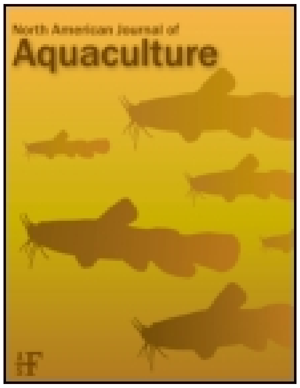

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ARTICLE

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Abstract
To investigate the environmental impact of fish farm discharge on the Zayandeh-Roud River in Iran, three trout farms (Dimeh, Hojat Abad, and Takab) with three different production capacities were studied by examining water physicochemical criteria and the macrobenthic invertebrate community. Six sampling sites were assigned to each farm: the inflow and outflow of the farm, the outfall into the river, 50 m upstream from the outfall (control), 50–100 m downstream from the outfall, and 1 km downstream from the outfall. Water and benthos samples were collected once during autumn, winter, and spring. Benthic samples were collected by using a quantitative technique (3 replicates/site). Results showed a significant (P < 0.05) increase in 5-d biochemical oxygen demand, chemical oxygen demand, and total suspended solids and a decline in dissolved oxygen concentration and pH in the outflow. However, concentrations of measured variables at each farm were generally within acceptable limits. Total taxonomic richness, abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT), and richness of EPT taxa were considerably lower at outflow stations than at inflow stations, especially for the farm with a higher production rate. In contrast to physicochemical results, the benthic community indices were significantly different between upstream and downstream samples. Sensitive taxa declined at downstream sites in comparison with upstream sites, while abundance of tolerant taxa (e.g., Chironomidae and Oligochaeta) increased at downstream sites. Although species richness and diversity improved farther downstream, the recovery was not complete within our study range. However, effluent effects were greatest during periods of low river flow. Thus, to reduce environmental impacts of aquaculture, production rates should be based on the lowest anticipated flow rate of rivers.

The worldwide increase in fish production to meet the growing global demand for fish consumption has consistently generated pollution problems. Although development of aquaculture generates profits, the industry should also be aware of the negative effects of farm effluents on a receiving water body. Pollution of water resources by pond effluents is probably the most common complaint, and this concern has attracted the greatest attention in most nations (Boyd 2003). Moreover, untreated effluents from flow-through systems that contain a high concentration of nutrients may have a serious environmental impact on water quality (Fornshell 2001) and consequently the biotic community.

Metabolic wastes and uneaten food result in an increase of both dissolved nutrients and suspended solids, thus exerting an oxygen demand on receiving waters that could decrease the dissolved oxygen (DO) concentration to a critical level for other aquatic organisms (Viadero et al. 2005). To avoid these negative environmental impacts, farm effluents should be monitored. Assessing the subtle effect of effluents on streams is often difficult if only chemical constituents are measured (La Point 1995). For that reason, the use of the benthic macroinvertebrate community structure for assessing the environmental impact of aquaculture effluents recently has received greater attention (Fries and Bowles 2002). Moreover, macroinvertebrate assemblages are usually good indicators of local conditions since they have limited mobility or are sessile, are common to abundant, and have great diversity (Plafkin et al. 1989; Barbour et al. 1999). They are particularly well suited for use in monitoring

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the effects of effluents, as measurements can be made upstream and downstream (site-specific impacts) from the discharge (Chapman and Jackson 1996; Barbour et al. 1999).

There has been much research on the effects of aquaculture on the benthos of aquatic ecosystems (Johannessen et al. 1994; Tsutsumi 1995; Loch et al. 1996; Fries and Bowles 2002; Pillay 2003; Yokoyama et al. 2007). Despite rapid expansion of commercial farming of rainbow trout *Oncorhynchus mykiss* in Iran, the potential effects of trout farms on river quality and the biological community are not fully understood. Therefore, the purpose of this study was to investigate the potential effect of rainbow trout farm effluents on the Zayandeh-Roud River ecosystem by using water physicochemical analyses and macroinvertebrate monitoring. Such monitoring could provide a strong screening tool for effluent characterization and management.

METHODS

*Study area.—*The Zayandeh-Roud River is the largest river on the central plateau of Iran. It rises in the Zagros Mountains and has a 41,500-km^2 basin. The river provides water for the growing population and industries in the region. Three fish farms (Dimeh, Hojat Abad, and Takab) representing different production capacities (250, 25, and 70 metric tons, respectively) were selected for study as they are in close proximity to the river (Figure 1). Dimeh Farm (32°50′N, 50°21′E) is spring fed, and its effluent is discharged into the river after passing through a 300-m-long, 4-m-wide canal. Dimeh Farm is considered to be a major source of water pollution in the river. Hojat Abad Farm (32°71′N, 50°79′E) is river fed; its effluent enters a lagoon, which serves as a sedimentation pond, before being discharged into the river. Takab Farm (32°37′N, 51°52′E) is also river fed, but this farm is located where an earthen dam has caused the river flow to be slow and somewhat stagnant. It should be noted that during this study, the inflow water quality of Takab Farm might have been degraded by a possible oil leakage from a pipe break that occurred upstream. Effluent from Takab Farm directly enters the river. All facilities are flow-through, are active year round, and maintain rainbow trout grower and brood stocks; the exception is Hojat Abad Farm, which engages only in rearing fingerlings to market-sized fish.

