Functional feeding groups of macroinvertebrates and their relationship with environmental parameters, case study: in Zarin-Gol River

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Abstract
Feeding strategies are typical traits reflecting the adaptation of species to environmental conditions. The aim of this paper was to find proof that functional feeding groups of benthic macroinvertebrates can be utilized as surrogates for biological assessments. The composition and abundance of functional feeding groups of the benthic macroinvertebrate assemblages were surveyed along the Zarin-Gol River, Iran during winter and spring of 2016. Benthic samples in 3 replicates were collected using a Surber sampler (900 cm² area) from 5 stations (in agricultural land, rural area, forest land, fish culture site and reference site). Ten environmental parameters including (temperature, dissolved oxygen, pH, Electric conductivity, etc.) of each station were assessed. A total of 901 individuals belonging to 30 families and 9 orders were identified. In terms of relative abundance, Collector/Gatherer and Collector/Filterers were the predominant groups at most sampling stations. Shredders had low abundance in all stations and were absent in station two. Principal component analysis and cluster ordination showed that there are two distinct groups of benthic macroinvertebrates and stations. The implication of the observed pattern of variation in the abundance of functional feeding groups was discussed regarding the functions of the ecosystem and water quality assessment.

Keywords: Functional feeding groups, Macroinvertebrate, Zarin-Gol River.

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Introduction
A multitude of general biological traits describe the ecological functions that reflect the adaptation of organisms to environmental circumstances (Townsend and Hildrew, 1994). Feeding strategies are typical traits reflecting the adaptation of organisms and they could form part of a unified measure across assemblages differing in taxonomic composition (Statzner et al., 2004). Classification of functional feeding in aquatic organisms increases our knowledge of trophic dynamics in aquatic systems by simplifying the benthic community into trophic functional feeding groups (FFG) (Cummins, 1995). Measures of feeding or trophic dynamics encompassing functional feeding groups provide information on the balance of feeding strategies (food acquisition) in the benthic macroinvertebrate community. Macroinvertebrates play important roles in many ecological processes in their ecosystems, being consumers at intermediate trophic levels and thus serving as channels by which bottom-up and top-down forces are transmitted (Wallace et al., 1999). Different food sources used by benthic macroinvertebrates comprise: the epilithic layer that grows on the surface of substrates (consumed by scrapers); the coarse detritus, composed chiefly of leaves falling down from riparian vegetation (consumed by shredders); the fine detritus, either deposited on the substrate (consumed by gatherers) or suspended in the water column (consumed by filterers); and finally, live animals (consumed by predators) (Merritt et al., 2002).

The functional composition of macroinvertebrate benthic fauna, quantified as the proportions of these different functional feeding groups (FFGs), has significant implications for ecosystem functioning (Minshall et al., 1992). Ecological patterns and processes in aquatic ecosystems have been revealed to vary at multiple spatial scales, between and within aquatic habitats (Merritt et al., 2002). However, there have been very few attempts on how the functional composition of macroinvertebrate assemblages changes with spatial scale in relation to habitat conditions.

The influence of land use change is a principal parameter on riparian vegetation and river biodiversity. Land-use changes have ecological consequences for the health of terrestrial and aquatic ecosystems (Bruno et al., 2014). In recent years, fluctuations in riparian vegetation, aquatic assemblages and the water quality of Zarin-Gol River, Iran, have been affected by different human activities. Indeed, agriculture, fish culture, and urban land-use changes often lead to decreases in diversity and shifts in relative abundance among the functional feeding groups (FFGs) of aquatic macroinvertebrate assemblages. In contrast, sites of good riparian quality (reference site) present higher abundances of some functional feeding groups (scrapers, predators, and collector-gatherers) (Mesa, 2014). The purpose of this research therefore, was to describe the general distribution of...
FFGs in the sampling site and to determine whether the observed pattern of distribution can be utilized as a basis for biological assessment of the sampling site.

