



OBSERVATION ON THE ROLE OF TEMPERATURE AND SALINITY ON THE DEVELOPMENT OF *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE) IN LABORATORY

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ABSTRACT

This article reviews the influence of various substrate salinity and temperature ranges on oviposition, larval survivability, and adult emergence during laboratory rearing. Habitat selection by the gravid females *Culicoides* spp. (Diptera: Ceratopogonidae) is influenced by physicochemical parameters such as temperature, pH, salinity, moisture, conductivity, and organic and inorganic compounds of substrates. These climatic and habitat related factors influence vectors' life history traits. The species-specific information will be useful in establishing a laboratory colony thereby contributing to our understanding of the vectorial capacity and competence amongst different species of *Culicoides*.

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INTRODUCTION

Hematophagous biting midges of genus *Culicoides* Latreille (1809) (Diptera: Ceratopogonidae) are distributed mainly in the tropics and temperate regions of the world, except a few scattered geographical areas. These tiny flies are implicated as vectors of at least 50 arboviruses, several protozoans, and nematodes. The livestock population is affected worldwide by epizootic haemorrhagic disease virus, bluetongue virus (BTV), epizootic hemorrhagic disease virus and African horse sickness virus¹. In India, lack of life history-related data, larval food, captive mating, and artificial blood feeding are the main bottlenecks impeding the studies on vector competence as well as the establishment of successful laboratory colonies of the vector species^{2,3}. Naturally, the immatures of these flies prefer areas like muddy fringe areas of stock ponds, shaded muddy pool margins, muddy areas of paddy fields, and cattle manure^{4,5,6,7}. Very few species have so far been laboratory-reared, and colonies maintained within the laboratory. Out of the 23 species attempted to rear under laboratory conditions^{2,3,8,9,10,11} only colonies exist for only two species of, *Culicoides sonorensis* Wirth and Jones and *Culicoides nubeculosus* (Meigen) are maintained². The rearing procedure and conditionalities of the most prevalent vector species, *Culicoides peregrinus* Kieffer, were performed in the laboratory with a good amount of success³. The growth and development of this vector species were studied mainly by following habitat-specific temperature ranges and other physicochemical parameters. However, information about species-specific rearing temperature ranges and suitable substrate salinity was unavailable. At different rearing temperature ranges, the rearing of several *Culicoides* spp. was examined, such as *Culicoides variipennis* (Coquillett)¹², *Culicoides arakawae* (Arakawa), *Culicoides maculatus* (Shiraki)⁸, *Culicoides brevitarsis* Kieffer¹³, *Culicoides (Avaritia) imicola* Kieffer¹⁴. Besides, the oviposition site selection of these vectors depends on the suitable temperature and the physicochemical factors of these habitats^{14,15,16}. Therefore, the peak abundance of these vectors and the appearance of bluetongue disease are related to suitable habitats as well as climatic variations¹⁷. Thus, in the southern states of India, peak abundance of *Culicoides* and BTD outbreaks was reported in the monsoon period¹⁸, but became low in abundance during post-monsoon¹⁹. The primary purpose of this

article is to explore the tolerance temperature range and substrate salinity of *Culicoides* spp. and how it affects oviposition, larval survivability, and adult emergence. This information provides evidence about the characteristics of the breeding sites and suitable parameters for their adequate survivability.

MATERIAL & METHODS

Investigation on the influence of rearing temperature was performed in laboratory and on field collected species^{19,22,26,27}. In laboratory different temperatures was selected for that purpose^{12,13,14,21,23}. To observe the effect of substrate salinity, gravid individuals were placed in oviposition glass vials and oviposition beds were prepared with different concentration of saline water^{3,30,33,34,35}. This review was on considered field collected species from different habitat with analysis of physiochemical parameters^{15,16,26,27,28,29}.

OBSERVATIONS

(i) Rearing Temperature

According to the preference performance hypothesis²⁰, gravid females can detect the ambient temperature to determine maximum larval survivability. It was observed that the development success and duration of different life stages of *C. variipennis* were influenced by rearing temperatures such as larval development was completed within 11-24 days at 25°C, 27°C and 30°C whereas fast development within 8-18 days at higher temperature (35°C). The larval development was delayed (23-48 days) at the low temperature of 20°C¹². In *C. brevitarsis* more than 80% of fourth instar larval survivability was recorded between 26°C and 30°C¹³. The development and duration of *C. arakawae* and *C. maculatus* were recorded at different rearing temperatures⁸. A higher percentage of pupation was observed in both species at 22.5°C. The duration from egg to adult in both species was prolonged (> 40 days), with a higher mortality rate of 20°C. Larval development ceased for both species in low temperatures (15-16°C), and *C. arakawae* and *C. maculatus* pupation did not occur at 35°C and above 30°C, respectively. The highest percentage of egg hatching and survivability occurred in *Culicoides circumscriptus* Kieffer and *Culicoides paolae* Boorman with a 1:1 sex ratio at 25°C and 30°C,