*Sampling procedures.—*Six sampling sites were established for each farm: (1) the farm inflow; (2) the farm outflow; (3) the outfall (where effluent discharges into the river); (4) the river upstream from the farm (50 m upstream from the outfall; used as a control); (5) approximately 50–100 m downstream from the outfall; and (6) approximately 1 km downstream from the outfall. Benthic macroinvertebrates were collected once in the middle of three seasons (autumn, winter, and spring) by using a quantitative technique (Barbour et al. 1999). Benthic samples were taken from similar points where water samples were collected. Macroinvertebrate samples were not taken from the outfall sites where the effluent canals were made of concrete. At Hojat Abad Farm, where the inflow and outflow canals were both concrete, benthic samples were taken from the outfall and upstream sites instead. Triplicate samples of the benthic macroinvertebrates

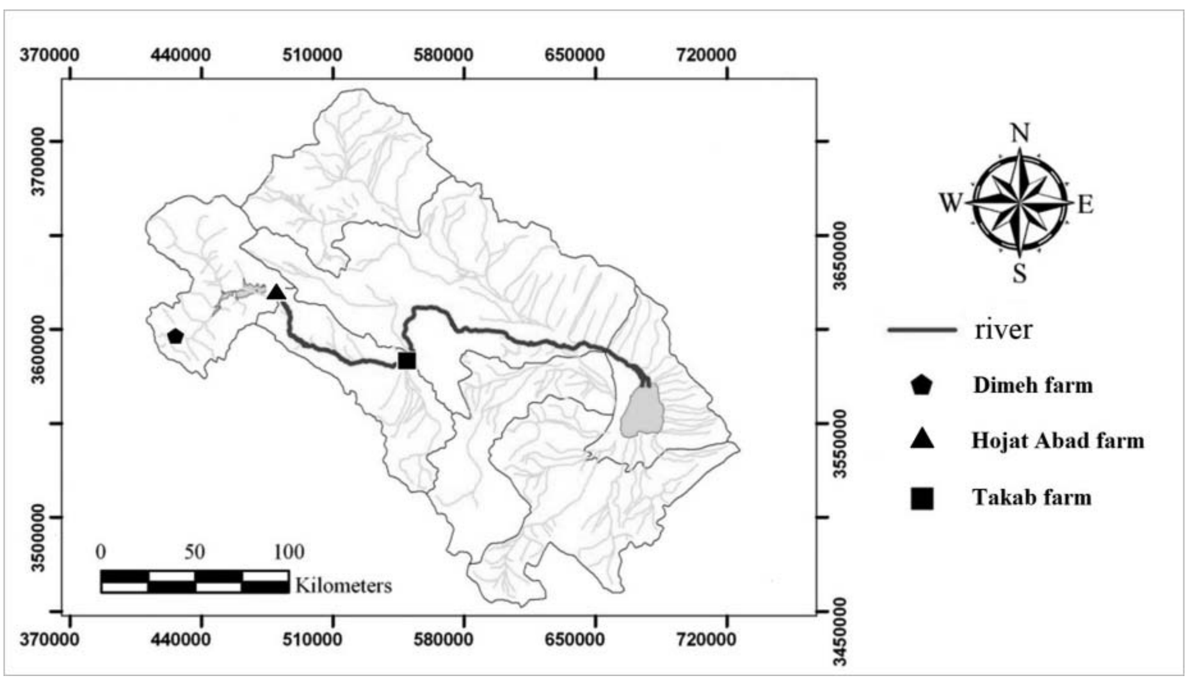

FIGURE 1. Map of the study area, showing rainbow trout farm locations along the Zayandeh-Roud River, Iran, where water quality and benthic macroinvertebrates were studied in relation to farm effluent.

were collected with a Surber sampler (0.062-m^2 sampling area, 60-μm mesh size) by using 2-min kicks (Barbour et al. 1999). After sorting, the collected benthos were identified to the taxonomic level required for each metric; for example, the benthos were identified to the genus level to calculate Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxonomic richness and EPT abundance and were identified to the family level for other calculated metrics. All organisms were identified by using a dissecting microscope and published taxonomic keys (Hynes 1977; Elliott et al. 1988; Milligan 1997; Pescador et al. 2004). Eight metrics were evaluated in this study: total taxonomic richness (number of taxa per sample), EPT taxonomic richness, EPT abundance (number of individual mayflies, stoneflies, and caddisflies), abundance of Chironomidae and Oligochaeta, mean benthic density (organisms/m^2), diversity, and Hilsenhoff's biotic index (HBI). Diversity was determined with the Shannon–Wiener diversity index (H'; Shannon and Wiener 1949):

$$H' = -\sum_{i=1}^{s} p_i \left[\log_e (p_i)\right],$$

where p_i is the proportion of each species i in the sample.

