no anthropoid contamination sources that directly release into the river upstream from the fish farm. Fish culturing with a flow-through system that uses oxygenated concrete raceways is a usual practice in the investigation place. The water resource is usually provided from a local river; returning to the river after crossing the farm without any filters. All feed (marketable pellets), is commonly hand-fed, twice a day, at 0.5–1% body weight per day. The land-based farm is used in the production of marketable (300-500 grams) rainbow trout (*Oncorhynchus mykiss*), with an annual volume of 10 tons.

Assessment of the Biological Characteristics

The locations of the sampling stations with different land use were shown in Figure 1. Water (temperature (°C), dissolved oxygen (mg/l), electrical conductivity (µs/cm), turbidity (NTU) and pH were measured using portable digital water-checker model (HACH sension MM 156 multiparameter meter)). NO_3 and PO_4 were measured following APHA (1998) methods in the laboratory in Gonbad Kavous University. Macroinvertebrates were sampled seasonally during 2018 from 4 stations (winter (january), spring (may), summer (august) and autumn (october)) (upstream (before fish farm), outfall (50 meter after fish farm), down1 (500 meter after) and down2 (1000 meter after)) using Surber sampler (0.09 m^3) with 3 replications. The benthic specimens collected after separating the unwanted material using standard sieves with a 500-micron mesh were fixed in 4% formalin and transferred to Gonbad Kavous University Laboratory. After transferring the specimens to the laboratory and washing for separation, the specimens were counted and identified using a loop and microscope with the help of family identification keys (Needham and Needham 1938; Pescador et al. 2004; Thorp and Covich 2009). The functional feeding group classification is based on known information related to how different macroinvertebrate groups obtain and consume food. The functional feeding groups of families identified macroinvertebrates can be viewed in (Table 1).

Statistical Analysis

The biodiversity indices including Margalef, Shannon-Wiener (H’), Simpson (1–D) and Pielou indices were calculated in terms of abundance by the PRIMER v6. Furthermore, the Hilsenhoff Biotic Index (HFBI) was calculated (Hilsenhoff, 1988). Kolmogorov-Smirnov and Levene's test was used for normality and homoscedasticity assumptions, before the analysis. Abundance and family richness of Trichoptera, Ephemeroptera, Plecoptera (EPT) and Chironomidae taxa metrics were measured. EPT is known as orders that are sensitive to pollution (Lenat, 1988), but Chironomidae is tolerant of pollution. One way ANOVA was done to calculate the significance of biotic indices among different stations and seasons. A heatmap was used to visualize the hierarchical clustering of the data. It is also called a false colored image, where data values are converted to a color scale. Heatmap allow to visualize clusters of samples and features simultaneously (performed in R.3.6.3 software). The difference of macroinvertebrates was investigated using non-metric multidimensional scaling (n.MDS) using the Bray-Curtis relative similarity index (by converting the second root data for all samples into the matrix of main macroinvertebrates in stations) in Primer software (version 6). Divergences of macroinvertebrates were tested according to the sampling technique using Analysis of Similarities (ANOSIM) and SIMPER. ANOSIM was used through the calculation of the global test-statistic “Rho” for the determination of differences between similarity matrices (generated using Bray-Curtis similarities) (Clarke and Gorley, 2006). The dbRDA classification test was used based on the DistLM model to determine how the environmental factors affecting

