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Habitat characteristics of *Anopheles* spp. larvae: Potential malaria vectors in the coastal areas of Gaura Village, West Sumba

Karakteristik habitat larva *Anopheles* spp.: Vektor potensial malaria pantai di Desa Gaura, Sumba Barat

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ABSTRACT

Malaria remains a public health burden in tropical coastal regions, where complex environmental conditions support vector populations and increase transmission risk, particularly among vulnerable groups. Understanding the environmental characteristics of *Anopheles* spp. breeding habitats is essential for explaining vector distribution and informing control strategies. This study analyzed the environmental characteristics of potential *Anopheles* spp. breeding habitats in the coastal areas of Gaura Village. An observational descriptive survey with a cross-sectional approach was conducted using purposive spatial sampling across two survey rounds, measuring physical, chemical, and biological variables. A total of 12 breeding sites were identified, with confirmed vectors including *Anopheles sundaicus* (Rodenwaldt), *An. subpictus* Grassi, and *An. barbirostris* van der Wulp. Larval densities peaked in confined anthropogenic microhabitats, particularly buffalo wallows (3.5 larvae/dip) and footprints (3.1 larvae/dip). The habitats exhibited wide physicochemical variability (temperature 29.0–39.4 °C; pH 7.3–9.2; salinity 0–20‰). Principal component analysis (PCA) explained 61.3% of total variation, distinguishing habitats along two gradients: PC1 (34.3%), driven by structural area, dissolved oxygen, and light intensity; and PC2 (27.0%), driven by pH and salinity. All highly productive habitats were located within 512 meters of residential areas, placing communities within the active flight range of vectors. These findings indicate that malaria transmission risk in coastal Gaura Village is driven by environmentally diverse yet spatially clustered larval habitats, particularly small, human-proximal sites associated with livestock, highlighting the need for targeted larval source management.

Key words: *Anopheles,* coastal areas, habitat, Indonesia, malaria

ABSTRAK

Malaria masih menjadi masalah kesehatan masyarakat di wilayah pesisir tropis, di mana kondisi lingkungan yang kompleks mendukung populasi vektor dan meningkatkan risiko penularan, terutama pada kelompok rentan. Pemahaman mengenai karakteristik lingkungan habitat perkembangbiakan *Anopheles* spp. penting untuk menjelaskan distribusi vektor dan mendukung strategi pengendalian. Penelitian ini bertujuan menganalisis karakteristik lingkungan habitat potensial *Anopheles* spp. di wilayah pesisir Desa Gaura. Penelitian menggunakan desain observasional deskriptif dengan pendekatan potong lintang melalui *purposive spatial sampling* dalam dua putaran survei, dengan pengukuran variabel fisik, kimia, dan biologis. Sebanyak 12 habitat ditemukan dengan vektor terkonfirmasi, yaitu *Anopheles sundaicus* (Rodenwaldt), *An. subpictus* Grassi, dan *An. barbirostris* van der Wulp. Kepadatan larva tertinggi ditemukan pada mikrohabitat antropogenik yang terbatas, khususnya kubangan kerbau (3,5 larva/ciduk) dan jejak kaki (3,1 larva/ciduk). Habitat menunjukkan variasi fisikokimia yang luas (suhu 29,0–39,4 °C; pH

7,3–9,2; salinitas 0–20‰). Analisis *principal component analysis* (PCA) menjelaskan 61,3% variasi total, dengan dua gradien utama: PC1 (34,3%) dipengaruhi oleh luas struktur, oksigen terlarut, dan intensitas cahaya; serta PC2 (27,0%) dipengaruhi oleh pH dan salinitas. Seluruh habitat dengan produktivitas tinggi berada dalam jarak ≤512 meter dari permukiman sehingga masih dalam jangkauan terbang vektor. Temuan ini menunjukkan bahwa risiko penularan malaria di Desa Gaura dipengaruhi oleh habitat larva yang beragam namun terkonsentrasi secara spasial, terutama pada habitat kecil yang dekat dengan aktivitas manusia sehingga diperlukan intervensi *larval source management* yang terarah

Kata kunci: *Anopheles,* habitat, Indonesia, malaria, wilayah pesisir

INTRODUCTION

Despite global efforts, malaria remains endemic in several tropical and subtropical zones, with infants, young children, and pregnant women bearing the greatest burden. The disease contributes significantly to anemia-related morbidity, decreased workforce output, and mortality rates. Five *Plasmodium* species are known to cause malaria in humans, which are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. Among these, *Plasmodium knowlesi* has recently emerged as a significant cause of malaria in Southeast Asia, including regions such as Sarawak, Malaysia (Turki et al. 2021).

In West Sumba Regency, East Nusa Tenggara (NTT) Province, malaria cases have shown a concerning trend. Data from the NTT Provincial Health Office (2018–2022) indicate a significant increase in malaria cases, from 3,027 cases in 2018 to 3,679 in 2019 and 3,912 in 2020. By 2021, the number of cases surged sixfold to 24,433, before significantly decreasing to 1,903 cases in 2022 (Dinkes NTT 2022). Within West Sumba, the highest number of cases was reported in the working area of the Gaura Community Health Center. Over the past five years (from 2019–2023), the Annual Parasite Index (API) in West Sumba has increased from 68.80‰ in 2019 to 113.24‰ in 2022 before decreasing to 47.42‰ in 2023 (Dinkes Sumba Barat 2022). To date, malaria case management still faces obstacles, including limited information on the morphological identification and bionomics of *Anopheles* spp. across areas with high, medium, and low endemicity. Moreover, Sumba's geographical and climatic conditions, characterized by numerous water puddles and high humidity, create an ideal breeding ground for mosquitoes. Furthermore, the remaining low utilization rate of bed-net and mosquito repellents plus outdoor activities during night hours add to the risk of transmission.