The HBI is a tolerance index that is used to measure the average individual sensitivity of the macroinvertebrate assemblage to pollution (Hilsenhoff 1987). The HBI metric represents the relative sensitivity of the sample to nutrient perturbation. This is calculated as an average tolerance value of all individuals in the sample (excluding those without tolerance values). The HBI is calculated as follows:

$$\text{HBI} = \sum (n_i \times a_i)/N,$$

where n_i is the number of individuals in taxon i, a_i is the tolerance value assigned to the taxon, and N is the total number of individuals in the sample with a biotic index value. Taxa are assigned tolerance values ranging from 0 for the most sensitive taxa to 10 for the most tolerant taxa. Therefore, we expected the HBI to increase with increasing environmental stress (Griffith et al. 2005).

Measured water quality variables were temperature, pH, DO, ammonium ($NH_4{}^+$), orthophosphate, total suspended solids (TSS), total dissolved solids (TDS), 5-d biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD). Water was sampled (three replicates at each sampling time) at the same sites where the macroinvertebrates were collected; however, TSS was only measured in the winter and spring. All samples were collected in 2-L, acid-washed bottles from a depth of 30 cm. Temperature and DO data were measured with a calibrated oxygen meter (Model OXI 196; WTW Measurement Systems, Fort Myers, Florida). Ammonium and nitrate concentrations were determined with a selective electrode (Nitrate Combination Ion Selective Electrode Model 3040; Jenway, Essex, UK). Phosphorus was measured as orthophosphate by means of the ascorbic acid method (APHA et al. 2005). The TSS, BOD_5, and COD were determined according to standard methods (APHA et al. 2005).

To compare the averages of all data collected at each site across seasons, one-way analysis of variance and Duncan's post hoc tests were used. Simple linear regressions were applied to assess the relationship between fish production rate (biomass [metric tons]; independent variable) and the macroinvertebrate indices and physicochemical parameters. To examine the influence of physicochemical parameters on biotic indices, multiple linear regressions were used. The Statistical Package for the Social Sciences (SPSS) version 15.0 was used for all statistical analyses.

RESULTS AND DISCUSSION

Physicochemical Characteristics

Physicochemical data (pH, DO, TSS, ammonium, orthophosphate, nitrate, TDS, COD, and BOD_5) for the three seasonal samples were averaged for each variable and are presented in Table 1. In general, concentrations of measured pollutants at each facility were within the acceptable ranges and were generally comparable with data reported by others (Fries and Bowles 2002; Pulatsu et al. 2004; Stephens and Farris 2004). Physicochemical variables (except for DO and pH) had higher concentrations at outflow sites than at inflow sites. Although pH values were within the regulatory range of 6–9 at all farms, an insignificant decrease in average pH was observed in outflow water. This reduction is probably related to the higher respiration rate and thus an excess of free CO_2 as a result of fish density and feeding activity (Teodorowicz et al. 2006). At all facilities, the average DO concentrations were above the regulatory requirement of 6 mg/L; however, a net decrease between average influent and effluent DO concentrations was observed at all farms. The differences were only significant at Dimeh Farm ($P < 0.05$), which could be attributed to the higher density of fish and the degradation of feces and feed remains in the raceways. Similar results were reported in a study by Kendra (1991). Owing to the river's large volume, farm effluents showed no significant effect on the DO concentration at downstream sites. The BOD_5 values were also below the regulated average limit of 30 mg/L; the maximum BOD_5 value of 13.74 ± 4.6 mg/L (mean ± SE) occurred at Dimeh Farm. Hinshaw and Fornshell (2002) reported average BOD_5 levels of 2.0 mg/L during normal operations, whereas levels increased by approximately 10 times as solids that had settled were disturbed during cleaning. However, BOD_5 concentrations never exceeded the regulatory limit of 30 mg/L at any time or at any site and remained within the reported safe limits of 3–20 mg/L (Midlen and Redding 1998; Boyd 2003). Moreover, the BOD_5 values at the downstream sites did not differ significantly from those measured at the upstream reference sites. In general, COD also showed a similar pattern of changes as reported for BOD_5. All values recorded for TSS were below the average regulatory limit of 30 mg/L. The highest mean TSS value of 19.27 ± 1.1 mg/L was recorded at Dimeh Farm, mainly because this

TABLE 1. Water quality data (means with SE in parentheses) from sampling stations at three rainbow trout farms located along the Zayandeh-Roud River, Isfahan, Iran (DO = dissolved oxygen; TSS = total suspended solids; BOD_5 = 5-d biochemical oxygen demand; COD = chemical oxygen demand; TDS = total dissolved solids). Within a given row, means followed by the same letter are not significantly different.

