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Anopheles arabiensis larval habitats characterization and *Anopheles* species diversity in water bodies from Jozini, KwaZulu-Natal Province

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Abstract

Background The South African government is now implementing winter larviciding as a supplementary vector control tool. To achieve effective larviciding programme there is a need to understand the distribution of the breeding sites of vectors and their corresponding ecology. This study aimed to determine larval breeding sites of anophelines and characterize the physicochemical properties of water that promote the proliferation of *Anopheles arabiensis* immature stages.

Methods A desktop survey of water bodies was carried out followed by a physical search of potential *Anopheles* breeding sites. Anopheline larvae were sampled from breeding sites in January and April 2021. At each breeding site, physicochemical characteristics of the water, including pH, electrical conductivity, total dissolved solids, salinity and turbidity, were measured. The collected *Anopheles* larvae were reared to adults and identified to genus and species level using morphological and molecular techniques. Factors associated with the presence of *An. arabiensis* larvae in the breeding sites were determined.

Results Out of the 72 water bodies identified using desktop survey only 53% (n = 38/72) were identified through physical search. Of these 84% (n = 32/38) were positive for *Anopheles* larvae. A total of 598 *Anopheles* larvae were collected, of which 59.4% (n = 355/598) emerged into adults. Morphological identification of these adults, showed that the *Anopheles gambiae* complex accounted for 70% (n = 250/355) of the collections. From the 250 *An. gambiae* complex collected, 94% (235/250) were identified to species level by PCR and 6% (n = 15/250) failed to amplify. Of the 235 *An. gambiae* complex that were identified to species level, 62.5% (n = 147/235) were from January collections and 37.4% (n = 88/235) were from April collections. Molecular identification of the *An. gambiae* complex to species level showed predominance of *An. arabiensis* in April, 91% (n = 80/88). All physicochemical parameters differed significantly between the breeding site classes ($p < 0.05$ in all instances), except for electrical conductivity ($p = 0.07$). The aquatic habitats surveyed showed that the impermanency of the water bodies, neutral to alkaline pH, moderate salinity and low total dissolved solids were associated with the occurrence of *An. arabiensis* larvae.

Conclusion This study showed that *An. arabiensis* primarily breed in small temporary water bodies characterized by neutral pH.

Keywords Malaria, Larviciding, Vector, *Anopheles*

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Background

In South Africa (SA) malaria is endemic in the low-lying areas of KwaZulu–Natal (KZN), Mpumalanga ([MP) and Limpopo Province (LP) [1]. In these areas, transmission generally occurs during rainy seasons [November to April) [2]. Malaria in SA is transmitted primarily by *Anopheles* [*arabiensis* after *Anopheles funestus* was nearly eliminated by the provincial indoor residual spraying (IRS) programmes [1, 3].

Indoor residual spraying using mosaic spraying approach of either dichlorodiphenyltrichloroethane (DDT) in traditional structures and or pyrethroids in modern structures is the main vector control strategy in SA [4]. Indoor residual spraying was first used in the 1940s and has been widely used [5, 6] resulting in a significant reduction in malaria burden [6]. Although this strategy remains effective, it fails to completely eliminate the malaria transmission as evidenced by ongoing residual transmission [4]. The IRS approach is experiencing numerous challenges such as the evolution of insecticide resistance in targeted vector populations [5] and the unsustainability of using IRS in low malaria settings [7]. This is coupled with the cosmopolitan feeding and resting behaviour of the primary vector *An. arabiensis* which is responsible for a majority of transmission [1]. *Anopheles arabiensis* feeds and rests both indoor and outdoor [1]. This makes the outdoor biting and resting segment of the population not fully amenable to IRS [1].

Against this background, additional vector control strategies are required to complement IRS. The KZN malaria control programme has started implementing yearly winter larviciding between May and July as a supplementary vector control strategy to augment IRS. Targeted larviciding, a component of larval source management (LSM), is more efficient and cost-effective in areas where aquatic habitats are fixed, scarce and findable [8, 9]. Larviciding is most effective when most of the productive breeding sites are targeted [10]. Therefore, in-depth knowledge on the larval distribution and ecological factors that are favourable to oviposition and subsequent mosquitoes' aquatic stages survival are needed before a larviciding programme is considered [11]. This information is required for planning, development and deployment of a larviciding programme [12]. Since breeding sites for *Anopheles* species differ, it is important to determine breeding sites of the different *Anopheles* vector species so that larviciding is tailored to the specific vector species dominant in a given locality.

Anopheles arabiensis larvae have been reported to occur in a variety of water bodies such as riverbeds, stagnant water, animal footprints, and man-made habitats [13]. In western Kenya, *An. arabiensis* were shown to prefer ovipositing in temporary pools [14]. Generally, temporary breeding sites are more productive for *Anopheles* mosquitoes [14]. This is because there is less predation in temporary breeding sites [15] and they tend to produce algae, which is the source of food for *Anopheles* larvae [16]. However, other studies have reported the occurrence of *An. arabiensis* in permanent water breeding sites such as ponds and perennial rivers characterized by irregular flow [17]. This shows that *An. arabiensis* is adaptable to different water bodies and its utilization of water bodies differ between geographic settings; therefore, it is important to identify and characterize breeding sites in any given locality before implementing control measures targeted to this species.

There is limited knowledge on the breeding sites of malaria vector species in SA particularly, in Mamfene, Jozini, KZN where *An. arabiensis* is the primary vector. Although, it is well known that rain puddles formed during rainy seasons are pivotal in creating potential *An. arabiensis* oviposition sites [1] their role as breeding sites in KZN has not been established. Such information is useful for implementing targeted larviciding. It is, therefore, important that evidence-based information on breeding sites distribution and larval ecology should be established in Mamfene to support the ongoing winter larviciding programme. Knowledge of the presence of potential breeding sites and their mapping is not sufficient for implementing a larviciding programme. The actual presence of larvae in these water bodies and subsequent physicochemical properties of the water that support anopheline oviposition and aquatic stages development is also critical [18].

Physicochemical factors of water bodies that are conducive to female oviposition and subsequent larval development include; total dissolved solids (TDS), dissolved organic and inorganic matter, turbidity, salinity, water temperature, electrical conductivity (EC), and hydrogen ion concentration [19]. Gouagna et al. showed that pH between 8.5 and 7.8 supported the growth of *An. arabienisis* larvae [20]. Moderate water turbidity was also found to be associated with high production from larvae to adults in Botswana and Ethiopia [21, 22]. Other separate studies showed a positive correlation between *An. arabiensis* larvae density and high water turbidity with algae and or larval density and absence of aquatic vegetation [23]. On the contrary, water EC had a negative correlation with *An. arabiensis* larval density [21]. Similarly, a study in Gambia found an association between abundance of *An. gambiae* sensu stricto *(s.s.)* and *An. arabiensis* larvae and low water EC, whereas high EC (i.e. 2000 micro Siemens (µS)/cm) was associated with low density of the larvae [23]. These data clearly show the role of physicochemical parameters of water bodies in determining breeding site productivity and to some instances

determine occurrence. Such information is useful in the implementation of targeted larviciding.

In this work, the occurrence of water bodies with physicochemical properties that are favorable to *An. arabiensis* larvae development were investigated. To achieve this mosquito-breeding sites in Mamfene, KZN province were surveyed and characterized. The occurrence of *An. arabiensis* was correlated with physicochemical properties. The data constitute the first comprehensive anopheline breeding sites mapping and characterization in Mamfene. Information obtained during this study established anopheline breeding site foci and can be used to inform larviciding priority areas for the KZN malaria control programme.

Methods

Study design and setting

The study was carried out in the Mamfene (S27°23′50.5″; E032°12′20.1″), Jozini Municipality, uMkhanyakude District, KZN Province (Fig. 1) [24] in January and April 2021. Mamfene is divided into ten administrative sections. Sections 2, 8 and 9 (Fig. 1], which fall within the KZN routine entomological surveillance sites, were the focus of this study. Most of Jozini's areas experience subtropical climatic conditions. The majority of malaria cases in this area are recorded during summer, from September to May, with *An. arabiensis* being implicated as the primary malaria vector [25]. The Jozini Local Municipality contains many non-perennial rivers, and tributaries [26] that are favourable for mosquito breeding. Balamhlanga is the main river that runs through Mamfene. In addition, the marshes in this area provide suitable mosquito larval breeding sites. Rainfall is highest from September to April, and the average rainfall is approximately 569 mm per annum [1].

Desktop survey for potential breeding sites in Jozini

All water bodies in the study area were first surveyed using a desktop survey. The water bodies were identified with Google earth Pro software. The identified points were imported from Keyhole Markup Language (KML) to the shapefile layer in ArcMap 10.6.1 software (Esri, New York, USA). The points were then converted to GPX and fed into a GPS Gamini (Olathe, Kansas, USA) device for navigation in the field. Subsequently, each of these water bodies were physically visited to assess the presence of larvae. Other water bodies that were not identified during the desktop analysis were also visited and considered during larval sampling.

