Research Article

Larval Anopheles Species Composition and Diversity at Different Habitats and Seasons of Gondar Zuria District, Ethiopia

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1. Introduction

Female Anopheles mosquitoes transmit deadly malaria disease-causing pathogens [1, 2]. To date, globally, about 500 Anopheles species are listed. Among the hundreds of identified Anopheles species, 30–40 species are responsible for malaria transmission, of which about 15 species are the major ones [3–5]. Anopheles gambiae complex, An. funestus, An. nili, An. pharoensis, and An. moucheti are malaria vectors in Africa [6]. An. gambiae complex and An. funestus complexes are the dominant malaria vector species widely distributed in Africa [4, 6]. An. gambiae complex accounts for the majority of malaria cases and deaths [7]. An. arabiensis is the major malaria vector widely distributed in Ethiopia [8–11]. Anopheles species have diversified larval habitats; in some cases, different species share the same habitats, while other species are more selective for habitats with specific characteristics [12–15]. Spatial and temporal distributions of Anopheles species larvae are affected by habitat types and environmental factors including temperature, humidity, and precipitation across seasons [16–19]. Studies reported associations between habitat types of different Anopheles species and their abundance varied across months [14, 16, 19–21]. However, in the current study area, there is no information on the species diversity and abundance of Anopheles mosquito larvae in relation to habitat types and seasons.

The objective of this study was to identify Anopheles species, determine their association to different habitats—swamp, pond, stream, sewage, and irrigation canal—as well as determine seasonal variation.

2. Materials and Methods

Anopheles larval sampling was made in potential breeding habitats at Gondar Zuria District (37° 24′ 24″ E–37° 45′ 43″ E and 12° 7′ 23″ N–12° 39′ 24″ N), 1500–2300 m above sea level (Figure 1). The district experiences mild temperatures from
11 to 32°C. Mean annual rainfall ranges between 900 mm and 1035 mm, which is bimodal, with short rains from March to May, long rains from June to September, post rains from October to November, and dry season from December to February [22]. Larvae of Anopheles species were collected using the WHO standard 350 ml dipper from breeding habitats of irrigation, pond, sewage, stream, and swamp. Sampling was conducted in the dry season (December/2019–February 2020) and the short rainy season (March–May/2020). Ten technical replicates (dips) on 10 subsites of each habitat type were made to collect Anopheles larvae monthly during the study period [16]. The collected larvae were preserved in 70% alcohol in separate coded vials within 24 h of collection. Species were identified using dichotomous keys and recorded with respect to habitat and season [23, 24].

2.1. Data Analyses. Data were subjected to analysis of variance using SAS software (version 9.4) for determining differences in larval abundance between habitats, between months, and their interaction, where appropriate. Detrended correspondence analysis was performed to determine the association between species and habitats and between species and sampling months. And finally, a number of species diversity indices were computed to establish the diversity of species at different habitats and months. Correspondence analysis and diversity indices were computed using PAST 4.03 software [25].

The following 11 diversity indices were used in the present investigation.

1. Dominance (D) = 1–Simpson’s index, which ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the entire community completely).

2. Simpson’s index (1–D) = 1–dominance, which measures the “evenness” of the community from 0 to 1.

3. Shannon index (entropy), a diversity index, takes into account the number of individuals as well as the number of taxa. It varies from 0 for communities with only a single taxon to high values (as high as 5) for communities with many taxa, each with a few individuals. It compares the diversity between various habitats.

4. Menhinick’s richness index is the ratio of the number of taxa to the square root of the sample size.

5. Margalef’s richness index is used for small samples. It can be measured as \( H = (S - 1)/\ln N \), where \( H \) stands for Margalef’s index, \( S \) for the number of species, and \( N \) for the total number of individuals.

6. Equitability (also known as Pielou’s evenness) is Shannon diversity divided by the logarithm of the number of taxa. This measures the evenness with which individuals are divided among the taxa present. \( J = H' / \log(S) \), where \( H' \) stands for Shannon index and \( S \) for the number of observed species in the community.