**Materials and methods**

**Study area**

Gorganrood River is one of the main rivers in the Caspian Sea basin in the Golestan Province due to its significance in agriculture, aquaculture, aquatic biodiversity and maintaining ponds and lagoons of the region (Abdoli and Rahmani, 2001). Zarin-Gol River is one of the stretches of the Gorganrood River basin at longitude of 37°57', latitude of 36°52' and altitude of 280 to 2800 m with a length of 22 km, catchment area of 342.82 m² and discharge of 75*10³ to 150*10⁶ m³ (Afshin, 1984; Power Ministration, 1991). Zarin-Gol River has a large gravel and sandy substrate appropriate for the occurrence of different fish species such as riverine stone loach, black fish and rainbow trout (Abdoli, 2000). Increasing anthropogenic activities such as agriculture, fish culture and deforestation alongside Zarin-Gol River necessitate the study of habitat requirements to manage its aquatic biodiversity (Fig. 1).

![Figure 1: Map of the study area, showing location of the sampling stations in Zarin-Gol River, Iran.](image)

**Sampling method**

Stations for monitoring and evaluation were selected systematically. This increased the probability of diversity in the current river section and biological parameters among the sampling stations and, therefore, led to a more accurate and inclusive study of changes in
biological and environmental parameters. Water physicochemical factors (temperature, dissolved oxygen, electric conductivity and pH), sediment particles and macroinvertebrate samples were collected during winter and spring in 2016 from 5 stations from downstream to upstream regions. Dominant substrate type was determined both visually and randomly by measuring the diameter of the riverbed stones in 20 selected quadrates (50×50 cm) based on Lotfi (2012), and then classified according to Johnston and Slaney (1996).

These seasons were selected for sampling due to river-flow stabilities and evidence that macroinvertebrate abundances are greatest during these periods. The research focused on five land use categories: agricultural land (station 1), rural waste water (station 2), native forest (station 3), rainbow trout farm (station 4) and reference site (station 5) (Gholizadeh et al., 2017).

Benthic macroinvertebrate samples were collected using a Surber sampler (900 cm$^2$ area and 250 µm mesh size) with 3 replicates. The samples were preserved in 4% formalin. In the laboratory, the samples were washed and all macroinvertebrates were sorted under a stereoscopic microscope. The individuals were identified to the lowest possible taxonomic level using the available keys of Quigley (1986), Needham (1976), and Merritt and Cummins (1996). Data from previous literature were used for recognition of the functional feeding groups (Merritt and Cummins, 1996; Mihuc, 1997; Graca et al., 2001; Polegatto and Froehlich, 2003).

**Data analysis**

The distributional differences of aquatic macroinvertebrates between stations and seasons were estimated by one-way ANOVA. Analysis of differences in feeding groups among all taxa was implemented using non-centered Principal correspondence analysis (PCA). Subsequently we applied a cluster ordination to the PCA results in order to determine the groups of taxa with similar dietary habits. Multivariate analyses and graphical outputs in this article were computed with R software, available free at https://cran.r-project.org and Excel 2013.

**Results**

The results of physicochemical factors studied are summarized in Table 1. Physicochemical features of the river did not differ significantly throughout the time of sampling, except discharge and temperature. Temperature ranged from a minimum of 11.5 °C in winter (station 5) to a maximum of 16.7 °C in spring (station 1). Discharge presented a considerable fluctuation throughout the study, with the highest value in winter (1.32 m$^3$ s$^{-1}$) in station 5 and a minimum value in spring (1.01 m$^3$ s$^{-1}$) in station 1. The values of pH (average=8.61±0.15) stayed approximately constant during the whole period, as well as conductivity that did not suffer significant peaks (average=2.09±0.22 ms cm$^{-1}$). The highest value of dissolved oxygen was observed in station 5 (9.1 mg L$^{-1}$) and minimum...
value was in station 2 (8.43 mg L\(^{-1}\)). Substrate size indicated coarse gravel in station 1, small cobble in stations 2, 3 and 4, and large cobble in station 5. The maximum depth (0.61 m) and width (4.3 m) were recorded in the forest area.