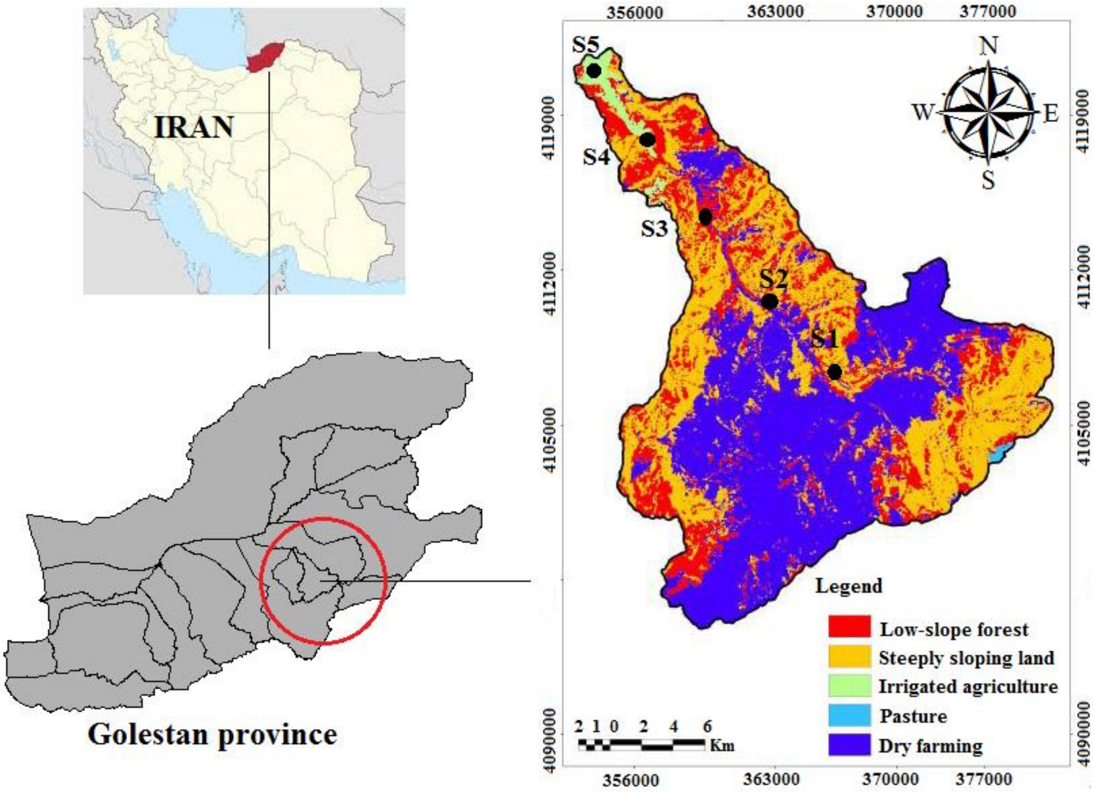

Figure 1. Location of the sampling stations under different land use in the Chehel Chai River.

Table 1. The macroinvertebrate orders and identified families are listed on their representative functional feeding group. Different food sources, fine particulate organic matter (FPOM), periphyton and prey

Order	Collector-gatherer	Filter-feeder	Scraper or Grazer	Predator
Ephemeroptera	Caenidae		Beatidae	
Trichoptera		Hydropsychidae		
Plecoptera				Perlidae
Diptera	Chironomidae; Psychodidae; Dixidae	Simuliidae		Ceratopogonidae
Mollusca	Physidae		Planorbidae	
Oligocheta	Tubificidae			
Food resource	FPOM; Periphyton	FPOM	Periphyton	Prey

the presence and distribution of macroinvertebrate at different stations. The DistLM analysis was performed using the Best method (the best predictive environmental parameters) and the determined AIC selection criterion. Before doing the analysis, those variables that were highly correlated ($r<0.9$) were excluded from the set of variables.

Results

Macroinvertebrate Composition and Abundance

2040 macroinvertebrate specimens belonging to 4 phyla, 6 orders, and 14 families were recognized (Table 2). Insect larvae included the highest number of families (9 families), with Diptera, Trichoptera, Ephemeroptera and Plecoptera (5, 1, 2 and 1 families, separately) as the main orders (Table 2). The most found taxa in the river were Chironomidae (29.78%) and Baetidae (28.84%). Platyhelminthes were detected only in the outfall station. One family belonging to the order Plecoptera were observed in the upstream station. The pupa of Chironomidae was observed only in down1 and down2.

Figure 2 shows that the highest abundance was observed in winter (38%) and autumn (25%). The lowest abundance was found in the summer (18%).

Biological Indicators and Physicochemical Factors

Mean amounts of indices according to the macroinvertebrates at each sampling station were summarized in Table 3 for the whole sampling period. In the study of diversity indices, the highest diversity was observed in the down1 in all seasons and the lowest in the upstream. The Shannon (H), Margalef (R) and Simpson (D) indices showed significant differences between seasons and stations (ANOVA; $P<0.05$). Shannon diversity index is not designed to evaluate the pollution level of study sites. Table 3 was a measure of diversity based on species richness and evenness. The maximum EPT/CHIR and EPT richness were detected in upstream and down2 that displayed significant

Table 2. List of all families (density (Ind/m^2)), and tolerance scores (FBI) in the Chehel Chai River

Phylum	Order	Family	HFBI	upstream	outfall	down1	down2
Arthropoda	Plecoptera	Perlidae	2	8.3	0	0	0
Class: Insecta	Ephemeroptera	Baetidae	5	700	66.7	216.7	300
		Caenidae	6	111.1	22.2	116.7	180.5
	Trichoptera	Hydropsychidae	4	197.2	13.9	52.8	383.3
	Diptera	Chironomidae	2	33.3	802.8	283.3	205.5
		Simuliidae	6	0	244.4	108.3	80.5
		Ceratopogonidae	6	5.5	0	25	8.3
		Psychodidae	8	0	44.4	22.2	5.5
		Dixidae	1	0	19.4	13.9	2.8
Mollusca		Planorbidae	7	0	0	0	5.5
Class: Gastropoda		Physidae	8	0	0	0	2.8
Annelida		Naididae	8	0	44.4	22.2	0
Subclass: Oligochaeta		Lumbriculidae	5	0	22.2	11.1	0
	Hirudinea			0	22.2	0	0
Platyhelminthes			4	0	5.5	0	0

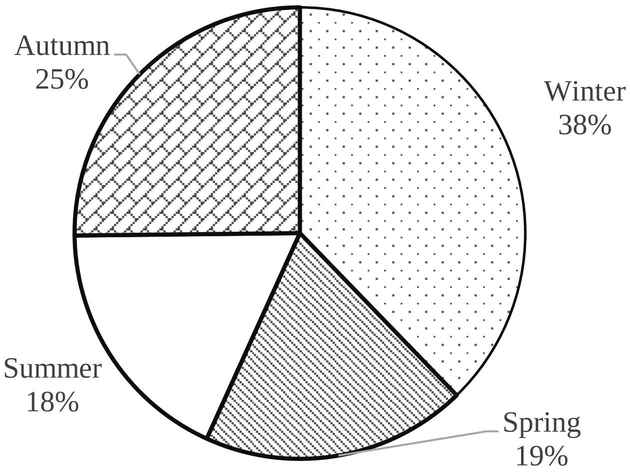

Figure 2. Percentage of macroinvertebrates at different sampling stations and seasons.

difference with other stations ($P<0.05$), while the lowest amounts were obtained in an outfall and downstream in down1.