7. Fisher’s alpha is a diversity index, defined implicitly by the formula \( S = \alpha \ln(1 + n/\alpha) \), where \( S \) is the number of taxa, \( n \) is the number of individuals, and \( \alpha \) is Fisher’s alpha. Fisher’s \( \alpha \) measures diversity within a population, and it is considered the best diversity index for many communities of species, including Lepidoptera.

8. Buzas and Gibson’s evenness: \( E = e^H / S \), where \( H \) is the Shannon index, \( S \) is the number of species, and \( E \) is the measure of evenness or equitability.

9. Brillouin’s index is more sensitive to species abundance and is calculated as \( HB = \ln(N!) - \ln(n_i!) \), where \( HB \) stands for Brillouin’s index, \( N \) for the total number of individuals in the sample, \( n_i \) is the number of individuals of species \( i \), and \( \ln(x) \) refers to the natural logarithm of \( x \).

10. Berger–Parker dominance: simply the number of individuals in the most dominant taxon relative to \( n \). A simple mathematical expression relates species richness and abundance and takes only the commonest species in the sample. \( d = N_{max}/N \), where \( N_{max} \) stands for the number of individuals in the most abundant species and \( N \) is the total number of species. It expresses proportional importance of most abundant species.

11. Chao 1, bias corrected: an estimate of total species richness based on the numbers of singleton and doubleton species. Formula: \( \text{chao1} = S_{obs} + N_{1}(N_{1} - 1)/(2 N_{2} + 1) \), where \( N_{1} \) and \( N_{2} \) are the counts of singletons and doubletons, respectively.

3. Results

3.1. Distribution of Larvae at Different Habitats. During this study, a total of 10195 larvae, i.e., 2025 from irrigated, 1987 from sewage, 2030 from pond, 2008 from stream, and 2145 from swamp were collected. Similarly, a total of 3697 An. gambiae complex, 1992 An. christyi, 1638 An. cinereus, 1509 An. demeilloni, and 1359 An. pharoensis larvae were collected.

According to the results of the analysis of variance, while the interaction effect of habitat and larval species was significant (\( F = 33.03, DF = 24,125, P < 0.0001 \)), it was not the case between months and species of Anopheles mosquito numbers (\( F = 0.98, DF = 16, P = 0.4802 \)).

None of the mosquito species significantly varied in abundance between different habitats (Table 1). Regardless of habitat, An. gambiae complex had the highest larval abundance, followed by An. christyi, An. cinereus, An. demeilloni, and An. pharoensis, in order of importance.

3.2. Seasonal Variation of Anopheles Larvae. According to the result of the analysis of variance, the number of larvae varied significantly with respect to months (\( F = 33.3, DF = 29,120, P < 0.0001 \)). Numbers were slightly higher in January and lower in March and April (Table 2).
Regardless of differences in sampling months, An. gambiae complex had the highest larval abundance, followed by An. christyi, An. cinereus, An. demeilloni, and An. pharoensis, in order of importance.

### Table 1: Mean of the number of larvae at different habitats.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Irrigation</th>
<th>Pond</th>
<th>Sewage</th>
<th>Stream</th>
<th>Swamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae complex</td>
<td>59.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>An. christyi</td>
<td>33.83&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>28.83&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>37.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.83&lt;sup&gt;b-d&lt;/sup&gt;</td>
<td>35.50&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>An. cinereus</td>
<td>27.50&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>27.66&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>28.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>27.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>29.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>An. demeilloni</td>
<td>27.50&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>23.66&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>26.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>25.33&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>24.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>An. pharoensis</td>
<td>16.83&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.50&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>24.16&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>17.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;c-e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) are not significantly different according to the Tukey honestly significant difference test at α = 0.05.

Regardless of differences in sampling months, An. gambiae complex had the highest larval abundance, followed by An. christyi, An. cinereus, An. demeilloni, and An. pharoensis, in order of importance.