		Sampling station					
Water quality variable	Farm	Inflow	Outflow	Outfall	50 m upstream of outfall	50–100 m downstream of outfall	1 km downstream of outfall
DO (mg/L)	Dimeh	8.56 (0.4) yx	6.75 (0.2) z	7.39 (0.1) zy	9.92 (1.1) x	9.52 (1.0) x	9.90 (1.1) x
	Hojat Abad	11.70 (0.3)	10.74 (0.5)	9.38 (0.7)	10.38 (0.8)	10.03 (1.1)	10.02 (0.8)
	Takab	8.85 (0.6) y	6.29 (0.2) z		9.45 (0.5) y	8.87 (1.2) y	9.92 (0.4) y
Temperature (°C)	Dimeh	10.31 (0.5) y	11.37 (0.3) y	10.68 (0.6) y	7.32 (1.7) z	7.12 (0.1) z	7.01 (1.3) z
	Hojat Abad	9.04 (3)	9.49 (3.1)	11.44 (2.4)	10.17 (2.1)	10.88 (2.2)	10.37 (2.1)
	Takab	13.31 (2.1)	13.85 (1.8)		14.02 (2.2)	13.61 (2.0)	13.70 (2.2)
pH	Dimeh	7.89 zy	7.61 z	7.82 (0.3) zy	8.14 (0.2) y	8.05 (0.2) y	8.05 (0.2) y
	Hojat Abad	8.08 (0.1)	7.80 (0.2)	7.92	7.89 (0.1)	8.01	8.00 (0.1)
	Takab	7.96 (0.1)	7.66 (0.1)		7.92 (0.1)	7.93 (0.1)	7.97 (0.1)
TSS (mg/L)	Dimeh	6 (2.7) z	19.72 (1.1) y	6.00 (1.5) z	9.44 (0.3) z	9.83 (0.9) z	11.7 (7.2) zy
	Hojat Abad	3.89 (0.3) z	11.11 (1.3) y	6.95 (1.8) z	5.25 (0.8) z	5.84 (0.5) z	4.11 (1.1) z
	Takab	4.67 (1.3) z	17.78 (1.8) y		9.50 (4.5) z	9.61 (5.7) z	8.95 (5.1) z
BOD_5 (mg/L)	Dimeh	2.81 (0.9) z	13.74 (4.6) y	9.57 (0.4) y	2.39 (0.7) z	3.10 (1.5) z	2.42 (1.4) z
	Hojat Abad	2.55 (1.5)	6.47 (0.5)	5.49 (0.9)	2.22 (1.1)	3.57 (1.4)	2.6 (1.2)
	Takab	2.69 (1.1)	7.52 (2.1)		3.94 (1.3)	3.21 (1.0)	2.83 (0.9)
COD (mg/L)	Dimeh	14.62 (6.3) z	48.50 (17.6) y	19.11 (2.9) z	7.72 (3.5) z	8.20 (4.5) z	7.90 (4.0) z
	Hojat Abad	7.77 (3.7)	25.17 (1.1)	12.00 (4.2)	8.22 (4.5)	10.50 (1.4)	12.50 (5.2)
	Takab	25.07 (7.8)	57.95 (12.5)		85.82 (15.3)	35.64 (22.6)	24.45 (11.8)
Ammonium (mg/L)	Dimeh	1.04 (0.3)	1.74 (0.7)	1.82 (1.2)	0.63 (0.1)	0.79 (0.1)	0.68 (0.3)
	Hojat Abad	0.59 (0.1)	1.08 (0.1)	1.12 (0.3)	0.59 (0.1)	0.63 (0.1)	0.56 (0.1)
	Takab	0.67 (0.1)	1.10		0.85 (0.1)	0.79 (0.2)	0.77 (0.2)
Orthophosphate (mg/L)	Dimeh	0.02	0.06	0.05	0.03	0.03	0.02
	Hojat Abad	0.02	0.04	0.02	0.02	0.01	0.02
	Takab	0.02	0.04		0.02	0.03	0.02
Nitrate (mg/L)	Dimeh	13.7 (6.2)	19.79 (10.1)	12.42 (7.4)	7.89 (1.5)	9.10 (2.4)	9.06 (1.9)
	Hojat Abad	16.89 (9.1)	19.66 (8.4)	12.95 (6.6)	13.38 (7.1)	13.72 (6.9)	13.81 (6.8)
	Takab	16.32 (8.9)	17.87 (8.9)		17.06 (7.8)	15.68 (7.9)	15.40 (7.6)
TDS (mg/L)	Dimeh	246.82 (1.3) y	248.54 (2.3) y	250.81 (3.2) y	157.39 (23.9) z	178.13 (25.6) z	166.64 (20.3) z
	Hojat Abad	172.59 (4.1)	177.39 (3.7)	216.18 (40.2)	169.74 (2.2)	171.31 (4.6)	172.09 (4.0)
	Takab	453.97 (103.9)	462.58 (105.5)		460.66 (99.9)	459.23 (109.4)	423.11 (139.7)