The other diversity indices including Margalef, Pielou's and Simpson showed an overall growth in the number of species and individuals. The values of HFBI were meaningful (ANOVA; $P<0.05$) higher at the outfall and down1 sampling stations than at upstream (Table 4). As the HFBI index, the results revealed a good water quality in the upstream where the water was cold and rich in oxygen. The outfall and down1 were respectively impacted by organic pollutants (rainbow trout farm), which revealed in a high HFBI index representing a fair/poor water quality. Furthermore, the HFBI was sensitive to typical stations against poor water quality (Table 3).

There was no significant difference in water temperature between stations, but there was a meaningful change among considered seasons ($P<0.05$). Minimum values were observed at upstream (10.6°C) in winter. The minimum pH was measured during the sampling period in autumn at station 2 (8/12) and its maximum was measured in autumn at down2 (8/56). The amount of this parameter was not significantly different at different stations but there was a significant difference between the summer and other seasons. There was no significant difference in the trend of electrical conductivity in different seasons ($P>0.05$). Minimum values were measured in spring at upstream (489 µs/cm) and maximum in winter at outfall (633 µs/cm). Minimum dissolved oxygen was measured in summer at outfall (8.9 mg/l) and maximum in autumn at upstream (11.4 mg/l).

The results of the Pearson correlation between diversity indices and environmental parameters in Table 4 showed that dissolved oxygen was not correlated with any environmental factors. TDS was significantly correlated with Shannon and Margalph index, EPT richness and EPT / CHIR. Turbidity and temperature have a significant correlation with EPT / CHIR index. Shannon-wiener and Simpson indices have a significant correlation with pH and EC. Minimum PO_4 was measured at the upstream station and maximum at the outfall station. PO_4 in different seasons and stations showed a significant difference ($P<0.05$). Changes in NO_3 levels were similar in different seasons and stations and increased after fish farming. Minimum NO_3 was observed in autumn in upstream and maximum in outfall in summer. A comparison of this parameter showed significant differences at different stations ($P<0.05$).

The ratio of annual fish production and average discharge in the dry season was observed 105 (Pr/Qmin; t per m3/s), because the influence of a fish farm on the receiver flow is most noticeable. Throughout the minimum discharge period, particularly since this period in Iran overlaps with the maximum water temperature, the highest number of fish in the farm and the highest

Table 3. Mean values of richness and diversity indices at the sampling stations and seasons of Chehel-Chai River

Season	Sample	Total Taxa	HFBI (Category)	Margalef	Pielou's	Shannon	Simpson	EPT/CHIR
Winter	upstream	5	4.33(Good)	1.33	0.91	1.46	0.78	18.23
	outfall	8	5.63(Fair)	1.99	0.92	1.92	0.85	5.33
	down1	10	5.67(Fair)	2.56	0.95	2.19	0.90	8.3
	down2	9	5.21(Fair)	2.31	0.90	1.98	0.87	13.8
Spring	upstream	6	4.39(Good)	1.72	0.80	1.44	0.73	23.7
	outfall	7	5.84(Fairly Poor)	1.93	0.89	1.73	0.82	4.6
	down1	10	5.55(Fair)	3.04	0.94	2.16	0.92	6.54
	down2	9	5.1(Fair)	2.7	0.93	2.08	0.91	8.8
Summer	upstream	3	4.41(Good)	0.73	0.84	0.92	0.59	7.43
	outfall	3	6.68(poor)	0.84	0.85	0.93	0.63	0.75
	down1	7	5.93(Fairly Poor)	2.26	0.93	1.82	0.88	1.3
	down2	6	5.31(Fair)	1.65	0.91	1.63	0.82	4.82
Autumn	upstream	6	4.28(Good)	1.66	0.94	1.69	0.84	37
	outfall	4	5.65(Fair)	1.05	0.78	1.09	0.61	5.1
	down1	6	5.27(Fair)	1.63	0.95	1.70	0.85	12.2
	down2	5	5.03(Fair)	1.31	0.9	1.53	0.80	19.5

Table 4. Pearson correlation of biological indices and environmental factors

Index/factors	DO	TDS	Turbidity	pH	EC	Temperature	NO_3	PO_4
Shannon-Wiener	0.2	-0.56*	-0.5	0.54*	-0.54*	0.13	0.63*	0.1
Simpson	-0.18	0.43	0.42	-0.65*	0.5*	-0.3	-0.6*	-0.33
Margalef richness	0.31	-0.46*	0.47	0.26	-0.49*	-0.2	0.44*	-0.17
Pielou	0.11	-0.29	-0.22	0.4	-0.25	0.43	0.51*	0.33
HFBI	-0.12	0.28	0.21	0.38	0.3	0.36	0.29	0.61*
EPT richness	0.38	-0.58*	-0.54*	0.39	-0.59*	-0.23	0.32	-0.11
EPT/CHIR	0.13	-0.62*	-0.57*	-0.1	-0.64*	0.53*	0.08	-0.2

*Significance of correlation at the level 0.05

amount of released contaminants. Average discharge of the recipient during the dry season was measured 0.17±0.006 (m^3/s). Annual production of fish farm was 20 ton of Chehel-Chai River.