The ecological conditions and their influence upon *Anopheles* spp. determine the risk transmission of malaria. For the survival and reproduction of these vectors, enviromental conditions that are favorable to larval habitats are another important input. Sunlit, standing shallow (a few feet deep) rice fields and buffalo wallows tend to be warm water bodies with a high nutrient content that speeds larval development, while shaded small streams or fast (but not shady) flowing waters usually inhibit breeding. In coastal areas, tidal pools with moderate salinity can favor brackish-water species such as *An. sundaicus* (Rodenwaldt) and *An. subpictus* Grassi (Surendran et al. 2011), whereas freshwater species like *An. barbirostris* van der Wulp thrive in irrigation canals or rain-fed puddles (Iwan 2018). Physical factors such as temperature, humidity, seasonal rainfall, wind exposure, topography, and light availability directly affect habitat suitability, while chemical variables including pH, salinity, and dissolved oxygen determine which species can successfully colonize a given site (Getachew et al. 2020). In addition, biological factors that cause some aquatic flora and fauna influence the number of mosquitoes. The primary function of aquatic plants like moss, grass, mangroves, algae and other forms of vegetation is to provide cover for horizontal movements in the environment, primarily these horizontal movements are a blockage/cover from sunlight as well as protection from predators for larvae. With these natural predators such as *Panchax* spp. (killifish), *Gambusia affinis* (mosquitofish), *Tilapia mossambica* (tilapia), *Oreochromis niloticus* (Nile tilapia), and dragonfly larvae also affects mosquito populations by serving as biological control agents (Adriyani 2019).

The coastal areas of Sumba Island, like Gaura Village, represent a unique ecological interface between monsoonal rainfall, tidal variation and changing land-use practices; these factors all interact to determine availability and quality of mosquito larval habitats. These habitats also rapidly change through seasonal shifts and extreme climate events such as floods and droughts, which can create new or remove existing breeding sites, thus modulating the transmission dynamics of malaria. In addition, Gaura Village have also recorded malaria cases over the last 5 years (2019–2023), highlighting additional imperative for improved larval habitat dynamics information to inform successful directed vector control methods.

A variety of *Anopheles* breeding sites including puddles, rice fields, irrigation ditches, swamps, buffalo wallows, gutters, abandoned fish ponds and brackish water near the estuarine zone have been characterized in previous studies in East Nusa Tenggara (Willa & Kazwaini 2015; Munthe et al. 2022; Kazwaini et al. 2018). Research conducted in Runut Village (Sikka District) and Mota'ain (bordering Timor-Leste) confirmed that fish ponds, swamps, rice fields, and river basins serve as common larval habitats in coastal areas, where species such as *An. barbirostris*, *An. subpictus*, and *An. vagus* Dönitz are found frequently (Munthe et al. 2022; Darma et al. 2022). The observed water variables from these habitats showed a variety of pH values (typically 7–9), salinity levels ranging from freshwater to brackish conditions, and varying degrees of sunlight exposure — all factors affecting larval survival and density (Willa & Kazwaini 2015; Darma et al. 2022; Mading & Kazwaini 2014). Spatial studies in West Kupang and East Sumba have also shown that larval habitats are often clustered within 0–1000 m of human settlements, indicating a high risk for local malaria transmission (Laumalay et al. 2019; Willa & Kazwaini 2015). Larval densities differ from sampling method, such as higher densities typically found in stagnant water with vegetation and few predators (Munthe et al. 2022; Mading & Kazwaini 2014).

However, the majority of studies in the region targeted environmental factors in isolation or focused on inland locals thus limiting available knowledge on integrated ecological dynamics between *Anopheles* larval habitats peculiar to an environmentally coastal local like Gaura Village. For instance, *An. sundaicus* larvae inhabit coastal ponds situated near the community settlements that are slightly acidic (pH 6.3–6.5), low salinity of 3–5% and clear waters with muddy substrates. For this reason, they are perfect habitats because the stagnant water, muddy bottom, and aquatic plants (grasses and mosses) promote the development of larvae. North Kalimantan provides additional evidence that water variables, such as pH, salinity, turbidity and substrate type, determined the distribution of *An. sundaicus* and *An. subpictus,* which are generally living in acidic and salinity-rich habitats like lagoons and disused fish ponds. It emphasizes the need for considering environmental covariates in vector surveillance to identify priority breeding sites for focused larval source management and environmental control. Notably, species such as *An. sundaicus*, which are known to dominate coastal and brackish habitats in Indonesia, have not been sufficiently documented in locality-specific vector surveillance in West Sumba (Sugiarto et al. 2016; Nurmalasari et al. 2019).

Given this gap, the objective of this study was to quantify the physical, chemical, and biological environmental characteristics of potential *Anopheles* spp. larval breeding sites. The results are expected to provide important data and information to support malaria prevention and vector control in areas at risk.

MATERIAL AND METHOD

Study site and samplings

This was a descriptive survey with cross-sectional study design, where all research variables were collected simultaneously (Notoatmodjo 2012). The study was done over two months, from February to March 2024, which consisted of two waves of data collection with a one-month interval. The sampling area focused on coastal areas of Gaura Village, West Sumba Regency. This village offers new ecological and human-related aspects of malaria risk. Local ecological conditions, such as the presence of permanent water bodies, create ideal habitats for *Anopheles* mosquitoes (Dejenie et al. 2011). It has been confirmed that malaria transmission occurs near these larval habitats, especially in lowland areas where such conditions are available (Barrera et al. 1999; Dejenie et al, 2011). In addition, the East Nusa Tenggara (NTT) province shows a pronounced monsoonal rainfall regime with months receiving <100 mm of rain in various years (Mulyani et al. 2013). This seasonal pattern determines when marine pools dry and become saline, subsequently supporting exceptionally high densities of *An. sundaicus* (Afghani et al. 2024; Soekirno et al, 1983). *An. sundaicus* high densities are frequently recorded during the dry season when brackish lagoons stabilize (Soekirno et al. 1983). Land use changes through anthropogenic activities that modify larval habitats, including the cases of seaweed farming, traditional salt-making, small fishponds or aquaculture and coastal ponds are prevalent in NTT specifically East and Southeast Sumba potentially contributing to an increased malaria transmission risk (Wirasantosa et al. 2011).

This study mainly targeted the identification of *Anopheles* spp. comprehensive data of potential breeding sites in coastal areas of Gaura Village showing association with blood-meals and larvae (preadult mosquitoes). Each potential breeding site were observed and recorded physical, chemical, and biological environmental variables associated with it. Data were recorded on either observation sheets or via digital applications. Standard dipper used to collect larvae from a 45-degree angle of areas with high larval aggregation. Larvae per dip were counted, and these specimens were pipetted into designated bottles. Each bottle had breeding site type, location and date written on it. Instars 1–4 were collected and taken to the Waikabubak Public Health Laboratory where larvae were raised to maturity for species identification following standard *Anopheles* spp. identification keys (O'Connor & Soepanto 1999). Morphological separation of *Anopheles* larvae can be challenging and would result in misidentification, so direct larval identification was not used.