Table 1: Characteristics of the sampling stations.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land use</td>
<td>Agriculture</td>
<td>Rural</td>
<td>Forest</td>
<td>Fish</td>
<td>Reference site</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>260.1</td>
<td>312.3</td>
<td>536.9</td>
<td>675.2</td>
<td>748.5</td>
</tr>
<tr>
<td>Distance from source (km)</td>
<td>21</td>
<td>15</td>
<td>9</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>Slope (%)</td>
<td>6.62</td>
<td>8.3</td>
<td>14.2</td>
<td>15.5</td>
<td>25.7</td>
</tr>
<tr>
<td>Discharge (m)</td>
<td>1.01</td>
<td>1.12</td>
<td>1.19</td>
<td>1.24</td>
<td>1.32</td>
</tr>
<tr>
<td>River width (m)</td>
<td>4.72</td>
<td>2.3</td>
<td>4.3</td>
<td>3.1</td>
<td>1.6</td>
</tr>
<tr>
<td>River depth (m)</td>
<td>0.22</td>
<td>0.35</td>
<td>0.61</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>16.7</td>
<td>15.1</td>
<td>13.5</td>
<td>12.9</td>
<td>11.6</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L(^{-1}))</td>
<td>8.55</td>
<td>8.43</td>
<td>8.83</td>
<td>8.61</td>
<td>9.1</td>
</tr>
<tr>
<td>pH</td>
<td>8.39</td>
<td>8.53</td>
<td>8.77</td>
<td>8.63</td>
<td>8.73</td>
</tr>
<tr>
<td>Electric conductivity (ms cm(^{-1}))</td>
<td>2.7</td>
<td>2.51</td>
<td>2.08</td>
<td>1.68</td>
<td>1.56</td>
</tr>
<tr>
<td>Maximum substrate size</td>
<td>Coarse gravel</td>
<td>Small cobble</td>
<td>Small cobble</td>
<td>Large cobble</td>
<td></td>
</tr>
</tbody>
</table>

To characterize general feeding habits of the studied taxa, the average percentage of each food type was computed for the 30 taxa (Table 2). However, the low abundance and frequency of some taxa in samples did not allow an accurate analysis. Nevertheless, we published all the results to facilitate future complementary studies. In a total of 901 specimens, belonging to 30 taxa (Table 2), Ephemeroptera and Diptera represented the dominant component of macroinvertebrate populations and accounted for 42.84% and 34.29%, respectively of the total organisms recorded. Amphipoda, Decapoda and Prosobranchiata were recorded at less than 1% each of the total macroinvertebrate population. Baetidae and Caenidae (collector-gathering), Chironomidae and Simuliidae (collector-filtering) and Ephemeroptera and Diptera were the most represented families in the study area.

Table 2: Functional feeding groups of macroinvertebrates in Zarin-Gol River.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Code</th>
<th>Number of individuals</th>
<th>FFG</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphipoda</td>
<td>Gammaridae</td>
<td>Gam</td>
<td>5</td>
<td>C.G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decapoda</td>
<td>Panopeidae</td>
<td>Pan</td>
<td>2</td>
<td>C.G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychomyiidae</td>
<td>Psy</td>
<td>35</td>
<td>C.G</td>
<td>3</td>
<td>18</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Trichoptera</td>
<td>Hydropsychidae</td>
<td>Hydr</td>
<td>27</td>
<td>C.F</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Glossosomatidae</td>
<td>Glas</td>
<td>46</td>
<td>Scr</td>
<td>5</td>
<td>3</td>
<td>17</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Philopotamidae</td>
<td>Phil</td>
<td>25</td>
<td>C.F</td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hydroptilidae</td>
<td>Hydr</td>
<td>17</td>
<td>Scr</td>
<td>8</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perlidae</td>
<td>Perl</td>
<td>7</td>
<td>Prd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plecoptera</td>
<td>Leuctridae</td>
<td>Leuc</td>
<td>2</td>
<td>Shr</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taeniopterygida</td>
<td>Taeni</td>
<td>1</td>
<td>Shr</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caenidae</td>
<td>Cae</td>
<td>141</td>
<td>C.G</td>
<td>13</td>
<td>8</td>
<td>58</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>Ephemeroptera</td>
<td>Heptageniidae</td>
<td>Hept</td>
<td>38</td>
<td>Scr</td>
<td>2</td>
<td>16</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baetidae</td>
<td>Bae</td>
<td>167</td>
<td>C.G</td>
<td>25</td>
<td>14</td>
<td>48</td>
<td>29</td>
<td>51</td>
</tr>
</tbody>
</table>
The Principal component analysis (PCA) and cluster analysis presented in Figure 2 display clearer separation of the macroinvertebrates and stations. The F1 and F2 axes of PCA, performed on variables listed in Fig. 2 a, and b describe 72.71 and 23.86%, respectively of the total inertia. Group I is composed of stations 5 and 3 and Baetidae and Caenidae (collector-gathering) with relatively higher in abundance representations at the sampling stations, while group II is composed of stations 1, 2 and 4, and Chironomidae and Simuliidae (collector-filtering).