So far quality variations in the considered factors have been investigated in the power analysis of the fish farms effect. We investigated the change between the amounts of the analyzed factors at the site immediately downstream of the output and those recorded at the control site to take into account the quantitative side as well as the amplitude of the changes. The recognized abiotic factors with a numerical significant variation were selected for analysis at least in one stream. Also, Pr/Qmin tested its predictive value.

The comparative frequency (%) of macroinvertebrate at considered stations is shown in Figure 3. According to the heat map of macroinvertebrate are divided into 2 groups in terms of sensitivity to pollution. The group with green color, including pollution-sensitive organisms such as Perlidae that was found in upstream. In the second group with red color, stations that belonged to Diptera and Oligochaeta (pollution-tolerant organisms). Heat map for the sampling stations and seasons showed 3 distinct groups. In the first group, which is far apart from other groups, the outfall of the winter (red color) is observable. In the second group, down1 and down2 in winter and down2 in spring are located slightly apart. In the third group, the rest of the sampling stations were relatively close to each other (Figure 3).

MDS plots of distances between season and stations are shown in Figure 4. Based on Figure 4, the station seems to cluster together, representing dissimilarities in macroinvertebrate communities. There

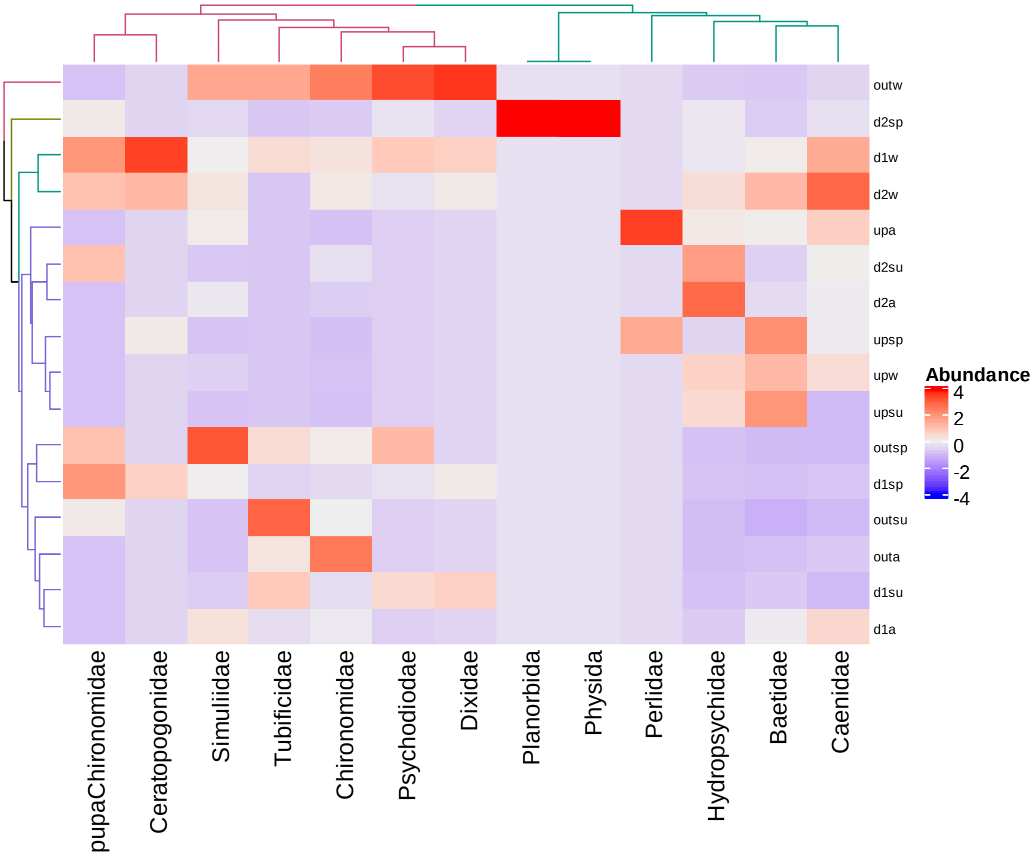

Figure 3. Heat map showing the abundance of macroinvertebrate taxa at sampling stations.