### 3.3. Detrended Correspondence between Mosquito Larval Species and Habitats.

In the case of habitats, the correspondence analysis explained 100% of the association between habitats and species, with 80% in dimension 1 and 20% in dimension 2 (Figure 2, top). Dimension 1 was a result of the opposite association between irrigation and sewage and dimension 2 was that of irrigation and pond or puddle and swamp. As to the species, most of them were found close to the centroid (origin) which means that they all had similar trends, although there was a tendency to align themselves on different sides of dimension 1, showing some differences between them. None of the species appeared to be associated with any of the habitats.

### 3.4. Detrended Correspondence between Mosquito Larval Species and Months.

The correspondence analysis generally accounted for 100% of the association between species and the habitats (Figure 2, bottom). Dimension 1 accounted for 99.9% of the association between time (month) and a number of individuals of each Anopheles species corresponding to January and February (left) vs. May and December (left). Dimension 2 explained nothing for the association.

Most of the Anopheles species appeared to be spaced not very far from the origin, indicating a similar contribution and none of them had an affinity to any month.

### 3.5. Diversity Indices.

According to the results of the Shannon–Weiner index, the species diversity of Anopheles mosquito larvae appeared to be similar across the different habitats (around $H' = 1.5$). This happened
because all habitats had the same number of species, i.e., 5. There was no dominance of any species in any habitat during this study ($D = 0.23$). A similar condition was found for Simpson’s index across the different habitats. Equitability ($J$) was high indicating that species were equitably represented in all habitats ($J = 0.94$). Other indices did not vary across the different habitats (Table 3).

When the same thing was seen for sampling date across the season, a similar pattern was observed as the one seen on habitats, with negligible differences (Table 4).

### 4. Discussion

In this study, we confirmed that the different habitats had similar abundance of *Anopheles* mosquito larvae, while the different mosquito species varied in abundance significantly, for example, *An. gambiae* complex was the most abundant of all.

The field collection of *Anopheles* larvae in the current study indicated the presence of *Anopheles* species including *An. gambiae* complex, presumably *An. arabiensis*, *An. christyi*, *An. cinereus*, *An. demeilloni*, and *An. pharoensis*.  

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**Table 2:** Mean of the number of larvae in months of the study season.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. gambiae</em></td>
<td>68.4a</td>
<td>68.2a</td>
<td>62.0bc</td>
<td>53.2bc</td>
<td>52.4bc</td>
<td>66.8bc</td>
</tr>
<tr>
<td><em>An. christyi</em></td>
<td>36.2de</td>
<td>39.2cd</td>
<td>32.4d-f</td>
<td>29.2d-g</td>
<td>29.4d-g</td>
<td>34.4d-f</td>
</tr>
<tr>
<td><em>An. cinereus</em></td>
<td>26.6d-h</td>
<td>30.4d-f</td>
<td>28.8d-h</td>
<td>27.8d- h</td>
<td>27.9d-h</td>
<td>26.8d-h</td>
</tr>
<tr>
<td><em>An. demeilloni</em></td>
<td>27.6d-h</td>
<td>25.6d-h</td>
<td>25.4d-h</td>
<td>21.8e-h</td>
<td>22.5e-h</td>
<td>29.6d-f</td>
</tr>
<tr>
<td><em>An. pharoensis</em></td>
<td>21.6e-h</td>
<td>21.4f-h</td>
<td>25.8d-h</td>
<td>14.4h</td>
<td>14.8g-h</td>
<td>21.0f-h</td>
</tr>
</tbody>
</table>

Means followed by the same letter(s) are not significantly different according to the Tukey honestly significant difference test at $\alpha = 0.05$.

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**Figure 2:** Detrended correspondence analysis between species abundance and time factor (months across the season).
Among the mosquito species recorded, *An. gambiae* complex was the dominant species and it had a stronger affinity to the stream habitat than the other habitats.

According to the detrended correspondence analysis, none of the mosquito species in the study area were found close to any of the habitats. Therefore, the present study indicated that all natural habitats contributed almost equally and no habitat was particularly associated with any of the mosquito species.