Figure 2: Principal component analysis (PCA) of five stations of the 30 analyzed taxa. (a) position of sampling stations, (b) distribution of different taxa at the F1 and F2 axes, (c) Cluster ordination of taxa based on the PCA results.

Five major FFGs were recognized in this research, these include; collector/gatherers (C.G), collector/filterers (C.F), Scraper (Scr), Predators...
(Pre) and Shredders (Shr). Analyzing the functional composition of the assemblage, it was found that collector/gatherers were the most abundant FFG (Fig. 3) with a total abundance of 468 accounting for 52% of the observed benthic macroinvertebrates. Collector/ filterers ranked second in total abundance (297) of individuals contributed, and constituted 33% of the total population recorded. A total abundance of 91 was recorded for the scrapers, while the predators had an abundance representation of 36, and both groups accounted for 10% and 4% of the total population respectively. Shredders were observed at the lowest abundance of 9 (1%) among all FFG.

There were differences in terms of abundance distribution of the FFGs at the spatial scales (sampling stations) (Fig. 4). Greater abundance of C.G and C.F were observed at stations 5 and 2, while that of scrapers were recorded at stations 1 and 5. The population of predators was highest in stations 3 and 6. The highest abundance of shredders was recorded in stations 4 and 5, but no shredders were recorded in stations 1 and 2 during this research.

![Figure 3: Overall percentage of the functional feeding groups in the sampling stations.](image)

![Figure 4: Percentage of macroinvertebrate functional feeding groups (FFG) sampled from the sampling stations.](image)
A brief summary of the taxa affinity to functional feeding groups is represented in the left part of Fig. 5. We state that omnivore, broadly defined as feeding on more than one trophic level, is a common feature of most taxa. This is the final result, combining our analysis and the available information of FFG from the literature. Most taxa were assigned to more than one FFG. Collectors-gatherers and collectors-filterers were the most frequent groups. Shredders were rarely assigned but often with a relatively high affinity.

**Figure 5:** Kite diagram showing general vertical distribution of functional feeding groups at stations.

**Discussion**

The result of physicochemical parameters showed relative fluctuations in the sampling stations. Rivers that drain agricultural and rural catchments are considered by their high levels of conductivity, nutrients, and suspended solids (Stefanidis et al., 2015; Gerth et al., 2017). The present study further established that high conductivity and nutrient values were associated with agricultural lands, rural area, and fish culture indicating that all of these land-use types can negatively affect physicochemical water quality. Another characteristic of rivers in agricultural catchments is comparatively elevated water temperatures (Gerth et al., 2017). Even though the current study displayed that station 1, draining agricultural catchments and station 2 in the rural area had higher water temperatures than other land-uses, likely as a result of scarce vegetative coverage, the recorded water temperatures were not excessively higher than the other sampling stations. The climate is influenced by the location of Golestan Province in the subtropical and humid area of the eastern Mediterranean. The Golestan Province includes mild weather and a temperate climate most of the year. The season influenced the structure and function of macroinvertebrates by accentuating changes in water quality and habitat characteristics. The abundance of most taxa was lower in the dry than in the time of high precipitation (Mathooko and Mavuti, 1992; Masese et al., 2009). Flow reduction during the spring season contributes to seasonal variability in physicochemical conditions that could affect benthic assemblages. For example, we recorded the lowest DO
and highest conductivity during the spring season in stations 1 and 2.