farm has the highest biomass and also because cleaning and feeding operations occurred at the time of sampling. Changes in the effluent quality during cleaning in hatcheries and farms have long been noted and reported. For instance, Kendra (1991) reported an increase in TSS from 1 mg/L to 88 mg/L during the cleaning activity at a hatchery. At Takab Farm, which has an intermediate level of production, the TSS concentration in the effluent was also high. This was mainly due to the circulation of untreated water at this farm because of the water supply shortage during winter sampling. However, the changes in TSS recorded during our study were all within the range (0–100 mg/L) reported in the literature (Yeo et al. 2004). Nonetheless, the differences between TSS concentrations at the inflow and outflow sites were generally significant at all farms ($P < 0.01$).

While orthophosphate concentration was higher at the outflow site than at the inflow site of Dimeh Farm, the difference between the two was not significant at the other farms. Similarly, the orthophosphate concentrations at sampling sites downstream did not differ significantly from those measured at the upstream reference sites. Mean orthophosphate values reported in our study were within the range of 0.01–0.17 mg/L, similar to values reported by others (Hinshaw and Fornshell 2002; Boardman et al. 2006). In general, our findings indicated that the ammonium and nitrate content of water did not significantly differ among sampling sites. However, for all three farms, the effluent water had higher levels of nutrients, including ammonium and nitrate, than the influent water. In contrast, other studies have reported significant increases in ammonium concentrations downstream of fish farms (Homewood et al. 2004). The increase in ammonium level is probably related to factors such as feeding metabolism, decomposition of leftover food and fecal materials on the pond's substrate, and general farm management (Carr and Goulder 1990; Kendra 1991; Bergero et al. 2001).

Macroinvertebrate Monitoring

The number of benthic taxa found during the present study is presented in Table 2. The aquatic macroinvertebrates identified were assigned to 10 classes, 17 orders, and 52 families.

No significant differences were detected between values of H' at inflow and outflow sites except at Takab Farm (Figure 2). However, for all farms, HBI increased significantly ($P < 0.01$) at the outflow and outfall sites in comparison with the inflow site. This was probably due to the increase in the number of tolerant taxa near the outflow at each farm and perhaps indicates the long-term existence of poor water quality at the outflow. A low value of H' at the Dimeh Farm inflow site was due to the dominance of Gammaridae at this site. Gammarids had the highest percentage abundance (91.44 ± 1.62% [mean ± SE]) relative to other identified families at this site. In contrast, the macroinvertebrates at the outflow site of Dimeh Farm were mainly dominated by members of Chironomidae, Oligochaeta, and Simuliidae, which also led to low diversity at that site. A significant increase in HBI and a decrease in taxonomic richness verify this fact ($P < 0.01$). The EPT taxonomic richness and EPT abundance decreased at the outflow site of Dimeh Farm compared with the inflow site, but the differences were not significant. However, it is commonly understood that these metrics are likely to decrease with increasing contaminant load or degradation of habitat quality.

A significant ($P < 0.05$) increase in chironomid and oligochaete density (overall density = 16,710 ± 3,801 organisms/m^2 [mean ± SE]; with 99.9 ± 0.1% dominance) was observed at the outflow site of Dimeh Farm (Figure 2). In contrast, mean density of Gammaridae was high at the inflow site (12,499.6 ± 4,408.2 organisms/m^2). While there were differences between macroinvertebrate indices at the Dimeh Farm inflow and outflow sites, benthic community responses measured as total taxonomic richness and EPT taxonomic richness showed minor differences between upstream and downstream sites. Values of H' were relatively similar, and no significant differences were observed between the upstream and downstream sites, thus indicating a good recovery of species diversity downstream. The HBI showed an increase at 50 m downstream from the Dimeh Farm outfall compared with the upstream site, followed by a significant decrease in EPT abundance ($P < 0.01$). Similar trends in taxonomic richness, diversity, and changes in the dominance of resistant organisms in response to fish farm discharge have been reported by others (Johnson et al. 1993; Loch et al. 1996; Yokoyama 2003). Although most macroinvertebrate indices showed improvement at 1 km downstream compared with 50 m downstream from Dimeh Farm, some indices (e.g., HBI) were still high at this sampling station, indicating that recovery was incomplete (Figure 2).