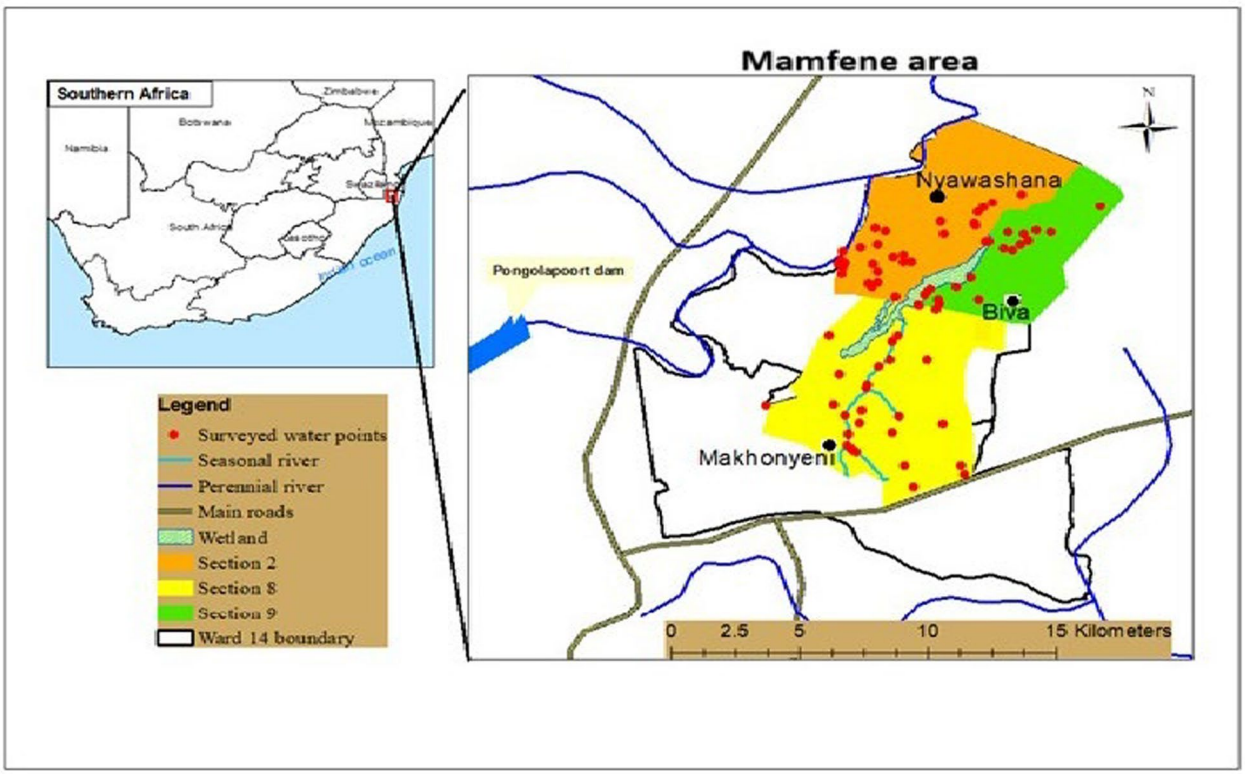


Fig. 1 Distribution of potential breeding sites identified through a desktop survey in Mamfene, Jozini, KZN, January 2021 stratified by section

Characterization of *Anopheles* larval habitats

At each breeding site, the physicochemical characteristics including water temperature, pH, EC, TDS, and salinity were measured on-site using a Consort C5020 meter (Consort, Turnhout, Belgium). Three replicate measurements were recorded from different sampling points per water body. To avoid bias, the Consort C5020 meter probes were cleaned with sterile water after each measurement. Turbidity was measured by placing water samples in glass test tubes and holding them against a white background and was categorized into three levels: low, medium, and highly turbid [27]. Breeding site types were first categorized into three broad classes as permanent, seasonal and temporary based on persistence of water in each water body. Based on this classification, each breeding site type was as assigned into one of these classes: spring, stream, riverbed, irrigation canal, marsh were categorized as permanent, seasonal breeding sites constituted marshes, while temporary breeding sites consisted of roadside, water tank, hoof or tyre print, rain puddle or artificial container. In addition, attribute information about the seasonality of the breeding sites was sourced from the community members and field workers who are stationed in the area. Exposure to sunlight was categorized as sunlit, semi-shade, well-shaded and deep shade. The presence of aquatic vegetation was visually inspected and classified into presence or absence. The distance to the nearest household was visually estimated. This was then categorized into four categories (0–99 m, 100–499 m, 500 m–999 m and more than 1 km).

Larval sampling and rearing

Global Positioning System (GPS) location of the breeding sites where larvae were collected was recorded and concurrently updated. The GPS points were transferred from the GPS device to Microsoft (MS) Excel file for data sorting and cleaning. The MS Excel file was imported into ArcGIS and then converted to the shapefile layer. To produce the thematic maps, the collected points were categorized according to the seasonality data obtained in the field and overlaid on top of a study area base map for elaboration and spatial analysis. The number of sampling points at the breeding sites was based on the size of the breeding site. On each water body that was classified as positive for larvae, sampling was performed using the standard dipping method [28]. In detail, a maximum of ten dips per sampling point were made depending on the size of the breeding site. Larvae collected were kept in containers with the same water from which they were collected [28]. Immediately after collection larvae were morphologically identified as *Anopheles* and any culicines were discarded. Predation of collected larvae was minimized by removing all visible organisms (predators) other than anopheline larvae. All collected larvae were transported to the field insectary for rearing into adults. Once in the insectary larvae were further re-examined and any residual culicines and predators were discarded [27]. The retained larvae now exclusively *Anopheles* were transferred to a clean 250 ml bowl according to the location of sampling. Larvae were reared to adults in 200 ml of distilled water at 25 °C and 75% relative humidity. The larvae were fed daily on a powdered larval diet consisting of a mixture of ground dog biscuits and yeast mixed at a ratio of 3:1 [29].

Anopheles larval species identification in different habitat types

Emerging adults were each assigned a unique identification number, morphologically identified using the dichotomous keys [30] and preserved individually on silica in 1.5 ml Eppendorf tubes. These specimens were subsequently transported to the National Institute for Communicable Diseases (NICD), Johannesburg, SA, for species identification using Polymerase Chain Reaction (PCR) as described by Scott et al., for the *An. gambiae* complex [31]

Statistical analysis

Data were captured on MS Excel and exported to STATA™ version 15 (StataCorp LLC, USA). Larval density was expressed as the number of larvae collected divided by the number of dips (containing approximately, 250 ml of water). Descriptive statistics were used in summarizing and visualizing data. Fisher exact test was used to determine the association between month of collection and larval sampling productivity and also the association between larval sampling productivity and breeding site type. The nonparametric Kruskal–Wallis test was used to infer differences in the physicochemical properties for breeding site class, breeding site type, *An. gambiae* complex members and other *Anopheles* species and differences in larval density between breeding sites. Dunn's test was used to compare differences in groups i.e. breeding site class, breeding site type, *An. gambiae* complex members and other *Anopheles* species. The point-biserial test was used to assess; the correlation between larval density for *Anopheles* species and breeding site class, and also the correlation between larval density for *An. arabiensis* and breeding site class. Temperature, pH, salinity, TDS and EC content are represented as medians.

Multivariable logistic regression was used to determine factors associated with the presence of *An. arabiensis* larvae. To identify the factors that influenced the presence of *An. arabiensis*, the outcome variable (species) was coded 1 if the mosquito species was *An. arabiensis* and 0 if it was any other species or the species could not be

identified. The prediction error of the models was evaluated using both the goodness of fit test and the Akaike Information Criterion (AIC). All the statistical analysis was performed in STATA, using a 5% level of significance ($\alpha = 0.05$).

Ethical consideration

Informed consent for the primary study was obtained from all household owners and community leaders involved in this study. Ethical approval to conduct this study was obtained from the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (257/2021) (Appendix 1 in ESM).

Results

Desktop survey for potential breeding sites in Jozini

A desktop survey revealed 72 potential water bodies (Fig. 1). The majority of these 38.9% (n = 28/72) were located in section 2, whereas 37.5% (n = 27/72) were in section 8 and 23.6% (n = 17/72) were in section 9 (Fig. 1). The desktop survey was only performed in January, since the time period between January and April was relatively short. Thus, we did not expect the number of potential breeding sites to differ significantly between January and April of the same year.

Physical survey and distribution of breeding sites

Out of the 72 water bodies identified using desktop survey only 52.8% (n = 38/72) were identified through physical search and these were subsequently surveyed for presence of anopheline larvae. Of the 38 water bodies 44.7% (n = 17/38) were found in the January survey and 55.3% (n = 21/38) in April. Among the larval breeding sites found in January, 88.2% (n = 15/17) were positive for *Anopheles* larvae, while in April 80.9% (n = 17/21) were positive (Supplementary Table 1). There was no association between month of collection and breeding site productivity (Fisher's exact test; p = 0.6) (Supplementary Table 1). Of the 15 breeding sites that were positive for mosquito larvae in January, 40% (n = 6/15) were classified as temporary, 40% (n = 6/15) as permanent, and the remaining 20% (n = 3/15) were classified as seasonal. In April, of the 17 breeding sites that were positive, 70.5% (n = 12/17) were temporary breeding sites, 17.6% (n = 3/17) were seasonal and 11.8% (n = 2/17) were permanent breeding sites (Table 1). There was no association between breeding site productivity and habitat class type (Fisher's exact test, p-value greater than 0.05 in all cases) (Supplementary Table 1).