Variables measurements

The physical, chemical, and biological characteristics of the *Anopheles* spp. larval breeding sites were observed and measured. Physical environmental variables included were measured using standard field equipment. Water temperature recorded using a Water Digital Thermometer With Long Probe ST-2400L (Gyma Instruments, Phillipines), light intensity measured using a InScienPro LT-4000 Lux Meter (InScienPro, Indonesia) to assess brightness, water flow (categorized as either flowing or stagnant), and habitat area (measured in square meters using a measuring tape) (Prussing et al. 2018; Orondo et al. 2022). Chemical variables were analyzed included salinity, measured with MASTER-S/Millα Salinity Refractometer (ATAGO, Japan) in parts per thousand, ‰, pH measured with a Digital pH Meter HI98127 (HANNA Instruments, Turkiye), and dissolved oxygen measured with a Waterproof Portable Dissolved Oxygen and BOD Meter HI98193 (HANNA Instruments, Turkiye) in milligrams per liter (Akeju et al. 2022). Biological variables were assessed by documenting the presence of aquatic flora (e.g., moss, algae, and water hyacinth) and fauna (e.g., fish, dragonfly larvae, frogs, and water beetles) (Prussing et al. 2018). All measurements and observations were documented using standardized forms.

Data analysis

Larval density was calculated as the percentage of positive dips relative to the total number of dips per habitat. The habitat index was obtained by determining the proportion of habitats with larvae to the total number of observed habitats (Kemenkes RI 2017). These analysis provide an overview of distribution and abundance analysis of *Anopheles* larvae from research down in the study site. Since 6 environmental variables were measured to encompass the major gradients we performed a principal component analysis (PCA) in order to derive the principal axes of variation in *Anopheles* larval habitats. All environmental variables were tested for normality before being mean-centered and scaled to unit variance prior to analysis. PCA was performed using Paleontological Statistics (PAST) version 5.0 (Natural History Museum, University of Oslo, Oslo, Norway). An eigenvalue cut-off of 1 was applied to retain Principal components (PCs) and the factor loadings were examined further to investigate which environmental variable contributed most strongly to identified PCs. Biplot displays clustered results for breeding sites and environmental variables relationships from PCA results.

RESULTS

Larval density and habitat indices

The larval survey of *Anopheles* spp. in coastal areas collected a total of 312 larvae across two survey rounds. Twelve breeding sites were identified, including water spring, freshwater puddle, rice field, river pools (1 and 2), estuaries (1 and 2), coastal puddles (1, 2, and 3), buffalo footprints, and buffalo wallows, which were distributed across coastal areas of Gaura Village, as presented in Figure 1. The measurements of larval density and habitat indices for *Anopheles* spp. larvae are presented in Table 1. Out of the total larvae collected, 199 individuals were successfully reared to adulthood. These consisted of *An. sundaicus* (n = 136), *An. subpictus* (n = 33), *An. vagus* (n = 18), *An. annularis* van der Wulp, (n = 5), *An. barbirostris* (n = 4), and *An. flavirostris* (Ludlow) (n = 3). *Anopheles sundaicus* was the most abundant *Anopheles* species found at the identified breeding sites (Table 1 – species after rearing). To confirm species-level identification, all successfully reared adults were examined using standard morphological keys for *Anopheles* spp.

Physical and chemical variables measurements

Measurements of physical variables revealed that the highest temperature at the breeding sites was recorded at the buffalo footprints site (39.4 °C), whereas the lowest temperature was found in freshwater puddles (29.0 °C). Light intensity was highest in rice fields (421 lux) and lowest at the buffalo footprints breeding site (103 lux). Generally, the water flow conditions at the twelve breeding sites were stagnant, except for the estuary habitats, which exhibited slow-moving to flowing water. The largest habitat areas were recorded at the estuaries, ranging from 250 to 500 m^2, whereas the smallest habitat area was recorded at the water spring, measuring 1 m^2 (Table 2). Additionally, the

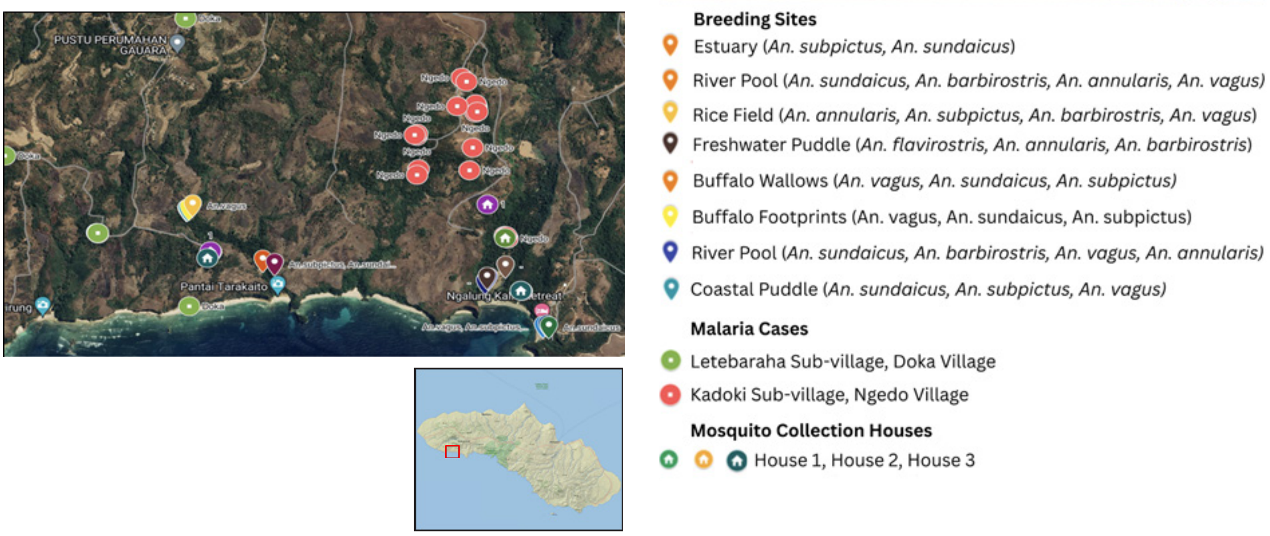

Figure 1. Spatial distribution of potential *Anopheles* spp. larvae breeding sites, reported malaria cases, and mosquito collection houses in the coastal areas of Gaura Village, West Sumba. The inset shows the location of the study sites within Sumba Island, Indonesia (Period: February-March 2024).