In line with the present study, various workers reported that *An. gambiae* complex was the most abundant species in different parts of Africa and Ethiopia [4, 6, 7, 26–29]. The better occurrence of the *Anopheles* species in January could be because the low temperature of the study area is not optimum for frequent adult emergence and the larvae stay a long time in breeding habitats [17–20, 30].

In contrast, Hinne et al. [29] reported that ponds of dug-out wells were the most productive habitats of *Anopheles* mosquitoes during the dry season, while swamps were also productive both in dry and rainy seasons in Africa. On the other hand, Getachew et al. [27] reported no association between the primary malaria mosquito, *An. gambiae* complex with swamps. Tire tracks, puddles, and collected rainwater supported the breeding of *Anopheles* mosquitoes temporarily [27].

On the other hand, irrigation-associated habitats were the major sources of *Anopheles* mosquitoes such as *An. arabiensis* and *An. pharoensis* [33, 34]. According to previous reports, *An. christyi*, *An. cinereus*, and *An. demeilloni* were mostly found in streams and swamps, whereas *An. pharoensis* were associated with swamp habitats [32].

In the current study, *Anopheles* species diversity was found to be average, i.e., $H' = 1.5$, indicating some signs of environmental degradation that failed to support more diversity. In another study carried out in Mara River and its tributaries, Kenya and Tanzania, Dida et al. [19] reported lower diversity than the current one. As a result, in the current study, no species were found particularly dominant (dominance $< 0.24$ overall), which is also supported by the high evenness and equitability values ($> 0.9$) indicating the lack of any dominant species.

In the current study, it was possible to underpin that it was not the habitats that mattered but the species themselves that dictated mosquito larval abundance because the larval abundance of any mosquito species did not vary between habitats. Significant differences in abundance were observed among the different species because of their intrinsic differences in their bionomic potential. Abundance slightly varied between months, whereby numbers were more in January than in March and April. Further study is required

### Table 3: Diversity indices of *Anopheles* mosquito larvae in different habitats.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Irrigation</th>
<th>Pond</th>
<th>Sewage</th>
<th>Stream</th>
<th>Swamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of species found (taxa)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Individuals</td>
<td>989</td>
<td>935</td>
<td>1118</td>
<td>947</td>
<td>1064</td>
</tr>
<tr>
<td>Dominance_D</td>
<td>0.2371</td>
<td>0.2375</td>
<td>0.2427</td>
<td>0.2358</td>
<td>0.2399</td>
</tr>
<tr>
<td>Simpson_1-D</td>
<td>0.7629</td>
<td>0.7625</td>
<td>0.7573</td>
<td>0.7642</td>
<td>0.7601</td>
</tr>
<tr>
<td>Shannon_H</td>
<td>1.523</td>
<td>1.526</td>
<td>1.516</td>
<td>1.527</td>
<td>1.521</td>
</tr>
<tr>
<td>Evenness_eH/S</td>
<td>0.9175</td>
<td>0.9201</td>
<td>0.9108</td>
<td>0.921</td>
<td>0.9156</td>
</tr>
<tr>
<td>Brillouin</td>
<td>1.51</td>
<td>1.512</td>
<td>1.504</td>
<td>1.513</td>
<td>1.509</td>
</tr>
<tr>
<td>Menhinick</td>
<td>0.159</td>
<td>0.1635</td>
<td>0.1495</td>
<td>0.1625</td>
<td>0.1533</td>
</tr>
<tr>
<td>Margalef</td>
<td>0.58</td>
<td>0.5847</td>
<td>0.5699</td>
<td>0.5837</td>
<td>0.5739</td>
</tr>
<tr>
<td>Equitability_J</td>
<td>0.9465</td>
<td>0.9483</td>
<td>0.942</td>
<td>0.9489</td>
<td>0.9452</td>
</tr>
<tr>
<td>Fisher_alpha</td>
<td>0.6876</td>
<td>0.6938</td>
<td>0.6744</td>
<td>0.6923</td>
<td>0.6797</td>
</tr>
<tr>
<td>Berger–Parker</td>
<td>0.3589</td>
<td>0.3668</td>
<td>0.3784</td>
<td>0.358</td>
<td>0.3712</td>
</tr>
</tbody>
</table>