Ecological description of *Anopheles* breeding site classes/types

Three breeding site classes (permanent, seasonal and temporary), were found in Jozini. The most frequent breeding site type, included marsh (permanent), stream (permanent), rain puddle (temporary), marsh (seasonal) and water tank (temporary). The most frequent larval breeding site type in January were marshes which contributed 41.2% (n = 7/17) of the total sites surveyed in that month. The least frequent were streams contributing (11.8%, n = 2/17) of the total sites. Conversely, in April, rain puddles constituted 52.3% (n = 11/21) of the total larval breeding sites found in that month, while water tanks accounted for 4.8% (n = 1/21), of the total larval breeding sites surveyed (Table 1).

The median larval density and interquartile range (IQR) for permanent breeding sites were lower (median = 3.3, IQR = (2–4.7)) compared to seasonal (median = 3, IQR = (2.6–18.3)) and temporary breeding sites (median = 3, IQR = (2.3–8.3)) (Supplementary Table 2). The point-biserial correlation coefficient ($r_{pb} = -0.4$) showed a very strong, positive correlation between the larval density for permanent and temporary breeding sites. Hedges's g indicated that the scores for permanent breeding sites was 0.7, which was lower than the score of temporary breeding sites. There was positive correlation ($r_{pb} = 0.5$) between the larval density and permanent and seasonal breeding sites. The score for permanent breeding sites was 0.9 for Hedges's g, which was lower than the score for seasonal breeding sites. There was a strong negative correlation ($r_{pb} = 0.3$), between the larval density for seasonal and temporary breeding sites. The scores for seasonal breeding sites were 0.6 for Hedges's g, which was lower than the score of temporary breeding sites (Supplementary Table 2). There were significant differences in larval density between different breeding site classes (Kruskal–Wallis test, $X^2 = 11$, $p = 0.004$) (Supplementary Table 3). A pairwise comparison revealed a significant difference in larval density between permanent and temporary breeding sites ($p = 0.006$), permanent and seasonal breeding ($p \leq 0.001$), but no difference were observed between seasonal and temporary breeding sites ($p = 0.2$) (Supplementary Table 4).

There was a significant difference in all physicochemical parameters between the breeding site classes (TDS (Kruskal–Wallis test, $X^2 = 163$, $p \leq 0.001$), pH ($X^2 = 80$, $p \leq 0.001$), salinity (($X^2 = 168.6$, $p \leq 0.001$), water temperature ($X^2 = 24.7$, $p \leq 0.001$), turbidity ($X^2 = 48.6$, $p \leq 0.001$) except for EC ($X^2 = 1.4$, $p = 0.5$ (Supplementary Table 3). The median TDS values of seasonal breeding sites (median = 13.3 g/l, IQR = (3.2 g/l–13.3 g/l)) were higher than that of permanent (median = 0.6 g/l, IQR = (0.5 g/l–0.8 g/l)) and temporary (median = 0.7 g/l,

Table 1 Characteristics of breeding sites characterized in section 2, 8 and 9, Mamfene, Jozini, KwaZulu-Natal, in January and April 2021 stratified breeding site class, breeding site type, section and month

Breeding sites		Section	January collection				April collection			
Breeding site class	Breeding site type		Number	Dips (larval count)	Larval density (larval count/dips (approximately 250 ml))	Perimeter (m)	Number	Dips (larval count)	Larval density (larval count/dips),	Perimeter (m)
Permanent	Stream	2	1	15 (42)	2.8	48	0	0	0	0
		8	0	0	0	0	0	0	0	0
		9	1	12 (69)	5.8	39	1	6 (7)	1.6	n/a
	Marsh	2	1	4 (8)	2	13	1	6 (9)	3	n/a
		8	1	9 (16)	1.7	22	0	0	0	0
			1	3 (6)	2	14	0	0	0	0
		9	1	6 (33)	5.5	20	0	0	0	0
Seasonal	Marsh	2	1	3 (13)	4.3	34	0	0	0	0
		8	1	13 (27)	2	22	1	3 (8)	2.7	11
			0	13 (109	8.3	136	1	6 (11)	1.8	15
			0	6 (22)	3.6	28	0	0	0	0
		9	1	3 (4)	1.3	72	1	3 (9)	3	n/a
Temporary	Puddle	2	1	12 (72)	6	30	1	3 (25)	8.3	4
			1	3 (2)	0.7	7	1	3 (24)	8	3.6
			1	3 (34)	11.3	5	0	0	0	0
		8	0	0	0	0	1	3 (1)	0.3	0
			0	0	0	0	1	3 (2)	0.7	14
			0	0	0	0	1	3 (6)	2	11
			0	0	0	0	1	3 (25)	8.3	11
		9	1	3 (24)	8	11	1	3 (28)	9.3	8
			1	3 (7)	2.3	11	1	3 (4)	1.3	0
			1	3 (7)	2.3	11	1	3 (2)	0.7	0
			0	0	0	0	1	3 (15)	5	0
			0	0	0	0	1	3 (7)	2.3	6
	Water tank	2	0	0	0	0	1	4 (6)	2	7
		8	0	0	0	0	0	0	0	0
		9	0	0	0	0	0	0	0	0

m meter, *n/a* not applicable, *Temp* temperature

IQR = (0.4 g/l–0.95 g/l)) breeding sites. Seasonal breeding sites had the highest water temperature (median = 30°c, IQR = (28.5 °C–30.2 °C)), whereas permanent breeding sites had the lowest temperature (median = 26 °C, IQR = (26 °C–31 °C)) (Fig. 2). Permanent and temporary breeding sites had neutral pH (median = 7, IQR = (7–7)) and (median = 7, IQR = (7–8)), respectively, whereas seasonal water had alkaline pH (median = 9, IQR = (8–9)). Salinity was high in seasonal breeding sites (median = 6.1%, IQR = (2.9%–14.3%)) and lowest in permanent water bodies (median = 0.5%, IQR = (0.5%–0.8%)) ((Fig. 2). The rank sum for seasonal (rank sum = 26,352) breeding site class was higher than that of permanent (rank sum = 12,856) and temporary (rank sum = 23,982) (Supplementary Table 3). A Dunn's test revealed a significant difference in TDS, temperature and pH between all the breeding site classes. On the other hand, there was a significant difference in salinity between all the classes except between temporary and permanent breeding sites (p = 0.1) (Supplementary Table 5). Moreover, there was a significant difference in turbidity between all the classes, except for seasonal and temporary.

There was a significant differences in the overall physicochemical properties of breeding sites ((TDS (Kruskal–Wallis test, $X^2 = 133$, $p \leq 0.001$), pH ($X^2 = 40$, $p \leq 0.001$), salinity (($X^2 = 140$, $p \leq 0.001$), water temperature ($X^2 = 102$, $p \leq 0.001$), turbidity ($X^2 = 31.7$, $p \leq 0.001$) between the different breeding site types but no significant difference was observed in EC ($X^2 = 8.5$, $p = 0.07$) between the different site types (Supplementary Table 3).