Table 1. Larval density and habitat index of *Anopheles* spp. larvae in coastal areas of Gaura Village (Period: February-March 2024)

Breeding site	Number of dips	Larval count	Presence of larvae	*LD	**HI (%)	Species	Number (Individuals)	Percentage (%)
Water spring	20	0	-	0.0	83.3	0	0	0.0
Freshwater puddle	20	3	+	0.2		*An. flavirostris*	1	33.3
						An. annularis	1	33.3
						An. barbirostris	1	33.3
Rice field	20	22	+/-	1.1		*An. annularis*	4	36.3
						An. subpictus	5	45.5
						An. barbirostris	1	9.1
						An. vagus	1	9.1
River pool 1	20	14	+	0.7		*An. flavirostris*	1	50.0
						An. vagus	1	50.0
River pool 2	20	14	+/-	0.7		*An. vagus*	1	11.1
						An. flavirostris	1	11.1
						An. barbirostris	2	22.2
						An. sundaicus	5	55.6
Estuary 1	20	39	+/-	1.0		*An. sundaicus*	15	79.0
						An. subpictus	4	21.0
Estuary 2	20	0	-	0.0		0	0	0.0
Coastal puddle 1	20	30	+	1.5		*An. vagus*	1	3.6
						An. sundaicus	24	85.7
						An. subpictus	3	10.7
Coastal puddle 2	20	39	+/-	2.0		*An. vagus*	3	14.3
						An. sundaicus	11	52.4
						An. subpictus	7	33.3
Coastal puddle 3	20	20	+	1.0		*An. sundaicus*	12	85.7
						An. subpictus	2	14.3
Buffalo footprints	20	61	+/-	3.1		*An. vagus*	3	10.3
						An. subpictus	7	24.1
						An. sundaicus	19	65.5
Buffalo wallows	20	70	+	3.5		*An. vagus*	7	11.3
						An. subpictus	5	8.1
						An. sundaicus	50	80.6

*LD = larval density (larvae per dip); **HI = habitat index (%), + = larvae were detected in the majority of dips, +/- = larvae were detected in only a few dips, - = no larvae were detected.

chemical variables revealed that the water pH at the breeding sites ranged from 7.3 to 9.2, salinity levels ranged from 0‰ to 20‰, and dissolved oxygen levels ranged from 1 to 8 mg/l. The highest pH and salinity levels were recorded in coastal puddle 1, while the highest dissolved oxygen level was recorded in the water spring (Table 2).

Biological variables measurements

This study recorded the presence of both aquatic and terrestrial flora and fauna at the breeding sites across the coastal areas. The aquatic and surrounding flora included moss (*Bryophyta*), grass (*Cyperaceae*), mangroves, taro (*Caladium*), coconut trees (*Cocos nucifera*), thorny pandan (*Pandanus tectorius*), and palm plants (*Cordyline*). The fauna observed at the breeding sites included water beetles (*Hydrophilidae*), golden apple snails (*Pomacea canaliculata*), fish species such as *Panchax* spp. and tilapia (*Tilapia mossambica*), frogs (*Fejervarya cancrivora*), water striders (*Gerris* spp.), dragonfly larvae (*Odonata*), snails, shrimp, and crabs. Moss and grass were the dominant types of flora, found at all 12 breeding sites. These are followed by coconut trees, which are present at five sites, and mangroves at four sites. Among the fauna, fish were the most prevalent, occurring at ten sites, while tadpoles are observed at nine breeding sites. Snails and crabs were also common, appearing in six and five breeding sites, respectively. The biological variables measured at these breeding sites are presented in Table 3.

Principal component analysis

The multivariate structure of physicochemical characteristics among multiple aquatic habitat types was determined via principal component analysis (PCA). PC1 and PC2 explained 34.3% and 27.0% of

Table 2. Physical and chemical variables of breeding sites in coastal areas of Gaura Village (Period: February-March 2024)

Breeding site	Physical variables				Chemical variables		
	Temp. (°C)	Light (Lux)	Water flow	Area (m^2)	pH	Salinity (‰)	Dissolved oxygen (mg/l)
Water spring	30.1	295	Stagnant	1	8.4	0.0	8
Freshwater puddle	29.0	225	Stagnant	12	8.3	0.0	4
Rice field	36.0	421	Stagnant	120	7.6	0.0	3
River pool 1	36.1	215	Stagnant	28	7.3	0.0	3
River pool 2	31.7	143	Stagnant	20	8.7	0.0	1
Estuary 1	34.1	290	Slow flow	500	8.7	0.1	3
Estuary 2	32.3	122	Flowing	250	7.8	0.1	1
Coastal puddle 1	36.3	129	Stagnant	10	9.2	20.0	1
Coastal puddle 2	34.9	122	Stagnant	6	7.5	0.1	1
Coastal puddle 3	33.6	115	Stagnant	3	7.3	0.1	2
Buffalo footprints	39.4	103	Stagnant	6	7.5	0.1	2
Buffalo wallows	35.5	165	Stagnant	35	7.6	0.1	2

Table 3. Biological variables of breeding sites in coastal areas of Gaura Village (Period: February-March 2024)

Breeding site	Flora	Fauna
Water spring	Moss, grass	Tadpoles, dragonflies, fish
Freshwater puddle	Moss, grass, taro	Frogs, water beetles, tadpoles
Rice field	Moss, grass, rice, coconut	Fish, tadpoles, frogs, dragonflies
River pool 1	Moss, grass, taro	Water beetles, fish, snails, tadpoles, shrimp
River pool 2	Moss, grass, coconut	Fish, tadpoles, shrimp, dragonflies
Estuary 1	Moss, grass, mangroves, coconut	Fish, snails, tadpoles, crabs, shrimp, dragonflies, butterflies, water beetles
Estuary 2	Pandan, moss, grass, mangroves, coconut	Fish, crabs, snails, dragonflies
Coastal puddle 1	Moss, grass, pandan, mangroves	Fish, crabs, snails, frogs
Coastal puddle 2	Moss, grass	Fish, snails, crabs
Coastal puddle 3	Moss, grass, coconut	Fish, tadpoles, crabs, snails
Buffalo footprints	Moss, grass, mangroves	Tadpoles
Buffalo wallows	Moss, grass	Fish, tadpoles

the total variance, respectively, with a cumulative contribution of 61.3% (Figure 2). These cumulative variances indicate that the first two axes represent major environmental gradients explaining most of the variation distinguishing the habitats.