Table 5. Summary of ANOSIM results for Chehel-Chai River (P<0.05 for all tests)

Test	*R* statistic
upstream, outfall	0.98
upstream, down1	0.81
upstream, down2	0.52
outfall, down1	0.37
outfall, down2	0.82
down1, down2	0.51
Global R=0.62	

The significance level of sample statistic: 0.1%

do not appear to be the change in communities among considered seasons.

Figure 5 illustrates the relationship among ecological variables and the macroinvertebrate abundance among different sampling stations of the Chehel-Chai River via the dbRDA graph based on the DistLM model. The vertical axis of dbRDA explains about 15.5% of the variance in the data compared to the horizontal axis of dbRDA (65%). The most important factor contributing to the first axis of plot, which separates upstream stations in seasons, is dissolved oxygen. The water temperature, NO3 and PO4 were most correlated with the second dbRDA axis.

The ANOSIM showed significant differences (P<0.05) among each pair of station groups (Table 5).

SIMPER results (Table 6) revealed the highest average dissimilarity (72.1%) to be observed between stations upstream and outfall with the top five

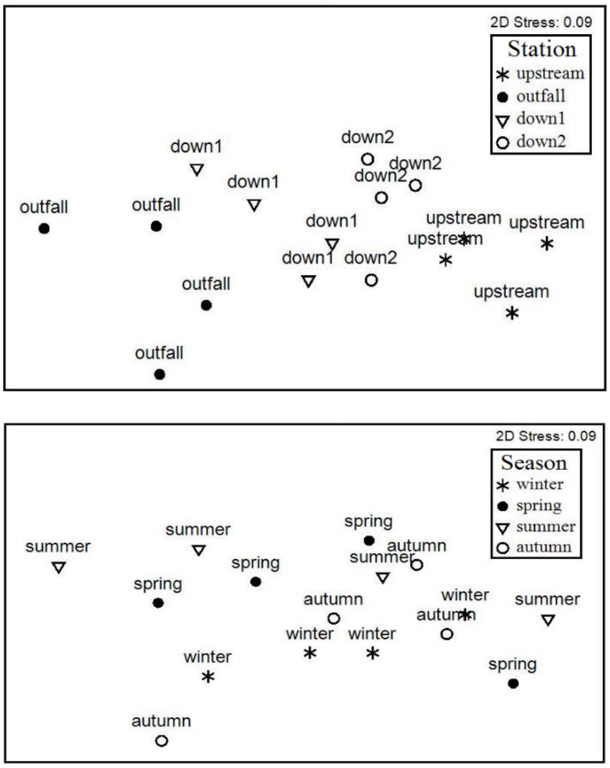

Figure 4. nMDS carried out on macroinvertebrate abundance based on the Bray-Curtis similarity index between season and stations.

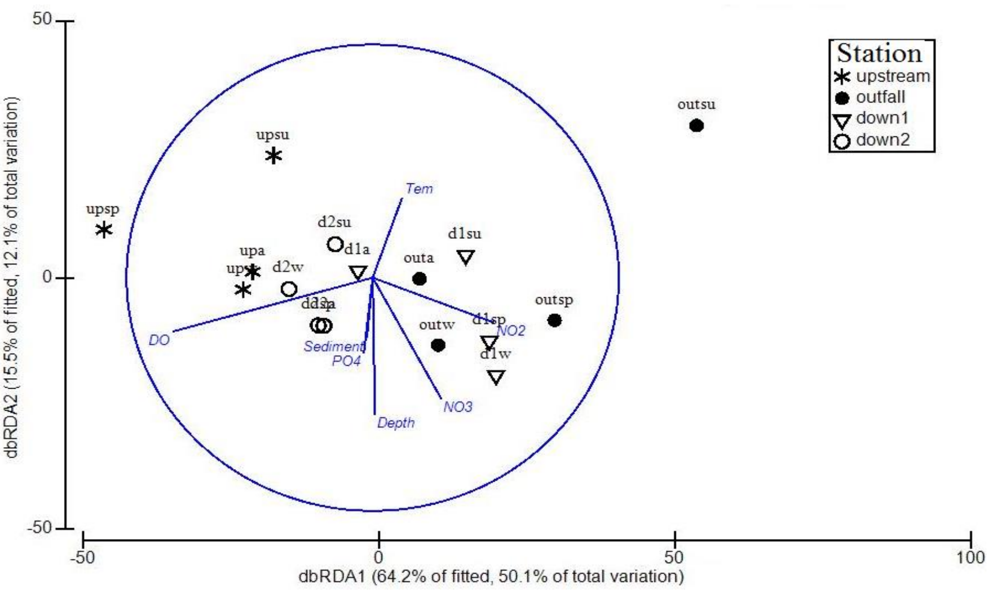

Figure 5. Ordination dbRDA between sampling station and environmental factors. (up=upstream, out=outfall, d1=down1, d2=down2, sp=spring, su=summer, a=autumn, w=winter).

contributor families including Baetidae (22.9%), Chironomidae (22.4%), Hydropsychidae (13.3%), Tubificidae (12.2%) and Simuliidae (12.1%). Sampling stations down1 and down2 revealed the lowest average dissimilarity (30.7%) with the top three contributor families Hydropsychidae (29.1%), Tubificidae (14%) and Caenidae (12.7%).