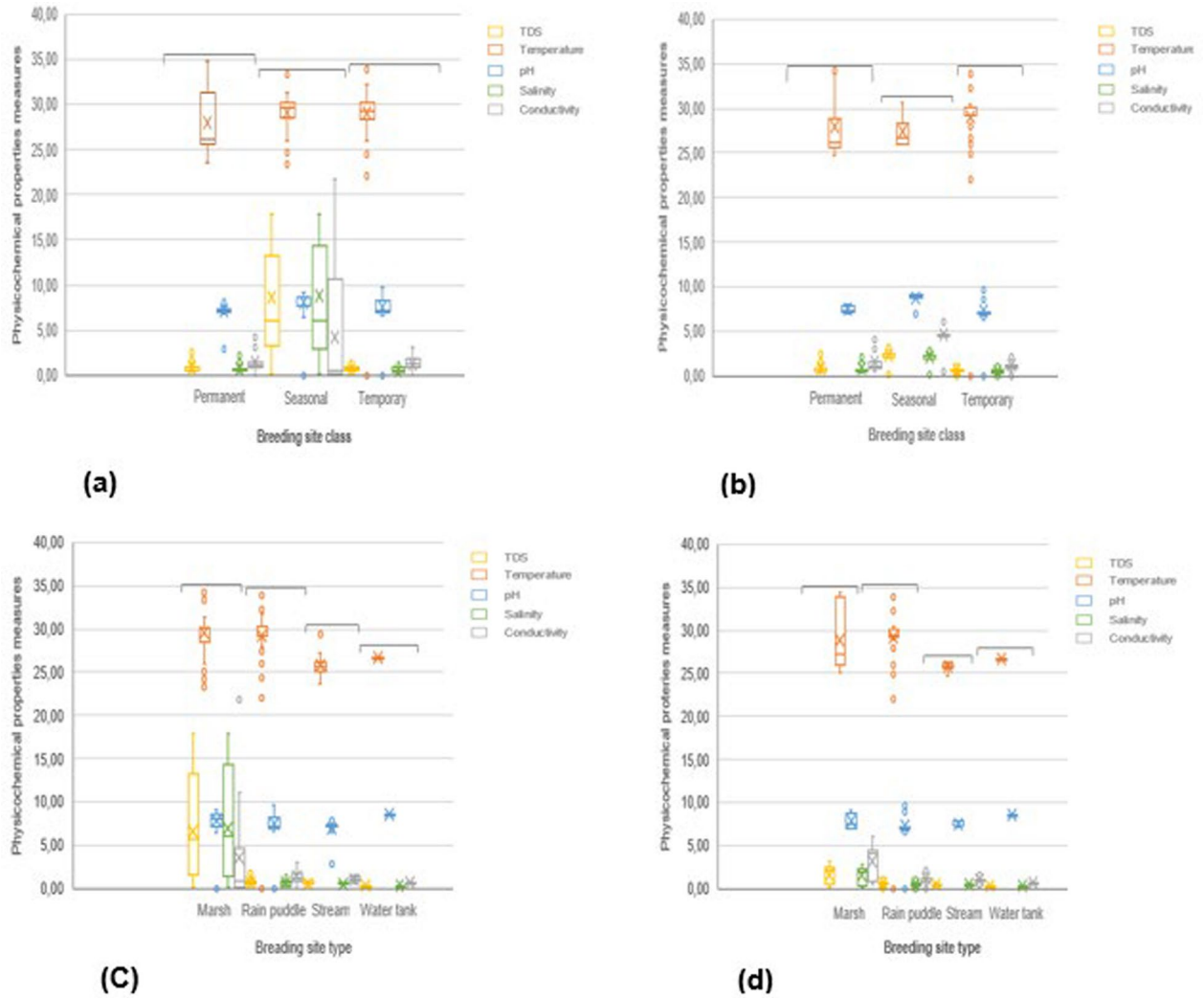


Fig. 2 Different physicochemical properties of *Anopheles* **(a)** breeding site class, **(c)** breeding site type, and *An. arabiensis* **(b)** breeding site class, **(d)** breeding site type characterized in Mamfene, Jozini, KwaZulu-Natal, 2021

The average TDS values of water tank (median = 0.37 g/l, IQR = (0.37 g/l–0.37 g/l)) and stream (median = 0.54 g/l, IQR = (0.5 g/l–0.79 g/l)) were lower than those of marsh (median = 6 g/l, IQR = (1.5 g/l–13.3 g/l)) and rain puddle (median = 0.6 g/l, IQR = (0.46 g/l–0.95 g/l)). Marshes had the highest water temperature (median = 30.2 °C, IQR = (28.5 °C–30.2 °C)), whereas stream water had the lowest water temperature (median = 25.6°c, IQR = (25 °C–26.1 °C)) (Fig. 2). Water tanks had alkaline pH (median = 8.6, IQR = 8.6–8.6), whereas streams had neutral water pH (median = 7, IQR = 7–7). Salinity was high in marshes (median = 6.0%, IQR = (1.4–14.2%)) and low in water tanks (median = 0.3%, IQR = (0.3–0.3%)) (Fig. 2). The rank sum for marsh (rank sum = 31,974) was higher than that of stream (rank sum = 7234), rain puddle (rank sum = 21,805) and water tank (rank sum = 2177) (Supplementary Table 3). There was a significant difference for TDS between different breeding site types but not between rain puddle and stream (Dunn's test, $p = 0.1$) and water tank and stream ($p = 0.05$). There was a significant difference in pH between all the breeding site types. Moreover, there was a significant difference in temperature between different breeding site types except rain puddles and marshes ($p = 0.41$) and water tank and stream ($p = 0.26$). On the other hand, there was a significant difference in salinity between all the groups except the rain puddle and stream ($p = 0.1$). There was a significant difference in turbidity between all the groups ($p \leq 0.001$) except the rain puddle and marsh ($p = 0.08$) (Supplementary Table 5).

Anopheles larval species identified, productivity between different breeding site classes/types and their physiochemical properties

In total 598 *Anopheles* larvae were collected. Of these 434 and 164 were collected in January and April 2021, respectively. Out of the 434, *Anopheles* larvae collected in January, 57.1% (n = 248/434) successfully emerged into adults, whereas of the 164 *Anopheles* larvae collected in April 65.2% (n = 107/164) emerged into adults (Table 2). After morphological identification of all emerged adults, the *An. gambiae* complex accounted for 64.1% (n = 159/248) of January collections. *Anopheles funestus* group accounted for 0.8% (n = 2/248). Other *Anopheles* species accounted for 35.9% (n = 89/248) of total collections. These include *Anopheles pharoensis* 22.2% (n = 55/248), *Anopheles rufipes* 10.1% (n = 25/248), *Anopheles squamosus* 1.6% (n = 4/248), *Anopheles coustani* 1.2% (n = 3/248). In addition, two specimens 0.8% (n = 2/248) were not identified morphologically. In April *An. gambiae* complex accounted for 85% (n = 91/107) of the total collections. Other *Anopheles* accounted 14.9% (n = 16/107). Of these *An. squamosus* contributed 9.3% (n = 10), *An. coustani* 4.7% (n = 5) and *An. pharoensis* 0.9% (n = 1) (Table 2).

Statistical analysis of the tolerances of all miscellaneous species to individual physicochemical properties revealed that there was a significant difference in tolerance of (*An. pharoensis, An. coustani, An. squamosus, An. rufipes)* to TDS (Kruskal–Wallis, $X^2 = 24.3$, $p < 0.001$), temperature ($X^2 = 10.1$, $p = 0.01$), pH ($X^2 = 41.4$, $p \leq 0.001$), salinity ($X^2 = 24.9$, $p < 0.001$), EC ($X^2 = 20.5$, $p < 0.001$) and turbidity ($X^2 = 12.2$, $p = 0.006$) in different breeding site classes (Supplementary Table 3). There was a significant difference in TDS between all miscellaneous species breeding sites, but not between *An. pharoensis* and *An. rufipes* (Dunn`s test, $p = 0.1$*)* and *An. coustani* and *An. squamosus* (Dunn's test, $p = 0.1$). (Supplementary Table 5). There was a significant difference in pH in breeding site classes where the different anopheline species were sampled, but not between *An. pharoensis* and *An. coustani* ((Dunn's test, $p \leq 0.001$, $p = 0.07$) and *An. coustani* and *An. squamosus* (Dunn's test, $p = 0.1$). There was a significant difference in temperature in breeding sites where the different anopheline species were sampled, but not between *An. pharoensis* and *An. rufipes* ($p = 0.45$), *An. pharoensis* and *An. squamosus* ($p = 0.54$) and *An. rufipes* and *An. squamosus* ($p = 0.4$). There was a significant difference in salinity in breeding sites where the different anopheline species were sampled, but not between *An. pharoensis* and *An. rufipes* ($p = 0.15$) and *An. coustani* and *An. squamosus* ($p = 0.1$). In addition, there was a significant difference in turbidity in breeding sites where the *An. pharoensis* and *An. rufipes* ($p = 0.002$) and *An. squamosus* and *An. rufipes (*$p \leq 0.001$) were sampled, but not between other species (Supplementary Table 5)..