The major contributors to PC1 were dissolved oxygen (DO), light intensity and habitat area with positive loadings on this axis, which constituted 56.0 of the total variance. Conversely, temperature was negatively associated with PC1. This means that PC1 indicates a gradient from larger, more or less well illuminated, oxygen rich habitats to relatively smaller and hotter water bodies. Habitats like water springs, freshwater puddles, estuary 1 and rice fields were located on the positive side of PC1 showing their association with greater dissolved oxygen concentrations, higher light exposure and larger surfaces areas. On the contrary were buffalo footprints, buffalo wallows and numerous coastal puddles which appeared on the negative side of PC1 at relatively higher temperatures and smaller dissolved oxygen levels.

The variation along PC2 was largely driven by pH and salinity (both with positive loadings) indicating that this axis represents a chemical gradient. Coastal puddle 1 clustered strongly separably along PC2, and was significantly positively associated with high salinity, distinguishing it from the mostly fresh water habitats. Conversely, river pool 2 and estuary 1 represented the moderate positions along PC2, indicating intermediate chemical properties.

Strong clustering patterns are observed ecologically in the biplot. We distinguished coastal habitats for which salinity was measured from inland freshwater habitats. Regardless, anthropogenic or semi-managed habitat types (e.g., rice fields) reflected tighter associations with light intensity and dissolved oxygen. Small, surficial and organic-rich habitats (i.e., buffalo footprints and wallows in aggregations) yielded similar physicochemical profiles at or near the surface with high temperatures and limited dissolved oxygen.

In conclusion, the PCA shows that habitat differentiation is largely structured by (1) a certain oxygen–light–area gradient along PC1 and (2) a salinity–pH gradient along PC2. Our results indicate that these environmental gradients likely underlie important measures of ecological suitability and the distribution of species in the studied aquatic systems.

DISCUSSION

The study found 12 breeding sites of *Anopheles* spp. The physical, chemical, and biological measurements of environmental variables at each of these sites showed how they differed. The predominance of *An. sundaicus* and *An. subpictus* in high-temperature habitats (e.g., buffalo wallows and coastal puddles) were strongly indicative of thermal tolerance

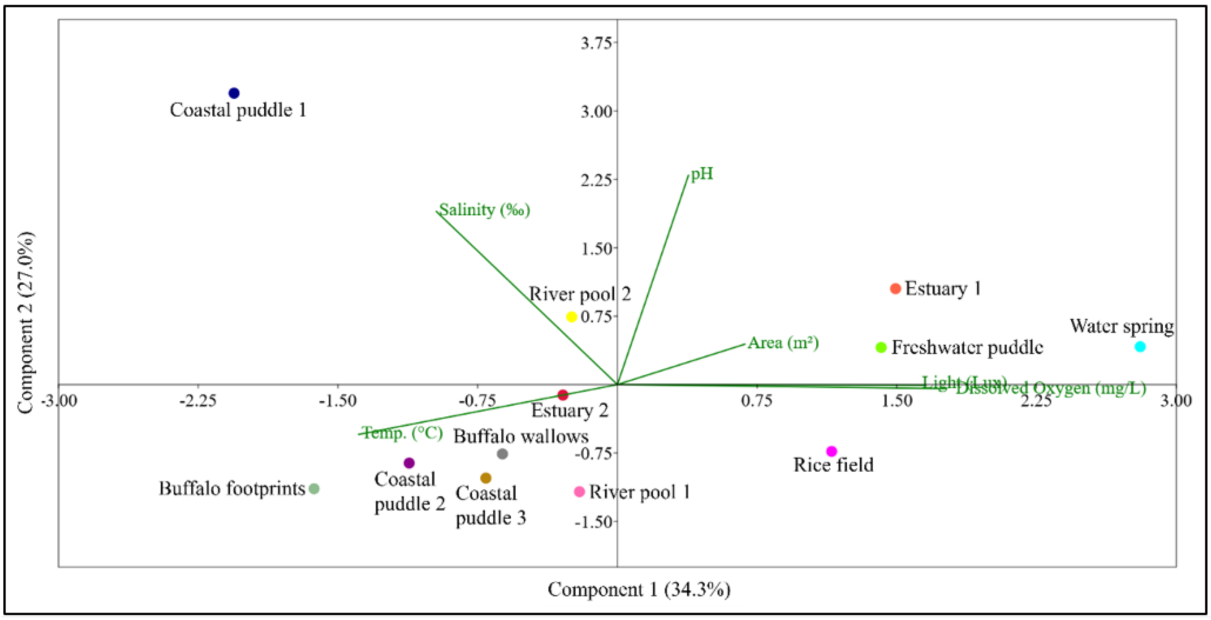

Figure 2. Principal component analysis (PCA) biplot of physicochemical characteristics across different *Anopheles* spp. aquatic breeding habitats in Gaura Village. PC1 and PC2 explained 34.3% and 27.0% of the total variance, respectively (61.3% cumulative). Vectors indicate the contribution and direction of each environmental variable (temperature, salinity, pH, area, and dissolved oxygen), while points represent the spatial clustering of the different aquatic habitat types. Habitats positioned in the direction of a vector are positively associated with that variable, whereas those located opposite show negative relationships.

consistent with their documented adaptation to coastal ecosystems within Southeast Asia (Sugiarto et al. 2016). Additionally, the thermal range observed (29.0–39.4 °C) exceeds the optimal developmental threshold for most Anopheles, which is typically 20–27 °C (Sugiarto et al. 2020). It suggests that local vector populations may have evolved physiological tolerance to climate-mediated temperature extremes, which could facilitate malaria transmission risk in Gaura Village under projected warming trends. These vectors are abundant in habitats such as buffalo wallows and coastal puddles where temperatures 8° C above the physiological threshold tolerated by other species due to high humidity (Sugiarto et al. 2016).