Within each season (Table 7), the highest dissimilarity of macroinvertebrate community was observed between winter and summer (SIMPER average dissimilarity=36.8%) with top four contributor families Caenidae (21.24%), Simuliidae (18.17%), Baetidae (15%) and Chironomidae (12.42%). Winter and autumn displayed the lowest average dissimilarity =26.6% with top four contributor families including Simuliidae (16.8%), Hydropsychidae (13.7%), Baetidae (12.3%) and Psychodiodae (11.2%).

Discussion

The current water systems are severely influenced by the freshwater fish industry (fish farm), whose effects can also be seen in aquatic communities. This effect is particularly evident in the macroinvertebrate (Hatami et al. 2017). These changes are predictable and specific structural patterns are evident along the stressful slope. These implications have been investigated in many kinds of research related to the biotic assessment of aquaculture contamination (Camargo 2019; Guilpart et al. 2012). In the study, aquatic insect larvae were the dominant fauna of the Chel Chai River. Numerous researchers in their studies have pointed to the predominance of aquatic insects in the benthic ecosystem composition of streams and rivers (Camargo et al. 2004; Loch et al. 1996). Five families of Diptera

Table 6. Average dissimilarities (SIMPER) between sampling stations according to the mean abundance and percentage of macroinvertebrates in Chehel-Chai River

Species	Av.abundance		Cont.%	Cum. %	Species	Av.abundance		Cont.%	Cum. %
Av.diss=72.1	upstream	outfall			Av.diss=54.3	outfall	down2		
Baetidae	7.8	2.1	22.9	22.9	Hydropsychidae	0.7	5.6	23.4	23.4
Chironomidae	1.7	8.1	22.4	45.3	Chironomidae	8.1	4.1	16.5	39.9
Hydropsychidae	4.1	0.7	13.3	58.7	Tubificidae	3.13	0	14.1	53.9
Tubificidae	0	3.1	12.2	70.8	Baetidae	2.1	4.92	12.16	66.1
Simuliidae	1.3	3.3	12.1	82.9	Simuliidae	3.29	2.49	12.1	78.2
					Caenidae	0.99	3.76	11.8	90.1
Av.diss=33.9	upstream	down2							
Baetidae	7.8	4.9	22.86	22.8	Av.diss=44.6	upstream	down1		
Chironomidae	1.7	4.1	16.76	39.6	Baetidae	7.81	4.3	21.6	21.6
Hydropsychidae	4.1	5.6	12.67	52.3	Chironomidae	1.72	4.99	17.8	39.4
Caenidae	2.7	3.7	12.61	64.8	Hydropsychidae	4.1	1.9	12.8	52.2
Simuliidae	1.3	2.49	10.03	74.9	Tubificidae	0	1.9	10.9	63.1
Av.diss=38.4	outfall	down1			Av.diss=30.7	down1	down2		
Simuliidae	3.3	2.9	18.6	18.6	Hydropsychidae	1.9	5.6	29.1	29.1
Chironomidae	8.1	4.9	18.6	37.2	Tubificidae	1.9	0	14	43.1
Baetidae	2.1	4.2	15.4	52.6	Caenidae	2.5	3.7	12.7	55.8

Table 7. Average dissimilarities (SIMPER) between seasons according to the mean abundance and percentage of macroinvertebrates in Chehel-Chai River

Species	Av.abundance		Cont.%	Cum. %	Species	Av.abundance		Cont.%	Cum. %
Av.diss=29.2	winter	spring			Av.diss=26.6	winter	autumn		
Caenidae	4.3	1.7	16.8	16.8	Simuliidae	3.6	2.5	16.8	16.8
Chironomidae	6.15	3.6	16.6	33.4	Hydropsychidae	3.7	3.4	13.7	30.5
Baetidae	6.1	4.4	15.9	49.3	Baetidae	6.1	4.5	12.3	42.9
Hydropsychidae	3.7	2.1	11.9	61.3	Psychodiodae	1.5	0	11.2	54.1
Av.diss=31.3	spring	summer			Av.diss=36.8	winter	summer		
Simuliidae	3.2	0.6	24.4	24.4	Caenidae	4.31	0.79	21.24	21.2
Hydropsychidae	2.1	3.1	14.5	38.9	Simuliidae	3.63	0.6	18.17	39.4
Caenidae	1.7	0.7	11.3	50.3	Baetidae	6.09	4.06	15	54.4
					Chironomidae	6.15	4.04	12.42	66.8
Av.diss=33.9	spring	autumn							
Simuliidae	3.2	2.5	22.6	22.6	Av.diss=34.1	summer	autumn		
Baetidae	4.4	4.4	13.8	36.5	Caenidae	0.79	3.1	20.8	20.8
Hydropsychidae	2.1	3.4	13.5	50.1	Baetidae	4.06	4.4	18.7	39.5
Chironomidae	3.6	5.1	11.6	61.7	Chironomidae	4.04	5.1	17.5	57.1
Caenidae	1.7	3.1	10.4	72.1	Simuliidae	0.6	2.5	15.4	72.4

consist of Chironomidae, Simuliidae, Ceratopogonidae, Psychodidae, and Dixidae were recognized. This family was the most diverse in comparison with other orders. Chironomidae was identified at all stations which the highest abundance observed after the outlet of the fish farm and the lowest abundance was at the upstream station (before the fish farm).