Stratifying *An. gambiae* complex occurrence by breeding site type, showed that the majority 62.3% (n = 99/159) of larvae collected in January were from marshes, and the least 14.5% (n = 23/159) were collected from rain puddles (Table 2)**.** In April, the majority 61.5% (n = 56/91) of the *An. gambiae* complex were collected from rain puddles, and the least 6.6% (n = 6/91) were collected from water tanks (Table 2). From the 250 *An. gambiae* complex collected, 94% (235/250) were identified to species level by PCR and 6% (n = 15/250) of the *An. gambiae* complex failed to amplify. From 235 *An. gambiae* complex that were identified to species level, 62.5% (n = 147/235) were from January collections and 37.4% (n = 88/235) were from April collections (Table 2). Species from the *An. gambiae* complex members grouped by collection time point showed that during January surveys *Anopheles merus* was predominant 56.5% (n = 83/147) followed by *An. arabiensis* 41.5% (n = 61/147) and lastly *Anopheles quadriannulatus* which contributed 2% (n = 3/147). During April surveys, *An. arabiensis* predominated at 90.9%

Table 2 Number of *Anopheles* specimens collected in sections 2, 8 and 9, Mamfene, Jozini, KwaZulu-Natal, January and April 2021 stratified by breeding site class, breeding site type, species, month and section

Collection date	Breeding site class	Breeding site type	Section	Different *Anopheles* species									Total
				Anopheles gambiae complex				Other *anopheles* species				*An. funestus* group	
				An. arabiensis	*An. merus*	*An. quadriannulatus*	No ID	*An. pharoensis*	*An. rufipes*	*An. coustani*	*An. squamous*	No ID	
January	Permanent	Stream	2	3 (4.9%)	11 (13.3%)	1 (33.3%)	0	6 (10.9%)	1 (4%)	1 (25%)	0	0	23 (9.3%)
		Marsh		0	0	0	0	4 (7.3%)	0	0	0	0	4 (1.6%)
	Seasonal	Marsh		0	0	0	0	0	0	0	1 (25%)	0	1 (0.4%)
	Temporary	Puddle		5 (8.2%)	1 (1.2%)	0	0	0	24 (96%)	0	0	1 (50%)	31 (12.5%)
		Water tank		0	0	0	0	0	0	0	0	0	0
	Permanent	Stream	8	0	0	0	0	0	0	0	0	0	0
		Marsh		11 (18%)	**5 (6%)**	1 (33.3%)	1 (8.3%)	10 (18.2%)	0	0	0	0	28 (11.3%)
	Seasonal	Marsh		0	66 (79.5%)	1 (33.3%)	7 (58.3%)	23 (41.8%)	0	2 (75%)	3 (75%)	1 (50%)	103 (41.5%)
	Temporary	Puddle		0	0	0	0	0	0	0	0	0	0
		Water tank		0	0	0	0	0	0	0	0	0	0
	Permanent	Stream	9	19 (31%)	0	0	3 (25%)	7 (12.7%)	0	0	0	0	29 (11.6%)
		Marsh		6 (9.8%)	0	0	0	2 (3.6%)	0	0	0	0	8 (3.2%)
	Seasonal	Marsh		1 (1.6%)	0	0	0	3 (5.5%)	0	0	0	0	4 (1.6%)
	Temporary	Puddle		16 (26.2%)	0	0	1 (8.3%)	0	0	0	0	0	17 (6.9%)
		Water tank		0	0	0	0	0	0	0	0	0	0
		Grand Total		**61 (24.6%)**	**83 (33.5%)**	**3 (1.2%)**	**12 (4.8%)**	**55 (22.2%)**	**25 (10.1%)**	**3 (1.2%)**	**4 (1.6%)**	**2 (0.8%)**	**248**

Table 2 (continued)

Collection date	Breeding site class	Breeding site type	Section	Different *Anopheles* species									Total
				Anopheles gambiae complex				Other *anopheles* species				*An. funestus* group	
				An. arabiensis	*An. merus*	*An. quadriannulatus*	No ID	*An. pharoensis*	*An. rufipes*	*An. coustani*	*An. squamous*	No ID	
April	Permanent	Stream	2	0	0	0	0	0	0	0	0	0	0
		Marsh		7 (8.7%)	0	0	0	0	0	0	0	0	7 (6.5%)
	Seasonal	Marsh		0	0	0	0	0	0	0	0	0	0
	Temporary	Puddle		6 (7.5%)	0	0	1 (33.3%)	0	0	0	6 (60%)	0	13 (12%)
		Water tank		6 (7.5%)	0	0	1 (33.3%)	0	0	0	0	0	7 (6.5%)
	Permanent	Stream	8	0	0	0	0	0	0	0	0	0	0
		Marsh		0	0	0	0	0	0	0	0	0	0
	Seasonal	Marsh		14 (17.5%)	6 (85.7%)	0	1 (33.3%)	0	0	0	0	0	21 (19.6%)
	Temporary	Puddle		30 (37.5%)	0	1 (100%)	0	0	0	1 (20%)	0	0	32 (29.9%)
		Water tank		0	0	0	0	0	0	0	0	0	0
	Permanent	Stream	9	0	0	0	0	0	0	2 (40%)	2 (20%)	0	4 (3.7%)
		Marsh		0	0	0	0	0	0	0	0	0	0
	Seasonal	Marsh		0	0	0	0	0	0	2 (40%)	0	0	2 (1.9%)
	Temporary	Puddle		17 (21.3%)	1 (14.3%)	0	0	1 (100%)	0	0	2 (20%)	0	21 (19.6%)
		Water tank		0	0	0	0	0	0	0	0	0	0
		Grand Total		80 (74.8%)	7 (6.5%)	1 (0.9%)	3 (2.8%	1 (0.9%)	0	5 (4.7%)	10 (9.3%)	0	107

No ID not identified

(n = 80/88) of the collection followed by *An. merus* 8% (n = 7/88) and lastly *An. quadriannulatus* 1.1% (n = 1/88) (Table 2). The odds of collecting *An. arabiensis* larvae were 10 (OR = 10, CI: 5.5–16.4, p ≤ 0.001) times higher in April than January (Table 3).

Anopheles arabiensis larval habitat characterization

Stratifying *An. arabiensis* occurrence by breeding site class and month of collection showed that most common *An. arabiensis* larval breeding site classes found in January were 83.3% (n = 5/6) permanent and the least frequent were seasonal 16.6% (n = 1/6) (Fig. 3). Meanwhile, in April, temporary breeding sites were the most abundant, at 77.8% (n = 7/9) while seasonal and permanent breeding sites accounted for 11.1% (n = 1/9) and 11.1% (n = 1/9) of the total breeding sites encountered, respectively (Fig. 3).

The median larval density for *An. arabiensis* and interquartile range (IQR) for permanent breeding sites were lower (median = 3, IQR = (2–4.7)) compared to seasonal (median = 3, IQR = (1.3–2.7)) and temporary breeding sites (median = 3, IQR = (2.3–8.3)) (Supplementary Table 2). There was a powerful positive correlation (r_{pb} = 0.3) between the *An. arabiensis* larval density for permanent and temporary breeding sites. The Hedges's g score for permanent breeding sites was 0.5, which was lower than the scores for temporary breeding sites (Supplementary Table 2). There was a strong correlation

Table 3 Results for multivariable regression model for ecological and physiochemical factors associated with the presence of *An. arabiensis*

Variables		Univariate		Multivariate	
		OR (95% CI)	P-value	aOR (95% CI)	P-value
Ecological parameters					
Section	9	Reference	Reference	Reference	Reference
	2	0.17 (0.09–0.30)	< 0.0001	0.02 (0.007–0.08)	< 0.0001
	8	0.17(0.09–0.30)	< 0.0001	0.3 (0.1–1.2)	0.10
Month	January	Reference			
	April	9.5 (5.5–16.4)	< 0.0001	6.6 (2.4–17.7)	< 0.0001
Exposure to light	Sunlit	Reference			
	Semi-shaded	2.7 (0.98–7.5)	0.054	2.9 (0.8–9.8)	0.075
Distance	100–499	Reference			
	0–99	2.3 (1.28–4.18)	0.005	0.8 (0.3–1.8)	0.7
Aquatic vegetation	Present	Reference	Reference	Reference	Reference
	Absent	3.2 (1.37–7.8)	0.008	2.6 (0.9–7.7)	0.06
Breeding site class	Seasonal	Reference	Reference	Reference	Reference
	Permanent	6.24 (3.2–12.2)	< 0.0001	57 (6.2–526)	< 0.0001
	Temporary	15.15 (7.8–29.4)	< 0.0001	46 (5.7–374.6)	< 0.0001
Breeding site type	Marsh	Reference	Reference	Reference	Reference
	Stream	2.46 (1.27–4.74)	0.007	0.3 (0.07–1.2)	0.102
	Rain puddle	6.5 (3.8–11)	< 0.0001		
	Water tank	1	–		
Perimeter	Perimeter	0.89 (0.86–0.92)	< 0.0001	0.9 (0.9–1)	0.1
Physicochemical parameter					
Physicochemical parameter	TDS	0.58 (0.48–0.71)	< 0.0001	0.97 (0.6–1.6–3.74e + 10)	0.9
	Temperature	0.84 (0.87–1.03)	0.33	16 (0.3–821)	0.1
	PH	0.8 (0.66–1.07)	0.17	2,288,874 (11,834.18–4.43e + 08)	< 0.001
	Salinity	0.57 (0.46–0.7)	< 0.0001	0.8 (0.4–1.5)	0.5
	EC	0.87 (0.8–0.94)	0.001	1.8 (1–3.2)	0.02
	Turbidity				
	Low	Reference	Reference	Reference	Reference
	Medium	1.95 (1.06–3.58)	0.030	0.7 (0.3–1.8)	0.5
	High	1.82 (0.9–3.62)	0.084	0.9 (0.3–3)	0.8