Turbidity is higher in buffalo wallows and coastal puddles than other types of puddles. At midday exposure to the sun, these puddles may be thermally stratified, with high surface temperatures due to absorbed solar radiation (Paaijmans et al. 2008). The ecological factors controlling turbidity in the lakes and reservoirs were soil type and rainfall in these water bodies. Tidal influence contributes to a unique sediment profile for each of the coastal areas in West Sumba. The sediment along the coast is dominated by medium and coarse sand (Nugroho & Putra 2019). Such temperature observations (29–39.4 °C) were similar to those of previously described sun-exposed, turbid water bodies found near buffalo footprints, puddles and rice fields in East Sumba matching previous reports with pH of 7–9 generally supporting high densities of larvae (Willa & Kazwaini 2015). Other coastal situations described in Motaain, NTT indicated that *Anopheles* larvae are concentrated in swamps, rice field pools and estuaries demonstrating the relevance of stagnant partially saline surface water for vector breeding (Darma et al. 2022).

The current study aligns with these patterns, especially in identifying *An. sundaicus* and *An. subpictus* as the dominant species in estuarine and animal-impacted sites such as buffalo wallows and footprints. In addition, previous studies conducted in North Kalimantan and Makassar also reported high frequences of these species, which were mainly found in muddy coastal habitats characterized by vegetation and aquatic biota such as grass, tadpoles and dragonfly nymphs (Sugiarto et al. 2016; Nurmalasari et al. 2019; Sindhania et al. 2020). Buffalo presence is strongly associated with maintaining *Anopheles* populations by promoting beneficial breeding conditions. Buffalo deposit dung in water bodies and create new habitats for aquatic organisms through the generation of habitat by contributing to creating more water-holding depressions (hoofprints) while compacting the soil and reducing water infiltration (Buxton et al. 2022; Östman et al. 2015). Grazing also creates small, dirty water pools that are ideal sites for mosquito breeding (Östman et al. 2015). The affinity for warm, shallow and biologically productive niches demonstrated by these coastal vector species underlines the commitment to monitoring transitional zones where estuarine, agricultural and livestock land uses converge. Cumulatively, this evidence reinforces the interpretation that buffalo-affected coastal habitats are consistently and substantially supporting areas of high-potential malaria vector populations across Nusa Tenggara and similar Southeast Asian environments.

Light intensity at these breeding sites sites were ranging from 103 to 421 lux, and even though trees and mangroves exists here, it was more correlated to their closeness along the coastline. Habitats exposed to direct sunlight are ideal for *An. sundaicus* to thrive. Direct sunlight increases water temperature, accelerates evaporation, and subsequently increases salinity–conditions under which euryhaline mosquitoes like *An. sundaicus* and *An. subpictus* competitively thrive (Surendran et al., 2011). Sunlight also promotes algal growth, providing abundant food and higher dissolved oxygen for *An. sundaicus* larvae. While previous study report optimal developmental temperature of 20 °C to 27 °C (Sugiarti et al. 2020), mosquitoes can survive at more extreme temperatures, though metabolic processes slow or cease below 10 °C or above 40 °C (Christiansen-Jucht et al. 2014). These findings align with Ernawati et al. (2012), which revealed that temperatures ranging from 26 °C to 33 °C remain suitable for *Anopheles* spp*.* larval development. Furthermore, Kermelita et al. (2024) reported that the optimal light intensity for larval growth ranges from 224 to 674 lux, which aligns perfectly with the peak light intensities recorded in our sun-exposed breeding sites.

The size of the breeding sites differed widely, from 1 m^2 to 500 m^2. Mulyadi (2010), also found that *Anopheles* spp. larval habitats have been recorded from 0.5 m^2 to >100m^2, displaying some spatial plasticity in these early life stages. This enormous breeding range (from small footprints of buffalo to extensive estuaries) allows for stable vector populations facilitating year-round malaria transmission in the village of Gaura, especially when these sites are located in close proximity to human dwellings so that they can remain undetected and thus unaddressed potentially targeting them for control. The physical, chemical, and biological characteristics contribute to the significant differences in *Anopheles* breeding sites size in Gaura Village. This difference in size is associated with physical factors (temperature, light, and water flow) that impose habitat stability, chemical variables (pH, salinity, DO) that dicatate developmnetal suitability. These differences are amplified by biological conditions, whereby aquatic vegetation serves as both habitat and food source. In the end, these preferences convey *Anopheles* mosquitoes adaptive behaviors of exploiting habitats which providing the greatest opportunity for survival.

The chemical features of the breeding sites in Gaura Village, especially their alkaline pH, low dissolved oxygen content and both fresh and brackish salinity greatly affect composition of *Anopheles* species. *An. vagus*, *An. subpictus*, and *An. sundaicus* are known to be consistently associated with coastal puddles with alkaline conditions (pH up to 9.2) and with moderate salinity (up to 20.0‰), all of which are also known to tolerate or prefer brackish, sun-exposed habitats. Estuarine zones and buffalo-impacted areas such as wallows and hoofprints, which are influenced by tidal intrusion and livestock activity, also supported *An. sundaicus* and *An. subpictus*, suggesting that both natural tidal dynamics and anthropogenic ecological features contribute to their presence. Meanwhile, although adaptable species like *An. vagus* and *An. subpictus* were also present, freshwater habitats like river pools and rice fields, characterized by zero salinity and neutral to slightly alkaline pH, were more frequently associated with species such as *An. barbirostris*, *An. annularis*, and *An. flavirostris*, which prefer non-saline environments. Together these results show the unique physicochemical profiles of larval habitats in Gaura Village that provide microhabitats to diverse *Anopheles* species, each associated with specific environmental conditions.

A previous study by Taher (2021) reported that *An. sundaicus* develops optimally in brackish water with salinity levels ranging from 12‰ to 18‰ but cannot survive at salinity levels above 40‰. Ecologically, coastal brackish habitats offer abundant microbial food and reduced competition that *An. sundaicus* to exploit. Physiologically, *An. sundaicus* adaption is likely supported by reduced cuticular permeability and specialized osmoregulatory responses in the rectum, permitting effective ionic balance in highly saline environments (Ramasamy & Surendran, 2011). In Kondamaloba Village, Central Sumba, *An. sundaicus* breeding sites have a salinity of 12‰ and a pH of 8.8 (Wayan & Adnyana 2011). In the current study, *An. sundaicus* demonstrated extreme flexibility; it was found in high-salinity coastal puddles (20.0‰), extremely low-salinity buffalo wallows and buffalo footprints (0.1‰), and even entirely freshwater river pools (0.0‰).