The variation in macroinvertebrate indices at the Hojat Abad Farm sites is shown in Figure 2. Generally, most of the biotic indices exhibited no significant differences among the sampling sites. Although benthic community responses measured as increased HBI and Oligochaeta percentage were significant ($P < 0.01$) at the outfall site of Hojat Abad Farm, increases in total taxonomic richness and H' were also observed. A suitable benthic environmental condition (sufficient flow and oxygenation) and enhanced food supply from fish farm effluent may be a possible reason for high densities of individuals and the large number of taxa in these circumstances (Yokoyama et al. 2007). In general, effluent from Hojat Abad Farm did not substantially affect water quality or benthic macroinvertebrate community structure except for the abundance of Chironomidae, which was high at 50 m downstream from the farm. Not only was benthic taxonomic richness unaffected by the farm effluent at all sites, but EPT taxonomic richness, EPT abundance, and total taxonomic richness also responded with an increase at 1 km downstream from this farm (see Figure 2). The similarity of H' values at upstream and downstream sites indicated that the number of taxa and the distribution of individuals did not considerably differ between these sampling sites. This may be attributable to the vegetation present in the ditch that acts as a sediment trap before effluent from Hojat Abad Farm is discharged into the river, as was reported in a similar study by Fries and Bowles (2002). On the other hand, Hojat Abad Farm is a small farm with a low production rate and low discharge, resulting in a minimal effect on the water quality and biotic indices of the fast-flowing, large-volume river receiving the discharge. In contrast, the effect of farm effluent on macroinvertebrate communities was more obvious during the period with the lowest flow rate. The HBI showed a significant ($P < 0.01$) increase at the outfall of Hojat Abad Farm under these circumstances. Furthermore, the increase in tolerant taxa was accompanied by a decrease in EPT taxa and EPT abundance at the outfall site compared with the inflow site.

The majority of macroinvertebrates at various stations of Takab Farm belonged to the families Tubificidae, Chironomidae, and Valvatidae. Except for H' and HBI, benthic community indices were not significantly different between inflow and outflow sampling sites (Figure 2). Moreover, physicochemical parameters such as TSS increased and the DO decreased at the outflow station, indicating a deterioration of water quality (Table 1). Interestingly, we found that the increases in oligochaetes and other tolerant taxa were not significant, but the abundances of sensitive taxa were noticeably reduced at the outflow of Takab Farm. Reduction in species richness, an increased density of tolerant taxa, and an increase in total faunal abundance with a high proportion of small-sized organisms are usually common features of a macroinvertebrate community that has been exposed to effluent from fish farms (Yokoyama et al. 2007). Analysis of variance showed no significant differences in EPT taxonomic richness and EPT abundance indices at inflow and outflow sites. This finding is probably due to impairment of the macroinvertebrate community by the poor quality of inflow water, which may have been caused by the low flow rate and by an upstream petroleum leakage (i.e., the broken oil pipe mentioned earlier).

Water quality variables (ammonium, BOD_5, and pH levels) and macroinvertebrate indices (HBI and H') were all

TABLE 2. Benthic macroinvertebrates collected from sampling sites (including the inflow, outflow, and upstream or downstream of the effluent outfall) at three rainbow trout farms located along the Zayandeh-Roud River.

Class	Order	Family	Genus
Crustacea	Amphipoda	Gammaridae	*Gammarus*
Malacostraca	Isopoda	Asellidae	*Asellus*
Insecta	Diptera	Simuliidae	
		Chironomidae	
		Ceratopogonidae	
		Empididae	
		Ephydridae	*Hydrella*
		Psychodidae	
		Tipulidae	*Antocha*, *Tipula*, *Dicranota*
		Tabanidae	
		Stratiomyidae	
Insecta	Ephemeroptera	Baetidae	*Acentrella*, *Heterocloeon*, *Centroptilum*, *Baetis*
		Caenidae	*Caenis*, *Cercobrachys*
		Heptageniidae	*Ecdyonurus*, *Heptagenia*, *Rhithrogena*, *Arthroplea*
		Oligoneuridae	*Oligoneuriella*
		Ephemerellidae	*Serratella*, *Attenella*
		Siphlonuridae	*Siphlonurus*
		Potamanthidae	*Potamanthus*
Insecta	Hemiptera	Corixidae	*Micronecta*, *Corixa*
Insecta	Trichoptera	Hydropsychidae	*Ceratopsyche*, *Potamyia*
		Hydroptilidae	*Hydroptila*, *Oxyethira*
		Philopotamidae	*Chimarra*, *Philopotamus*
		Phryganeidae	*Agrypnia*
		Polycentropodidae	*Polycentropus*
Insecta	Coleoptera	Elmidae	*Elmis*, *Stenelmis*
		Hydraenidae	
		Hydrophilidae	
		Curculionidae	
		Dytiscidae	
		Gyrinidae	
Insecta	Odonata	Gomphidae	
		Calopterygidae	
Gastropoda	Pulmonata	Physidae	
		Lymnaeidae	
		Ancylidae	
		Planorbidae	
Gastropoda	Ectobranchia	Valvatidae	
		Hydrobiidae	
Bivalvia	Veneroida	Sphaeriidae	
Hirudinea	Pharyngobdellida	Erpobdellidae	
	Rhynchobdellida	Glossiphoniidae	
Oligochaeta	Haplotaxida	Naididae	
		Tubificidae	
		Enchytraeidae	
		Lumbriculidae	
		Lumbricidae	
		Haplotaxidae	
Arachnida	Actinedida	Hygrobatidae	
		Limnocharidae	
Turbellaria	Seriata	Planariidae	
Entognatha	Entomobryoidea	Entomobryidae	