### Table 4: Diversity indices of *Anopheles* mosquito larvae across the season (months).

<table>
<thead>
<tr>
<th>Month</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>December</th>
<th>February</th>
<th>January</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxa_S</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Individuals</td>
<td>732</td>
<td>729</td>
<td>893</td>
<td>903</td>
<td>872</td>
<td>924</td>
</tr>
<tr>
<td>Dominance_D</td>
<td>0.2397</td>
<td>0.2376</td>
<td>0.2408</td>
<td>0.2433</td>
<td>0.2313</td>
<td>0.2409</td>
</tr>
<tr>
<td>Simpson_1-D</td>
<td>0.7603</td>
<td>0.7624</td>
<td>0.7592</td>
<td>0.7567</td>
<td>0.7687</td>
<td>0.7591</td>
</tr>
<tr>
<td>Shannon_H</td>
<td>1.517</td>
<td>1.522</td>
<td>1.519</td>
<td>1.514</td>
<td>1.541</td>
<td>1.517</td>
</tr>
<tr>
<td>Evenness_eH/S</td>
<td>0.9113</td>
<td>0.9161</td>
<td>0.9138</td>
<td>0.909</td>
<td>0.9339</td>
<td>0.9119</td>
</tr>
<tr>
<td>Brillouin</td>
<td>1.499</td>
<td>1.504</td>
<td>1.505</td>
<td>1.5</td>
<td>1.526</td>
<td>1.503</td>
</tr>
<tr>
<td>Menhinick</td>
<td>0.1848</td>
<td>0.1852</td>
<td>0.1673</td>
<td>0.1664</td>
<td>0.1693</td>
<td>0.1645</td>
</tr>
<tr>
<td>Margalef</td>
<td>0.6064</td>
<td>0.6068</td>
<td>0.5887</td>
<td>0.5877</td>
<td>0.5908</td>
<td>0.5858</td>
</tr>
<tr>
<td>Equitability_J</td>
<td>0.9423</td>
<td>0.9455</td>
<td>0.944</td>
<td>0.9407</td>
<td>0.9575</td>
<td>0.9427</td>
</tr>
<tr>
<td>Fisher_alpha</td>
<td>0.7223</td>
<td>0.7228</td>
<td>0.6989</td>
<td>0.6977</td>
<td>0.7016</td>
<td>0.6951</td>
</tr>
<tr>
<td>Berger–Parker</td>
<td>0.3634</td>
<td>0.3594</td>
<td>0.374</td>
<td>0.3787</td>
<td>0.3555</td>
<td>0.369</td>
</tr>
</tbody>
</table>
to find out the year-round species composition of *Anopheles* mosquitoes and molecular identification of sibling species of *An. gambiae* complex in the area. Moreover, further study is required to determine the insecticide resistance level of the primary malaria mosquito, *An. gambiae* complex at a broader geographic area.

5. Conclusion

The present study gave information about *Anopheles* species diversity at different habitats during the different months. The results indicate that permanent and semipermanent larval habitats exist for malaria transmission, where vector management tools could be applied. Seasonal variation was noticed as larval population declined in March and April. *Anopheles* species diversity was rather low (about \( H' = 1.5 \)) as well as dominance (about 0.24 across all habitats and months). As a result, evenness and equitability were high (above 0.9) indicating the lack of any dominant species. The detrended correspondence analysis showed associations between habitats and or months, but none of the mosquito species had an affinity for any habitat or month.

Data Availability

The data used to support this study are available from the corresponding author upon request.

Consent

All parties including the authors agree on its publication in the current form.

Disclosure

The funder had no role in this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

YA initiated the research agenda, supervised the study, and prepared a draft manuscript. SA conducted the field and laboratory works, collected data, and wrote the draft thesis. MW conducted the statistical analyses and critically revised the final manuscript. All authors read and approved the final version of the manuscript.

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