Additionally, previous studies also revealed that *Anopheles* spp. larvae tolerate pH values ranging from 7.9–8.9. Water pH influences aquatic organisms and water fertility, with optimal microbial activity generally occurring at pH values between 6.5 and 8.3. Extreme pH levels can deactivate or kill these microorganisms, creating an intolerable environment by reducing the availability of food for *Anopheles* larvae. Larvae predominantly feed on microorganisms–including algae, bacteria, and protozoa–as well as organic detritus suspended in water. When unsuitable pH conditions suppress microbial growth and survival, then resulting decline in food availability negatively impacts larval survival and abundance. Furthermore, dissolved oxygen is crucial for larval survival and is inversely related to water temperature (Mading & Kazwaini 2014; Febriani et al. 2019).

Anopheles subpictus was found to thrive in both saline and non-saline aquatic habitats. Its presence in rice fields with zero salinity demonstrates its high adaptability to various breeding conditions, further highlighting its role as a widespread malaria vector. A previous study by Laumalay (2022) reported that *An. subpictus* is broadly distributed and successfully inhabits both freshwater and high-salinity environments.

Anopheles barbirostris was found exclusively in freshwater puddles, rice fields, and river pools, with no salinity detected. As a strict freshwater mosquito, *An. barbirostris* lacks of the osmoregulatory adaptation found in euryhaline mosquitoes. In particular, it does not possess specialized salt-excreting structures, such as modified rectal segments, rendering its larvae unable to effectively eliminate excess salts. As a result, exposure to saline water leads to osmotic imbalance and larval mortality (White et al. 2013). This aligns with Jastal (2005), who reported that *An. barbirostris* larvae inhabit lowlands, hills, and mountains, predominantly utilizing rice fields, freshwater pools, springs, fishponds, and swamps.

Anopheles vagus exhibited broad ecological tolerance, being found in river pools, buffalo footprints, buffalo wallows, and coastal puddles–even in high-salinity environments reaching 20‰. Laumalay (2022) identified *An. barbirostris*, *An. subpictus*, *An. vagus*, *An. vagus* var. *limosus*, and *An. indefinitus* in Lifuleo Village. This confirms that coastal ecosystems, with their mosaic of fresh and brackish micro-habitats, can support a highly diverse vector community with varying degrees of salinity adaptability.

The presence of *An. sundaicus*, *An. subpictus*, *An. barbirostris*, and *An. vagus*—confirmed malaria vectors in East Nusa Tenggara—highlights the urgent need for a multifaceted vector control strategy to prevent malaria transmission in the coastal areas of Gaura Village (Jastal 2005; Laumalay 2022). Larval source management, through the targeted filling, draining, or modification of coastal puddles and stagnant water bodies can reduce breeding habitat availability. In localized areas, establishing biological control agent, such as predator reservoirs, may offer supplementary suppression. Furthermore, selective larviciding should be deployed in habitats that are persistent or unsuitable for physical modification. Simultaneously, standard adult-focused interventions, including the use insecticide-treated nets and indoor residual spraying, are critical to block transmission from anthropophilic species such as *An. vagus* and *An. barbirostris.* Finally, community education aimed at reducing outdoor activities during peak biting periods and minimizing exposure to breeding sites will further bolster these integrated interventions (Ndoen et al. 2010).

The biological variables of the 12 breeding sites (Table 3) confirmed the important ecological functions performed by aquatic flora and fauna affecting *Anopheles* spp*.* larval habitats. Aquatic vegetation is an important oviposition site and offers shelter for mosquito larvae to resist predation whereas aquatic fauna act as predators and natural competitors (Huang et al. 2006; Culler & Lamp 2009). For instance, Febriani et al. (2019) reported that the presence of larvivorous fish inversely affects larval density, acting as a direct biological control–higher fish populations lead to lower larval densities, and vice versa. In addition, aquatic plants affect dissolved oxygen concentrations which in turn determines the extent to which habitats are suitable for certain other aquatic organisms.

Previous research shows that these ecological drivers exert influences on larval development but also have carry-over effects with adult traits–size, fecundity and longevity–that shape the population dynamics of mosquitoes (Roux & Robert 2019). While this study did not directly address the emergence of adults, the long-term occurrence of supportive aquatic flora and fauna suggests that these sites function as multilayered developmental sites providing critical ovipositioning, shelter, foraging resources, and refuge from predation (Depkes RI 2004). The aquatic vegetation forms a niche and also provides adult mosquitoes with much more microhabitats for rookery fattening in order to complete gonotrophic cycles.

The highest larval densities were recorded in animal-impacted habitats, specifically buffalo wallows (3.5 larvae per dip) and buffalo footprints (3.1 larvae per dip), both of which predominantly hosted *An. vagus*, *An. subpictus*, and *An. sundaicus*. Coastal puddles similarly hosted these three species with moderate densities ranging from 1 to 2 larvae per dip. In contrast, agricultural and natural freshwater habitats exhibited lower productivity; rice fields had a density of 1.1 larvae per dip (hosting *An. vagus*, *An. annularis*, *An. barbirostris*, and *An. sundaicus*), estuaries recorded 1 larva per dip, and river pools recorded the lowest density at 0.7 larvae per dip. These findings reinforce that animal-impacted, spatially confined habitats are the most highly productive breeding sources in the village.

Spatial analysis found that the 12 breeding habitats were found located between 6 and 159 m from the coastline, and between 108 and 512 m to residential areas. The close association of these breeding places with human habitation greatly increases transmission risk of malaria, especially with the presence of confirmed malaria vectors (*An. subpictus*, *An. sundaicus*, and *An. barbirostris*) and suspected vectors (*An. vagus*, *An. annularis*, and *An. flavirostris*). Based on these results, coastal areas in Gaura Village are considered to be at high risk for malaria transmission as all potential breeding site recorded were well within the flight range of *Anopheles* spp. This aligns with the findings of Heriyanto et al. (2011), that these mosquitoes usually fly as much as aerodynamically would paint out approximately 1–2 km from their breeding grounds, following the fact that each of the residential buildings are at a most distance of only 512 meters.