OR odds ratio, *CI* confidence interval, *aOR* adjusted odds ration

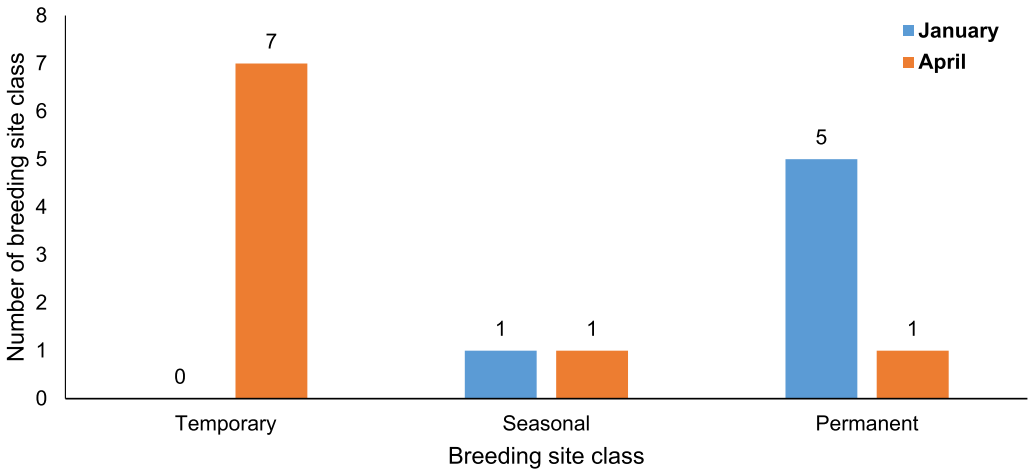


Fig. 3 Summary of breeding site classes productive for *An. arabiensis* in Mamfene, Jozini, KwaZulu-Natal, January and April 2021 stratified by month of collection

($r_{pb}=0.5$) between the *An. arabiensis* larval density for permanent and seasonal breeding sites. The Hedges's g score for permanent breeding sites was 0.8, which was lower than that of seasonal breeding sites. There was strong, positive correlation ($r_{pb}=0.5$) between the larval density for seasonal and temporary breeding sites. The Hedges's g score for seasonal breeding sites was 0.8, which was lower than for temporary breeding sites (Supplementary Table 2). There were significant differences in *An. arabiensis* larval density between the different breeding sites (Kruskal–Wallis test, $X^2=73$, $p\leq0.001$) (Supplementary Table 3). There was significant difference in larval density between other groups of breeding site classes ($p\leq0.001$), but no difference was observed between seasonal and temporary (Supplementary Table 4).

Stratifying *An. arabiensis* by breeding site type showed that most *An. arabiensis* larvae occurred in all breeding site types and co-existed with other species (Fig. 4). Overall, most *An. arabiensis* were collected from rain puddles 52.5% (n=74/141) followed by marsh 27.7% (n=39/141), stream 8.5% (n=12/141), and lastly water

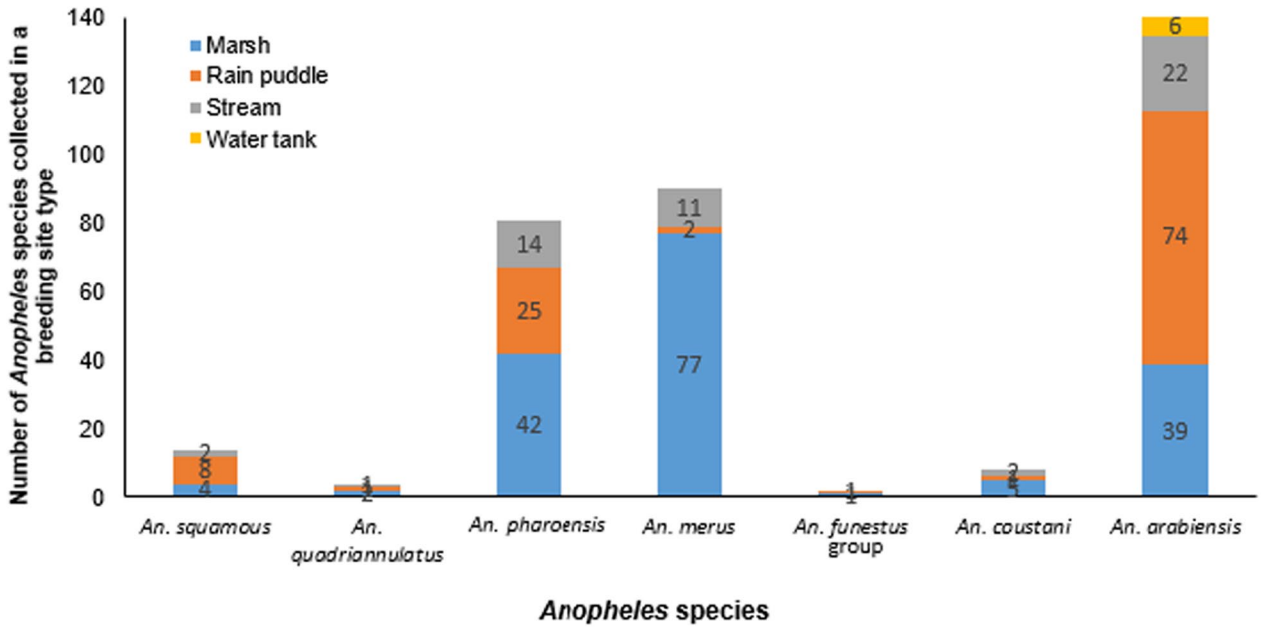


Fig. 4 Distribution of different *Anopheles* species by breeding site type in Mamfene, Jozini, KwaZulu-Natal, January and April 2021

tanks 4.3% (n = 6/141) (Fig. 4). The odds (unadjusted) of collecting *An. arabiensis* larvae from streams were three times higher (OR = 3, 95% CI: 1.2–4.74, p = 0.007) than those from the marsh. Similarly, the odds of collecting *An. arabiensis* larvae from rain puddles were seven (OR = 7, CI: 3.8–11, p ≤ 0.001) times higher than collecting them from marsh (Table 3). Furthermore, the multivariable logistic regression model showed that, the odds of collecting *An. arabiensis* larvae from temporary breeding sites were 47 (OR = 47, CI: 5.7–374.6–526, p ≤ 0.001) times higher than those collected from seasonal breeding sites. On the other hand, the odds of collecting *An. arabiensis* larvae from permanent breeding sites was 57 (aOR = 57, CI: 6.2–526, p ≤ 0.001) times higher than collecting from seasonal breeding sites (Table 3).

The pH in *An. arabiensis* larval habitats were neutral, ranging from 7.1 to 8.1. The concentration of TDS was highest in the breeding sites containing *An. merus* (13–43 g/l). Salinity was significantly high (12%) in *An. merus* breeding sites as compared to 0.55% in *An. arabiensis* breeding sites (p = 0.001) (Supplementary Table 3). These individual physicochemical properties showed a significant difference in tolerance of each member of *An. gambiae* complex to TDS (Kruskal–Wallis test, $X^2 = 131$, p < 0.001), temperature ($X^2 = 7.5$, p = 0.02), $_{p}H$ ($X^2 = 27$, p < 0.001) and salinity ($X^2 = 133$, p < 0.001) but not EC ($X^2 = 1.1$, p = 0.57) and turbidity ($X^2 = 0.8$, p = 0.6) (Supplementary Table 3). There was a significant difference in TDS of breeding site classes favoured by *An. arabiensis* compared to those that are preferred by *An. merus* (p = 0.001). In addition, there was a significant difference in pH of water that *An. arabiensis* larvae preferred and where *An. merus* larvae mainly occurred (p < 0.001). Moreover, the difference in water temperature between *An. arabiensis* breeding site classes and *An. merus* larval breeding site class was also significant (p < 0.001). There was also a significant difference in the salinity of water preferred by the two species (p = 0.001) (Supplementary Table 5). Temporary breeding sites with no vegetation found in April were strongly associated with the presence of *An. arabiensis* larvae (p < 0.01) (Table 3). On the other hand, physicochemical properties of breeding sites, such as TDS, salinity and EC were also significantly associated with the presence of *An. arabiensis* larvae (p < 0.01). Moreover, the physicochemical properties (i.e. EC and pH) of those breeding sites were also associated with the presence *An. arabiensis* larvae (Table 3).

Discussion

This study aimed to identify and characterize *Anopheles* larval breeding habitats in Mamfene, KZN, and to understand the ecology of *An. arabiensis* aquatic stages. The first step constituted a desktop survey of all the water bodies in the study area. This was followed by a physical search and characterization of all potential breeding sites that were positive for *Anopheles* species.

The number of breeding sites identified through desktop surveys was more than those physically characterized. This was expected as some of the water bodies identified through the desktop survey were inaccessible. This highlights the limitation of using conventional ground surveys of mosquito breeding sites compared to geospatial mapping using remote sensing techniques. Geospatial mapping is more accurate, less labour-intensive and exhaustive. However, this approach has its own drawbacks because they need a level of expertise that is not readily available in most malaria control programmes. In addition, even after desktop surveys are done there is still a need to do physical searches to confirm productivity of a water body.