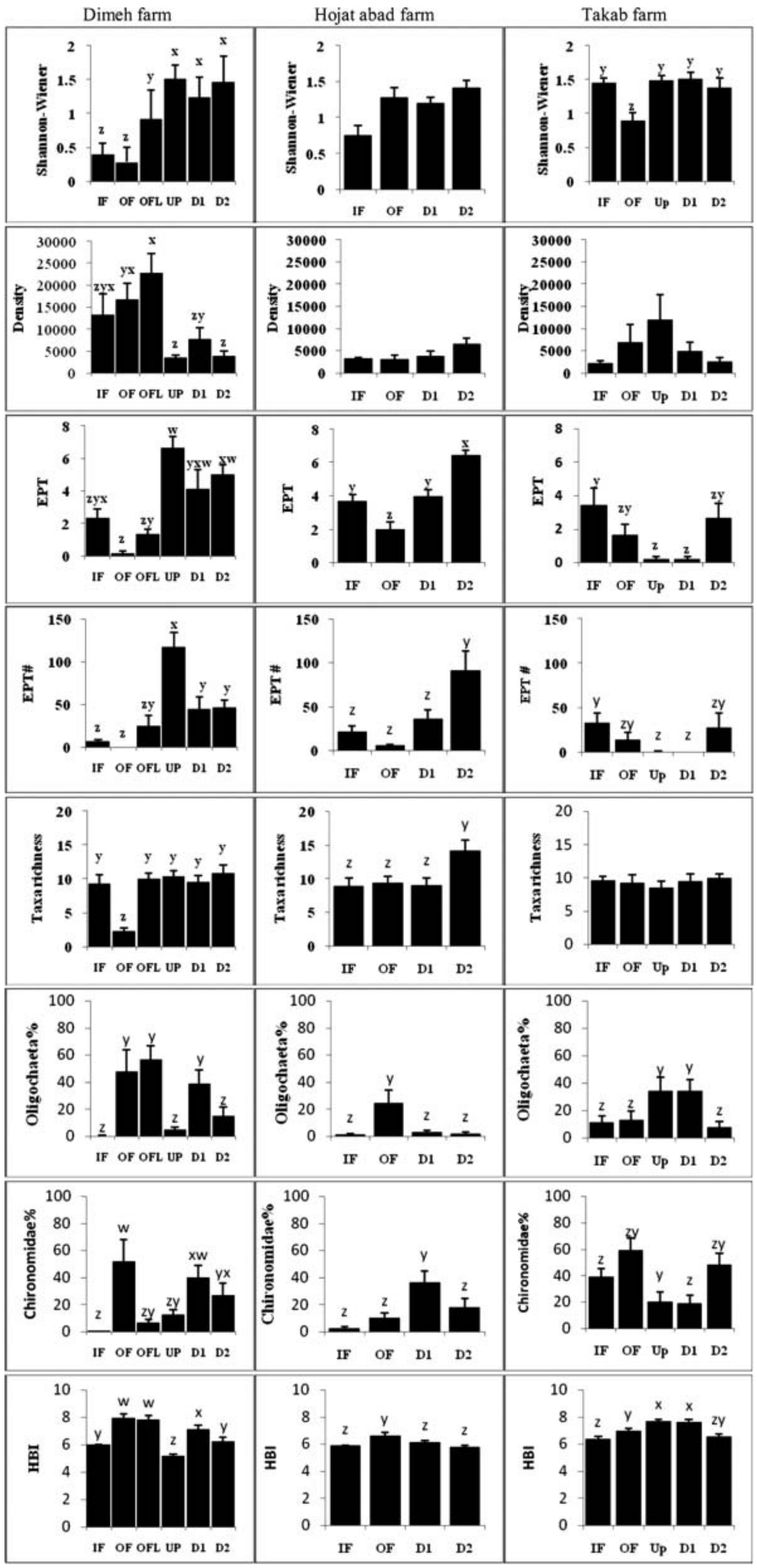

FIGURE 2. Benthic macroinvertebrate community metrics (mean + SE) calculated for the six sampling stations (IF = inflow, OF = outflow, OFL = outfall, UP = upstream, D1 = 50–100 m downstream, D2 = 1 km downstream) at three rainbow trout farms on the Zayandeh-Roud River (for Hojat Abad Farm, UP = IF and OF = OFL; Shannon–Wiener = Shannon–Wiener diversity index; density = number of macrobenthic organisms/m^2; EPT = richness of Ephemeroptera, Plecoptera, and Trichoptera taxa; EPT# = abundance of EPT individuals; taxa richness = total number of taxa observed; Oligochaeta% = percentage of sampled individuals that were oligochaetes; Chironomidae% = percentage of sampled individuals that were chironomids; HBI = Hilsenhoff's biotic index). Significant differences ($P < 0.05$) between stations for a given farm are indicated by different letters above the columns.