Baetidae, Caenidae, and Hydropsychidae families were present at all stations. The effluent of fish farms is of the greatest imperative factors which hurt the structure, changing the population of macroinvertebrate, increasing the number of tolerant groups and declining the number of sentient groups (Soofiani et al. 2012). The highest abundance of macroinvertebrate species was observed in outfall and down2 stations that belonged to Baetidae and Chironomidae families. This may be due to the entrance of the nutrient to the river through the farms after and before these considered stations. Dixidae family had the lowest abundance at the down2 station and the Ceratopogonidae family had the lowest abundance at the station before the fish farm.

Jafari et al. (2011) examined the structure of macroinvertebrate populations in the Casilian River located in Mazandaran province. During their research, 31 macroinvertebrate families were identified. Three orders, including Ephemeroptera, Trichoptera, and Diptera were dominated in the studied stations. The obtained result indicated that the frequency of the Chironomidae family increased significantly because of urban pollution at the two stations. The results also revealed that Trichoptera had the maximum abundance which is in agreement with the results of the present study.

The EPT/CHIR and maximum EPT richness indices were detected in upstream and down2 that showed significant changes with other studied stations ($P<0.05$), whereas the lowest amounts of indices were obtained in further downstream in down1and an outfall. The results of this study showed a trend of increasing and decreasing of this index in the stations before and after the fish farming workshop. An increase in the Chironomidae population compared to sensitive groups will result in a decrease in the amount of EPT/CHIR, indicating the impact of environmental stressors. The quantity and quality of organic matter inputs from fish farm activities can affect the energy structure and communities of macroinvertebrate, thereby disrupting ecosystem functioning. Because in areas where a lot of organic matter is being loaded, the amount of EPT/CHIR decreases and the filtering group increases (Rosenberg, 2004). The increase in TDS and turbidity and the decrease in DO levels at outfall can be associated with the farm and its output into the river.

The influence of FPOM to downstream sites of the fish farm is one of the factors that often changes but is categorized by quick improvement in following downstream sites. Because the superior share of FPOM in the fish farm sites is due to the deposition of undigested food.

The Chehel Chai River is located in a mountainous area with rocky beds that has five sub-basins. Periodic overflows (in winter) in this region cause homologous sediment creation (Assine and Silva 2009). The survival of Chehel Chai River has been dependent on the development of agriculture, horticulture and fish culture. These happenings have besides altered the water characteristics. The pH changes were completely under standard conditions at all stations because it lacked major manufacturing procedures and self-purification in the upstream part. In the present study, macroinvertebrate (Chironomidae, Simuliidae, and Oligochaeta) resistant to organic contamination were more abundant in the station after the farm in the compared to the upstream station. Fish farm effluent is one of the effective factors in changing the composition of benthic populations and can result in increased resistance and reduced susceptibility (Fries and Bowles, 2002).

Conversely, the species number and information-based diversity indices were changeable, and their developments changed at the post-farm fish station. In this case, increased values of total species (S), frequency and Shannon-wiener index (H ') decreased with the interval from farm output and had the lowest values at control sites. Chironomidae and Oligochaeta desire contaminated environments and have very varied populations, so the effect can be increased.

At the outfall station, the percentage and richness of EPT were low, which may indicate the impact of fish farming activity (about 15 tonnes production) on this station. Anomalous increases in the number of Chironomidae and Oligochaeta relative to susceptible organisms (decreasing EPT/CHIR levels at the outfall) indicate environmental stress. Thus, the quantity and quality of organic matter input from fish farm activity into the river can affect the energy structure and communities of macroinvertebrate and cause disturbances in ecosystem function. Because the ratio of EPT to Chironomidae is reduced in high organic matter load areas and filter feeder groups increase (Rosenberg 2004). Therefore, the trend of macroinvertebrate population fluctuations at pre- and post-farm stations is due to the disruption of ecological equilibrium created under appropriate biological conditions and is not irrelevant to the reasons stated.

In the present study, the study sites were classified into four categories of good, appropriate, relatively weak and poor based on HFBI biomarker. Based on this, the outfall station alone fell into a poor-quality class (very significant organic contamination) due to the activity of the rainbow trout farm in the area. According to this index, the other downstream stations of the farm were classified as having good quality which indicates self-purification in the river. Rainbow trout farms are currently active in upstream areas. Therefore, the identification of other locations should be made under the active capacity of these farms.

Conclusion

On the basis of the obtained results, it can be concluded that the trout farm at the Chehel-Chai River had serious and clear influence on the water chemistry parameters and macroinvertebrate community in the receiving watercourse. With increasing NO_3, PO_4 and turbidity at the fish farming outfall station, a decrease in dissolved oxygen was observed. The fish farm with higher season biomass and density and upper amounts of the Pr/Q_{min} factor produced the maximum of NO_3 and PO_4 concentration into the recipient river. The HFBI results, organic water pollution at different stations were classified as good (upstream) and poor (outfall). Although the development of aquaculture plays an important role in commercial and community development, irrespective of the ecological potential it causes irrecoverable loss. The anthropological pressures related to aquaculture and water consumption for agriculture have caused unhealthy water quality in the Chehel Chai River. Therefore, appropriate management practices should be considered under the flow and capacity of the river and the water needs for the aquaculture industry.