Different breeding site classes such as permanent, seasonal, and temporary were found during this study. Categorizing breeding sites into these classes allows the understanding of the persistence of each breeding site and how they contribute to the seasonal abundance of different *Anopheles* species. Different *Anopheles* mosquitoes breed in different breeding sites [32, 33]. Various breeding sites were found during the ground surveys, these included rain puddles, marsh, water tanks and streams. Rain puddles were the most predominant breeding sites observed mainly because this study was done during the rainy season. Generally, rain puddles are formed during the rainy season, and they create potential oviposition sites for gravid female *Anopheles* mosquitoes [1]. This tally with reports from different studies that have reported the occurrence of numerous rain puddles during the rainy season [34]. The availability of numerous different types of breeding sites show that larviciding may be less effective when conducted during January or April (rainy season) since they will be too numerous to effectively manage [9]. It is therefore necessary to consider larviciding during winter seasons when the number of breeding sites is few and findable [9]. Occurrence of fewer breeding sites in winter was observed in Botswana and Zimbabwe by Mpofu et al. who reported a low baseline larval density in areas where larviciding was implemented in winter [35]. However, the impact of this winter larviciding was not conclusive as it was not clear if other vector control measures were responsible for the significant reduction in mosquito density and malaria cases [35].

In-depth knowledge of the correlation between *Anopheles* larval density and breeding site classes is important to establish when considering larviciding. Such information is important because it provides understanding of the association or relatedness between different breeding

site classes. The finding on strong correlation between *Anopheles* larval density and some breeding site types is important to malaria control programmes because it informs the programmes on the type of breeding site to consider when conducting larviciding targeted to primary and secondary malaria vectors in a particular area.

This study showed that different *Anopheles* species prefer different water bodies. Marshes and rain puddles were the most productive *Anopheles* breeding sites in Mamfene. This contradicts findings of Mereta et al. who reported high larval productivity in puddles compared to marshes in Ethiopia [28]. On the other hand, in a study done by Hinne et al., swamps and furrows were the most productive habitats [36]. This demonstrates that anopheline mosquitoes are adaptable to different breeding sites and indeed sampling productivity is specific to particular environmental, ecological factors and geographical settings, making it difficult to generalize [36]. The productivity of rain puddles in Mamfene could be explained by the fact that rain puddles usually do not support a wide range of predators and competitors compared to permanent breeding sites [28]. Therefore, seasonal and temporary breeding sites are more likely to support the development of *Anopheles* mosquito than other breeding site classes, hence the need to target this class of breeding site during larviciding. Another interesting observation was the presence of members of the *An. funestus* group in temporary water puddles and seasonal marshes. *Anopheles funestus* is traditionally associated with breeding in permanent water bodies [39]. However, because of the small sample size these findings warrant further investigation to see if indeed *An. funestus* group member are slowly adapting to breed in seasonal breeding sites.

The knowledge of ecology and physicochemical properties of breeding sites that support the development of *Anopheles* larvae is essential in designing a LSM programme. This information can be used when designing a larviciding programme [42]. Physiochemical parameters such as temperature, TDS, pH, turbidity, salinity and EC were different in all breeding sites. This difference may be attributable to the characteristics of their soil particles and edaphic factors, which refer to soil-related factors that influence food production. Further studies are required to validate this hypothesis [37].

Water temperature is one of most important physicochemical parameter because it affects both the larval development [38] and the effectiveness of insecticides used during larviciding activities. It has been established that efficacy of insecticides depends not only on the active ingredient, but also on ambient temperature [39]. Water temperature was significantly different between the different breeding site types surveyed. The median water temperature was high in marshes and rain puddles. These findings particularly for rain puddles agrees with those reported in Iran by Soleimani-Ahmadi et al. [40]. Rain puddles are generally characterized by high water temperature because of their relatively small size and are normally exposed to direct sunlight and have few emergent vegetation [27]. This explains their larval productivity more than other types of breeding sites. Moreover, high temperatures are known to play a significant role in rapid development of larvae allowing *Anopheles* to develop before these temporary breeding sites dry [41] explaining why most fresh water breeding anophelines have adopted in ovipositing in such habitats.

Mosquito larvae have different tolerance for TDS and this in turn determine their density in a given habitat. In this work TDS was significantly different between the different breeding site types surveyed. Total dissolved solids observed in this study ranged from [0.2–1.2 g/l) in rain puddles and (0.2–17.8 g/l) in marshes. This falls within a range reported in other similar studies. For example, Akeju et al. reported TDS levels ranging between 10 and 27 ppm for breeding sites where *Anopheles* larvae were sampled [42]. On the other hand, Abai et al. recorded a high TDS range of (1261.40 ± 1214.31 ppm) which were associated with *Anopheles* mosquito larvae [43]. These observed differences confirms the ability of *Anopheles* larvae to adapt to high levels of TDS over time [42]. However, this tolerance is only up to an optimal point in which it becomes retrogressive. It has been reported that breeding sites with high TDS levels can be harmful to larvae of some mosquito species due to accumulation of toxic substances, which may interfere with their development [44]. Marshes had the highest median TDS level. This could be due to stagnation and or slow water flow, which gives more time for interaction between the water column and the underlying sediments, increasing the concentration of anions and cations.

pH is widely considered as a predictor of occurrence of *Anopheles* larvae [45]. Both an increase (alkaline) and a decrease (acidic) of pH decreases mosquito species diversity in breeding sites. Usually neutral pH is ideal for most anophelines especially for African malaria vectors [45]. In our findings the water pH level recorded varied across the different breeding sites and ranged from neutral to slightly alkaline. However, most mosquito larvae were found in neutral pH waters, which confirms that neutral aquatic habitats provide optimal environments for mosquito larvae to develop. *Anopheles* larvae require a well-balanced pH since it affects their homeostasis [45]. The pH range in anophelines breeding sites found in this study (7–9) is closely related to that recorded by Akeju et al. [42], where breeding sites pH ranged from 6.05 to 8.23 [42]. Although water pH is directly related to and may limit the distribution of aquatic organisms,

some species are tolerant to pH changes. Thus, when pH fluctuates, some species adopt to other mechanisms that allow them to survive at higher or lower pH values, resulting in a decrease in species diversity in such conditions [45].

Another physiochemical property investigated was salinity. The importance of having understanding on this parameter in breeding sites is illustrated by the complex interactions between natural and anthropogenic activities, which in turn affect this parameter [45]. Salinity differed across the breeding sites surveyed. High salinity level was recorded in marshes compared to other larval breeding sites in Mamfene. High salinity levels in semi-permanent breeding sites are often associated with rain-dissolving salts deposited at the bottom of these water bodies increasing salinity levels [46]. In essence, saline breeding sites contain high nutrient levels and most predators cannot survive in this environment due to the osmotic effect, which prevents predator development [48]. There is, however, limited information on how salinity level is associated with mosquito and predator population dynamics and this presents an opportunity for more research in this area. *Anopheles merus* was predominant in January because the breeding sites were slightly salty, however after the rains most breeding sites were diluted resulting in a decline in *An. merus* populations. During the dry season, salinity in semi-permanent water bodies starts to increase because of evaporation, this creates a suitable environment for *An. merus* a known salt tolerant species [46]. These finding are further supported by a study in Tanzania, where salt tolerant members of *An. gambiae* complex were abundant in the dry season, whereas freshwater members were shown to be abundant in the wet season [47].

Mosquito breeding sites are characterized by different ranges of EC and the breeding types are also a major factor that can influence this difference. An important parameter used to estimate the level of dissolved salts in water and soil is EC. Generally, an introduction of pollutants in water bodies tend to increase the EC levels [42]. The EC reported was not significantly different amongst all the water bodies that were productive for *Anopheles* larvae. The EC recorded in this study was low compared to that reported by Olusi et al. who reported EC which was 100 times higher than what was recorded in this study [48]. In their study EC was low in the rainy season compared to dry seasons [48]. This could explain the low EC values recorded in this study, which was conducted during the rainy season.

Turbidity was significantly different between the different breeding site types and it was high in marshes. However, in other studies high turbidity was recorded in River, rain water was observed to have low turbidity [49]. Although, water turbidity affects the basal temperature of a breeding site. It has also been observed that turbidity affects the distribution of female species for example the *An. gambiae senso lato* seem to prefer water with high turbidity [38]. Additionally, higher water turbidity is known to potentially decrease the possibility of being preyed upon because of decreased visibility [38]. However, there are limited studies done on turbidity of marsh as a breeding site for *Anopheles* mosquito.

A clear knowledge of vector species involved in sustaining malaria transmission is important in areas at risk of malaria. Larvae from the *An. gambiae* complex were most predominant species sampled from the breeding sites. From this species complex, *An. arabiensis* was the most abundant. This was not surprising as *An. arabiensis* has been reported as the predominant species in this area [1]. It remains the primary vector, following the near eradication of *An. funestus* [50]. Although there is a lot of information on the adult distribution and population dynamics of this vector species in Jozini, KZN [1, 50], this study focused on larval distribution and corresponding ecology.