Over the coastal areas, 12 potential breeding habitats were recorded in total during both survey rounds. Of the 12 potential breeding sites surveyed, 10 were larvae positive giving an overall habitat index of 83.3%. The potential from this risk is also reflected by the majority of malaria vector confirmed in East Nusa Tenggara (*An. subpictus*, *An. sundaicus*, and *An. barbirostris*), alongside suspected vectors (*An. vagus*, *An. annularis*, and *An. flavirostris*) (Kemenkes RI 2017; Heriyanto et al. 2011).

Principal component analysis (PCA) showed that environmental differentiation of *Anopheles* larval habitats in the coastal ecosystem of Gaura Village was mainly explained by two large ecological gradients: habitat area as the main gradient of Component 1 and water pH as the main gradient of Component 2 (Figure 3). The revised PCA revealed that light intensity (LI) and the majority of the physicochemical variables had only a small effect on habitat segregation, in contrast to some preliminary results. In these nursery habitats, species-specific structural and chemical properties– not sun exposure or temperature – are more central to making up larval habitat heterogeneity in this landscape.

Strong positive loadings on habitat area were associated with Component 1, which accounted for 34.3% of the total variance. Rice fields, estuary pools, freshwater puddles and river pools cluster on the right side of this axis, indicating that they share structural traits and larger habitats. Smaller, more localized habitats such as buffalo footprints (BF), buffalo wallows (BW), and coastal puddles (P) clustered at the negative end of Component 1. This gradient is relevant to ecology, because habitat area influences hydrological stability, predator assemblages and resource distribution. Broader water bodies accommodate more complicated food webs and higher densities of natural predators to mosquitoes (Minakawa et al. 1999). In contrast, smaller habitats can be predator-poor and very favorable for larval development. The ecological context from our findings is consistent with more broad evidence for habitat mediated patterns of larval competitive interactions (Juliano 2009). This size-related pattern is also consistent with observations in coastal environments, where spatially constrained habitats regulate larval production (Mutuku et al. 2006).

Component 2 which explained 27.0% of the total variance was influenced by both water pH and salinity. This axis separated alkaline and saline habits such as coastal puddles from those with lower or neutral pH such as buffalo-associated habitats and water springs. The relationship between larval development and pH has been well established because pH affects microbial community structure, algal productivity, and nutrient availability—all crucial resources for larval foraging. Relationship between some physicochemical characteristics (pH, ionic concentration, etc.) have also been previously shown with larval abundance in field studies (Avramov et al. 2024). While salinity does play a role in this PCA output, its expression is not as strong but remains an important ecological feature of coastal ecosystems subject to tidal exchange. The wide range of salinity tolerance of coastal malaria vectors has been demonstrated by early studies on the *An. sundaicus* complex (Dusfour et al. 2004). Field observations of larval development in brackish water provided further evidence of significant quantitative differences in salinity tolerance and physiological plasticity among populations of *An. sundaicus* (Surendran et al. 2011). This greater tolerance to environmental conditions aligns well with the use of habitats by dominant malaria vectors at regional and continental scales as described in large-scale distributional analyses (Sinka et al. 2011).

Notably, results from the ordination indicated that light intensity, temperature and dissolved oxygen were traits with short eigenvectors which contributed very little to the main gradients of habitat differentiation. This indicates that whichever breeding sites were sampled had levels of light, thermal and oxygen conditions consistent enough to not exceed the range habitual tolerances in *Anopheles* larvae. While in some inland environments, light exposure may be critical to algal productivity and larval food availability the coastal habitats of Gaura Village appear to be more influenced by size and water chemistry than energy from sunlight or heat. Among the habitat types, Coastal puddle 1 was unique and distinctly isolated from all other habitats along the positive Component 2 axis. This implies that its physicochemical conditions, massively high or low in pH and salinity relative to other aquatic habitats, distinguishes it clearly from all others. These outlier habitats may host distinctive larval compositions and/or have different rates of development, but require more ecological scrutiny.

On the other hand, river pools and water springs are distributed near the higher pH and dissolved oxygen areas where they are less influenced by tidal wave, revealing their relative water quality stability. In wrapping up, the PCA reports that habitat area and water chemistry drive heterogeneity of *Anopheles* larval habitats in this coastal ecosystem. These results suggest that vector management efforts in Gaura Village should characterize habitats based on habitat size, stability and chemical conditions rather than just openness or sunlight exposure. Conversely, highly saline habitats, particularly coastal puddles and spatially isolated buffalo-associated microhabitats, represent important larval sources and should be prioritized in larval source management strategies. Furthermore, the structural and chemical gradients are critical for the development of more ecologically-based vector suitable control interventions in coastal, malaria-endemic areas.

CONCLUSION

This investigation of 12 *Anopheles* breeding sites in the coastal area of Gaura Village confirmed a significant and ongoing risk of malaria transmission. This risk is driven by a diverse vector community–including confirmed carriers such as *An. sundaicus*, *An. subpictus*, and *An. barbirostris*–with larval densities reaching up to 3.5 larvae per dip in habitats proximate to human dwellings. Although the habitats exhibited considerable environmental variability (temperature 29.0–39.4 °C; salinity 0–20‰; pH 7.3–9.2), Principal

Component Analysis (PCA) reduced this complexity into two principal gradients explaining 61.3% of the total variance. PC1 (34.3%) represented a physical and structural gradient driven by habitat area, dissolved oxygen, and light intensity, whereas PC2 (27.0%) a chemical gradient driven primarily by water pH and salinity. The spatial distribution of sampling sites indicates that the higher larval densities tend to cluster in smaller, spatially confined habitats (positioned on the negative side of the PC1 structural gradient), such as buffalo footprints and wallows. Furthermore, the extreme physiological flexibily of *An. sundaicus*–demonstrated by it's ability to thrive in both entirely freshwater environments and highly saline coastal puddles–combined with the proximity of these breeding sites to residential areas (maximum 512 meters, well within the vector flight range), underscores the urgent need for targeted Larval Source Management (LSM) programs to specifically address small, temporary, and livestock-associated microhabitats, as well as alkaline coastal puddles, to effectively reduce vector populations and mitigate malaria risk in Gaura Village.

Ethical approval

This research was ethically approved by the Health Research Ethics Committee Faculty of Public Health Diponegoro University (No. 637/EA/KEPK-FKM/2023).

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