References

Aazami J., Moradpour H., Zamani A., Kianimehr N., 2019. Ecological quality assessment of Kor River in Fars Province using macroinvertebrates indices. International Journal of Environmental Science and Technology 16(11), 6935-6944. https://doi.org/ 10.1007/s13762-018-2107-y

Amirkolaie A. 2011. Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. Reviews in Aquaculture 3, 19–26. https://doi.org/ 10.1111/j. 1753-5131.2010.01040.x

Assine M.L., Silva A., 2009. Contrasting fluvial styles of the Paraguay River in the northwestern border of the Pantanal wetland, Brazil. Geomorphology 113(3-4), 189-199. https://doi.org/ 10.1016/j.geomorph.2009.03.012

Camargo J.A., 2019. Positive responses of benthic macroinvertebrates to spatial and temporal reductions in water pollution downstream from a trout farm outlet. Knowledge & Management of Aquatic Ecosystems (420), 16. https://doi.org/10.1051/kmae/2019010

Camargo J.A., Alonso A., De La Puente M., 2004. Multimetric assessment of nutrient enrichment in impounded rivers based on benthic macroinvertebrates. Environmental Monitoring and Assessment 96(1-3), 233-249. https://doi.org/10.1023/B:EMAS.0000031730.78630.75

Clarke K, Gorley R. 2006. Primer v6. 1.10: user manual/tutorial PRIMER-E. Plymouth Routines in Multivariate Ecological Research, Plymouth.

Dauda A.B., Ajadi A., Tola-Fabunmi A., Akinwole A.O., 2019. Waste production in aquaculture: Sources, components and managements in different culture systems. Aquaculture and Fisheries 4(3), 81-88. https://doi.org/10.1016/j.aaf.2018.10.002

Fao F., 2012. Agriculture Organization of the United Nations. 2012. FAO statistical yearbook.

Fries L.T., Bowles D.E., 2002. Water quality and macroinvertebrate community structure associated with a sportfish hatchery outfall. North American Journal of Aquaculture 64(4), 257-266. https://doi.org/10.1577/1548-8454(2002)064<0257:WQAMCS>2.0.CO; 2

Guilpart A., Roussel J.M., Aubin J., Caquet T., Marle M., Le Bris H., 2012. The use of benthic invertebrate community and water quality analyses to assess ecological consequences of fish farm effluents in rivers. Ecological Indicator 23, 356-365. https://doi.org/10.1016/j.ecolind.2012.04.019

Hatami R., Paul W., Soofiani N.M., Asadollah S., 2017. Rapid bioassessment of macroinvertebrate communities is suitable for monitoring the impacts of fish farm effluents. Aquaculture 468: 19-25. https://doi.org/10.1016/j.aquaculture.2016.09.042

Loch D.D., West J.L., Perlmutter D.G., 1996. The effect of trout farm effluent on the taxa richness of benthic macroinvertebrates. Aquaculture 147(1-2), 37-55. https://doi.org/10.1016/ S0044-8486(96)01394-4

Needham J.G., Needham P.R., 1938. Guide to the Study of Fresh-water Biology.

Pescador M.L., Rasmussen A.K., Harris S.C., 2004. Identification manual for the caddisfly (Trichoptera) larvae of Florida. Revised Edition. State of Florida, Department of Environmental Protection.

Rosenberg D., 2004. Taxa tolerance. values. Bull. Entomol. Soc. Can, 30, 144-152.

Sharma M.P., Sharma S., Gael V., Sharma P., Kumar A., 2006. Water quality assessment of Behta River using benthic macroinvertebrates. Life Science Journal 3(4), 68-74.

Soofiani N.M., Hatami R., Hemami M., Ebrahimi E., 2012. Effects of trout farm effluent on water quality and the macrobenthic invertebrate community of the Zayandeh-Roud River, Iran. North American Journal of Aquaculture 74(2), 132-141. https://doi.org/10.1080/15222055.2012.672367

Staicu G., Bănăduc D., Găldean N., 1998. The structure of some benthic macroinvertebrates and fishes communities in the Vişeu Watershed, Maramureş, Romania. Travaux du Museum National d Histoire naturelle Grogore Antipa 587-608.

Thorp J.H., Covich A.P., 2009. Ecology and classification of North American freshwater invertebrates: Academic press.

Turcios A., Papenbrock J., 2014. Sustainable treatment of aquaculture effluents—what can we learn from the past for the future? Sustainability 6(2), 836-856. https://doi.org/10.3390/su6020836

Wells F., Metzeling L., Newall P., 2002. Macroinvertebrate regionalisation for use in the management of aquatic ecosystems in Victoria, Australia. Environmental Monitoring and Assessment 74(3), 271-294. https://doi.org/10.1023/A:1014235211968