Most of the *An. arabiensis* larvae were collected from temporary breeding sites. The preferred *An. arabiensis* breeding sites recorded during this study tally with the findings of Tarekegn et al. who also showed that *An. arabiensis* primarily breeds in small temporary water bodies that are less turbid [51]. This is further supported by Hamza who reported *An. arabiensis* breeding in rain pools and puddles [52]. *Anopheles arabiensis* prefer breeding in fresh temporary water pools because of the absence of predators and their exposure to sunlit which provide warmer water for rapid development of larvae to pupae [53]. In addition, small temporary water bodies have high algal density which act as a source of food for larvae [54].

Determining the accurate nature of the relationship between the breeding site class and the larval density of *An. arabiensis* is crucial because it provide information on the breeding sites that are preferred by this species. The strong correlation observed in this study, between some breeding site classes and *An. arabiensis* larval density gives information on the breeding site types that should be targeted when implementing winter larviciding targeted for this species. Since in this season temporary breeding sites preferred by this species are limited. Therefore, other water bodies such permanent and seasonal which had strong correlation in this study should be considered when implementing winter larviciding. However, there are limited studies conducted on this aspect.

Anopheles arabiensis co-existed with other *Anopheles* species meaning that a larviciding programme has the

potential of also controlling secondary vectors, such as *An. merus* which are not necessarily targeted by IRS. A comprehensive understanding of the aquatic immature stages of the vector's ecology is necessary for the proper implementation of mosquito larviciding in particular setting [55].

pH affects the permeability of the cell membrane and is directly related to cell function of the larvae, it is a significant factor that restricts the abundance and spread of aquatic organisms [45]. One of the physicochemical properties of water that was associated with *An. arabiensis* occurrence was a neutral pH. This was expected, previous studies have shown that mosquitoes larvae prefer water bodies with pH that ranges between 6.8 and 7.2 [21]. A study in Kenya showed that the developmental time of larvae to pupation was faster in water with a pH of 6.8 [56]. It has been hypothesized that the neutral pH is optimal in weakening the egg shells of *Anopheles* larvae increasing hatching efficiency [57]. However, there are some exceptions in which *An. arabiensis* has been found breeding in alkaline water habitats [52] and in some instances it has been associated with breeding sites that have a low level of acidity [58], demonstrating its adaptability. Nevertheless, water bodies with a pH range of below 4.5 (Acidic) and or above 10 (Alkalinity) do not support *An. arabiensis* breeding as it causes mortality of its larvae [19].

Salinity has an indirect effect in the control of the metabolism of aquatic organisms and the productivity of ecosystems [45]. Our results also show that salinity is also an important parameters driving the occurrence and distribution of immature mosquito species. Consistent with literature *An. arabiensis* larvae from this study were mainly found in waters with low levels of salinity. Interestingly, *An. arabiensis* larvae have also been reported to occur in highly saline water [59]. This cosmopolitan breeding site preference was reported as far back as 1947 when it was shown that immature stages of *An. arabiensis* can survive in water containing 0.00444 ppt of sodium chloride (NaCl) [60]. These findings were corroborated by Lemrabott et al., who demonstrated that approximately 86.5% of *An. arabiensis* larvae were able to survive in water containing 0.0175 ppt NaCl, confirming their ability to exist in saline environments [59]. In the same study, *An. arabiensis* was reported to exist in freshwater habitats, indicating the species ability to adapt to different ecotypes [59]. These findings are consistent with the results of the studies that showed that normally saltwater tolerant mosquito larvae can survive in both fresh and saline habitats [60].

Many aquatic biological processes for *An. arabiensis* such as growth and development are significantly influenced by water temperature [38]. Results from this work showed that *An. arabiensis* larvae were abundant in waters with warmer temperatures. This tally with literature, which insinuates that *An. arabiensis* larvae prefer warm temperatures [61, 62]**.** For example, Lyons et al. reported an optimal temperature of 32 °C for the development of *An. arabiensis* larvae [62]. The ability to survive warm temperatures is advantageous to *An. arabiensis*, because it is often associated with faster development ensuring its development in short lived temporary water bodies [63]. In essence, at high temperature the enzyme-catalyzed reaction of organisms increases, resulting in an increased growth rate [64]. On the other hand, low temperatures reduce larval development and adult activity of some *Anopheles* species, whereas, higher temperatures are associated with excessive mortality [62].

Total dissolved solids have a significant impact on niche partitioning of *An. arabiensis* larvae in their breeding sites [65]. That is, TDS influence the density of *An. arabiensis* larvae in a given breeding site. *An. arabiensis* in this study was observed to breed in water bodies with low TDS. Low TDS may increase the efficiency of larviciding since dissolved particles are few and may not interfere with insecticides used for larviciding.

Although, water turbidity affects the basal temperature of a breeding site. It has also been observed that turbidity affects the distribution of female species for example in the *An. gambiae* sensu lato seem to prefer water with high turbidity [38]. Additionally, higher water turbidity is known to potentially decrease the possibility of being preyed upon because of decreased visibility [38]. *Anopheles arabiensis* was mostly found breeding in sites that had medium turbidity. The result were contrary to those reported in other studies that showed a positive correlation between *An. arabiensis* and *An. gambiae s.s.* and high water turbidity with algae and or no aquatic vegetation [66]. Turbidity is mainly caused by the presence of food particles which explains why habitats with high turbidity are suitable for *An. arabiensis* larval development [67]. Although the general hypothesis is that gravid female *Anopheles* prefer to breed in turbid water bodies [27] there are some exceptions. For example, *An. arabiensis* larvae have been previously recorded in clear water [44] and moderate turbidity breeding sites [22] showing how this species is adaptable.

Electrical conductivity also has a significant impact on niche partitioning of *An. arabiensis* larvae in their breeding sites [65]. The EC for water bodies in which *An. arabiensis* larvae were found breeding was low and could not be associated with *An. arabiensis* larvae. It was lower than that reported by Chirebvu and Chimbari, who found larvae of this species in breeding habitats with water EC in the range 880.4–1641.8 µS/cm [21]. These findings were also confirmed in Gambia by Fillinger et al., who

found the association between the abundance of *An. gambiae s.s.* and *An. arabiensis* larvae with low water EC [66]. This is probably because *An. arabiensis* being an osmoconformer lacks the ability to regulate osmolarity and the ion content of its internal body fluids [68]. It is possible that excess ions derived from ingestion creates problems for the maintenance of homeostasis. On the other hand, the cuticle of fresh-water species is more permeable to water than that of saline-water mosquito larvae. High water EC can increase the permeability to ions, leading to increased effluxes and eventually larval death [68].

Despite several ecological and physicochemical factors being shown to play a role in the occurrence of *An. arabiensis* individually, when assessed together only a few factors were significantly associated with the presence of *An. arabiensis* larvae in breeding sites surveyed. Such factors included the type of breeding site, EC and pH. This is probably because in nature these factors interact with each other therefore taking them individually is an oversimplification of natural processes.

It is important to note that this study provides a basis for designing control interventions targeted to malaria, especially in areas where *An. arabiensis* plays a major role in malaria transmission. This study suggests that temporary water bodies play a major role in the ecology of *An. arabiensis*. There were a few limitations to this study, i.e. some physicochemical properties of water, such as temperature, usually fluctuate throughout the day and this had an impact in determining the suitable temperature ranges optimal for the survival of mosquito larvae. Some water bodies could not be characterized due to inaccessibility. The study was conducted in the summer months only and therefore information about the ecology and distribution of *Anopheles* larvae in the dry season is limited.

Conclusion

This study showed that *An. arabiensis* primarily breed in small temporary water habitats characterized by neutral pH. Larval abundance is influenced by warm water temperature. It is therefore recommended that malaria vector control programmes should target these temporary water bodies in order to optimize the efficacy of larviciding. Sympatric occurrence of different anophelines in the same breeding sites provides an opportunity to control other secondary vectors using larviciding. A similar study is recommended in winter to provide information on ecology and distribution of *Anopheles* larvae during this season. Such studies will help inform if winter larviciding, is effective.

Abbreviations

µS/cm	Micro Siemens
AIC	Akaike Information Criterion
DDT	Dichlorodiphenyl-trichloroethane
EC	Electrical conductivity
GPS	Global positioning system
IQR	Interquartile range
IRS	Indoor residual spraying
KML	Keyhole markup language
KZN	KwaZulu-Natal
LP	Limpopo province
LSM	Larval source management
MP	Mpumalanga
MS	Microsoft
NaCl	Sodium chloride
NICD	National institute for communicable diseases
PCR	Polymerase chain reaction
SA	South Africa
TDS	Total dissolved solids

Supplementary Information

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Additional file 1

Additional file 2

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Author contributions

G.M. designed the study, performed data collection, data analysis. E.E.M. Performed data collection, collate and analysed data. A.M. and I.M. Performed data analysis. D.D. and N.M. performed data collection, larvae rearing and morphological identification of adult mosquitoes. S.W. performed data collection and molecular identification. T.M. Performed data collection, generated spatial maps (Fig. 1) and molecular identification. E.E.M. and G.M. developed the manuscript and all authors reviewed the manuscript critically for critical intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethical approval to conduct this study was obtained from the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (257/2021).

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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