This is the first major study in Zarin-Gol River examining the functional structure of benthic macroinvertebrates as a bio-assessment tool. In terms of the distribution of functional feeding groups, the benthic community revealed few fluctuations across the study stretch, collectors (gathered) being the dominant functional group in all the sampling stations. The results of the conducted research support the negative impact that land-use variations can have on freshwater ecosystems, particularly in catchment areas. Significant alterations were detected in the physicochemical parameters for water quality, in riparian vegetation quality, and in macroinvertebrate assemblages. Studies across Zarin-Gol River show that agricultural practices have intensified in intermediate and lower catchment areas, extending to river banks where water quality is then negatively impacted (Sadeghi et al., 2015). Stations in the agricultural and rural land-use catchments were significantly more affected than stations in other land-use catchment areas. For instance, aquatic ecosystem health was best at stations within the forest and reference area. Considering that most land-use variations in the catchment regions of Zarin-Gol River occur due to destruction of native forest trees, agricultural lands, rural waste water and rainbow trout farm (Gholizadeh and Pakravan, 2017).

The representation of collector gatherers and collector filterers in all the study sites across the river indicates the importance of seston transport (suspended sediment) in the water column (Minshall et al., 1992). As all the stations were open and a great part of the river basin drains agriculture land (station 1), fish culture site (station 3) and forests (station 4) where particulate organic matter tends to be high providing enough filterable materials, this research revealed a positive and important relationship between abundance of collectors-gatherers/filterers and the total dissolved solids in the water column. The importance of fine detritus as food resources for benthic fauna has been emphasized by Tomanova et al. (2006) in other lotic ecosystems. The fact that the collectors-gatherers (Baetidae and Caenidae) and collector filterers (Chironomidae and Simuliidae) were abundant in most of the sampling stations responded to an important habitat complexity that possibly improved organic matter retentions and availability of suspended organic matter. The poor representation of collectors-gatherers at station 1 and collector-filterers at station 3 could be related to the discontinuities in seston supply and poor water circulation in the muddy sediment (Uwadiae, 2009). In this research, shredders had the lowest relative participation (1%). Irons et al. (1994) recommended that shredding may be less significant in subtropical systems because there are alternative decomposition pathways for leaves, such as faster microbial processing due to higher temperatures and humidity. The nature of sediment size is a significant parameter in shaping
macroinvertebrate assemblages, both in structural and functional composition. Substrate was coarse gravel in station 1, small cobbles in stations 2, 3 and 4, and large cobbles in station 5. Generally, the FFG ratios provided evidence of widespread human effects in the sampling site. Selected ecosystem attributes can be the most sensitive measures of the state of ecosystems. Using various macroinvertebrate functional-group ratios as surrogates for these attributes can provide critical data with much less effort.

The results of this research strongly support that physicochemical factors and biological features are significantly modified as the result of anthropogenic land-use variations. The assessed land-use variations resulted in deteriorated riparian vegetation, and subsequently, significantly alter the physicochemical and biological characteristics of aquatic ecosystems. This is of great importance in a country such as Iran, where there is a general tendency to replace native forests with forest destruction, urbanization and agricultural/grazing lands. Macroinvertebrate communities were applied as a powerful tool in establishing the ecological effects of diverse land-use. Study stations in the forest catchment area and the reference site had the greatest diversity of macroinvertebrates and sensitive species. In contrast, stations in the agricultural and rural catchment area presented lower macroinvertebrate richness and a complete lack of some sensitive species. However, each provides a distinct understanding of existing ecological conditions, providing measurements for very different variables. As such, these indices should be utilized in conjunction with biological and physicochemical assessments to ensure gaining a clear understanding for the status of aquatic and terrestrial ecosystems. Since both indices work in diverse ways, the development of a multimetric index able to evaluate the overall ecosystem in a single value would be important for future studies.

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