TABLE 3. Relationship between total fish production (biomass [metric tons, values are the slopes (*b*) of the regressions]; independent variable) at three rainbow trout farms located along the Zayandeh-Roud River and the measured macroinvertebrate indices and physicochemical characteristics (dependent variables), as evaluated by simple linear regression (BOD_5 = 5-d biochemical oxygen demand; density = number of macrobenthic organisms/m^2; H' = Shannon–Wiener diversity index; HBI = Hilsenhoff's biotic index).

Parameter	pH	BOD_5	Ammonium	Density	H'	HBI
Biomass	−0.002	0.053	0.009	112.04	−0.006	0.008
r^2	0.50	0.68	0.56	0.645	0.90	0.626
P-value	0.03	0.006	0.02	0.009	<0.001	0.01

significantly related to the total biomass of fish held at the facilities (Table 3). Results of multiple linear regressions between each biotic index and physicochemical variables are presented in Table 4. The BOD_5 was retained in all models except for the EPT abundance, EPT taxonomic richness, and total taxonomic richness models, whereas pH was retained only in these three latter models. The positive relationship of BOD_5 with percent Chironomidae, percent Oligochaeta, and HBI and the negative association of BOD_5 with H' imply that pollutants measured as physicochemical conditions influence the macroinvertebrate community. Similarly, Teodorowicz et al. (2006) recognized that a change in fish production rate can be one of the main indicators of the environmental impact of trout farming.

The association between tolerant groups and the physicochemical variables suggests that existing macroinvertebrate populations were exposed to high levels of pollution. The increase in BOD_5 was related to a high load of organic matter that nourished chironomid and oligochaete populations, resulting in an increased abundance of these two families and a high total density of macrobenthos. Results also revealed a significant positive correlation between sensitive group indices (EPT taxonomic richness and EPT abundance) and pH. In fact, as expected, these indices demonstrated significant negative relationships with nitrate concentration and TDS. In general, concurrent with a reduction in water quality, the numerical dominance of pollution-tolerant taxa (e.g., Chironomidae, Simuliidae, and Oligochaeta) and a decline in sensitive taxa (e.g., EPT) were observed. Farm effluent quality is influenced by many factors, including influent water quality (Clarke 2003), flow rate (Axler et al. 1997), stocking density, and farm management (Boardman et al. 1998). It should be noted that each farm is unique and would be expected to differ in its discharge volume and characteristics. Therefore, one should be cautious when using the data from this study to extrapolate applications for other production units.

Conclusions

Although results of the physicochemical analysis showed differences in water quality characteristics between inflow and outflow sites, the overall effects of farm effluent on the water quality of the Zayandeh-Roud River were negligible. High water flow of the river diluted the effluent and caused a reduction in nutrient concentrations, thus maintaining the water quality constituents within acceptable ranges. However, the responses of macrobenthic organisms to farm discharge reflected the environmental deterioration. Effluent from the trout farms (especially the farm with a biomass greater than 100 tons) had a definite effect on the river's macrobenthic community; changes in macrobenthic indices implied that water quality was diminished up to 50 m downstream from the outfall but that the community had begun

TABLE 4. Minimal models of multiple linear regressions between biotic indices (dependent variables) and physicochemical characteristics (independent variables; physicochemical attributes are defined in Table 1; HBI = Hilsenhoff's biotic index; density = number of macrobenthic organisms/m^2; EPT# = abundance of Ephemeroptera, Plecoptera, and Trichoptera individuals; richness = number of taxa observed [EPT taxa or total taxa]; H' = Shannon–Wiener diversity index). Values represent slope values from the multiple regressions.

	Biotic index							
Physicochemical characteristic	Percent Oligochaeta	Percent Chironomidae	HBI	Density	EPT#	EPT richness	Total richness	H'
Nitrate	0.73			–0.022	–0.36			
pH					2.245	0.217	8.354	
TDS						–2.793		
COD			0.015					
BOD_5	2.939	4.314	0.125	0.67				–0.57
r^2	0.398	0.296	0.528	0.570	0.781	0.699	0.338	0.238
P-value	0.002	0.002	<0.001	0.002	<0.001	<0.001	<0.001	0.007

to recover 1 km farther downstream. Nonetheless, we maintain that a distance of 1 km is not sufficient for complete recovery of the macrobenthic community. We recommend that proper husbandry practices, suitable distance between farms (Loch et al. 1996), treatment of farm effluents before discharge (Tacon and Forster 2003), and the river's capacity for self-purification be taken into consideration for maintaining ecologically sustainable aquaculture.

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