respectively²¹. The most suitable rearing temperature for *Culicoides obsoletus* (Meigen) with the highest developmental success was 24°C, but the sex ratio was 3:1, male: female²². This sex ratio became 1:1 at 16-20°C with decreased pupal development. *Culicoides imicola* Kieffer showed the highest larval survivability at a lower temperature 20°C, with delayed development time¹⁴. Such an experiment was performed with vector species of *C. peregrinus*, and it was noted that 26°C was the ambient temperature for the highest oviposition (70%) and survivability. Duration of pupation was extended (25-39 days) in lower temperatures; 15°C, and 20°C²³. From these observations, it can be stated that a non-linear relationship exists between rearing temperature and larval survivability. At low-temperature pupation was recorded as 0% and it peaked up to 81% at 26°C later when the temperature increased to 35°C the pupation dropped to 4%. This information could give insight into the possible thermal preference range (20-56°C) of populations to environmental conditions, thereby increasing the understanding of vector distribution and population changes over time. The thermal tolerance in the field conditions may vary greatly depending on the environmental conditions, and geographic location determines the abundance of these vectors.

(ii) Substrate Salinity

Immature distribution of *Culicoides* spp. depends on habitat availability as well as the physicochemical factors of habitats, i.e., moisture, dissolved oxygen, pH, salinity, organic contents and conductivity^{15,16}. Thus, the occurrence of species richness and distribution of *Culicoides* spp. determined by the interactions of the physicochemical parameters. Worldwide *Culicoides* species showed a widespread habitat distribution from freshwater to coastal areas such as *Culicoides crepuscularis* Malloch, and *Culicoides bermudensis* Williams found in freshwater in the United States, but in Bermudas, these species are associated with a salinity range of 1.2 to 36.2 parts per thousand (ppt)²⁴. The *Culicoides furens* (Poey) were also reported from salt marsh and freshwater while *Culicoides mississippiensis* Hoffman from salt marsh areas of Florida in low abundance²⁵. *Culicoides melleus* (Coquillett), *Culicoides hollensis* (Melander and Brues), and *C. furens* were isolated from the salt marshes of Georgia²⁶. The levels of dissolved salts influence the suitability of aquatic habitats for immature populations of the *C. variipennis* complex¹⁵. However,

the highest abundance of the larval habitat of *Culicoides distinctipennis* Austen was in the freshwater lake edges²⁷. *Culicoides furens* showed a wider habitat range in both salt marsh and freshwater habitats²⁵. The soil chemistry characteristics substrate was examined for *Culicoides peliliouensis* Tokunaga, of Hooghly estuary²⁸, while a similar investigation was done for *C. variipennis*, *C. sonorensis* and *Culicoides occidentalis* Wirth and Jones throughout their geographic ranges²⁹ and various *Culicoides* spp.¹⁶. Habitat parameters show variations within seasons and for species^{29,16}.

DISCUSSION

Laboratory-based studies were also reported for the oviposition site preference and the emergence of *Culicoides* are stimulated by different salinity of habitat i.e., in *C. imicola*³⁰, *C. obsoletus*³¹ and *Culicoides impunctatus* Goetghebuer³². Salt concentration below 0.06 g 10 ml⁻¹ was selected in *C. imicola* as the preferable oviposition substrate³⁰ whereas *Culicoides variipennis*sonorensis (Coquillett) females also chose to oviposit in 0‰ rather than 19‰. Still, it was noticed that no eggs were laid on 34‰³³. Oviposition and survivability of *C. peregrinus* were gradually affected when exposed to high salinity; 30-40 ppt³⁴. Many oviposited eggs were deposited in 0 ppt but reduced sharply in 5 ppt and then continuously decreased to 20 ppt. Oviposited eggs were not observed in 30 ppt to 40 ppt salinity. Egg retention was observed in stressful high saline concentrations. The hatching rate of eggs was highest at 0 ppt (without salt) and gradually decreased with increasing salinity. The hatching duration has not differed with salinity. The larval survivability and pupation recorded maximum in 0 ppt and significantly reduced in 25 ppt³⁴. The developmental period from egg to adult was about 18-23 days in the control condition (without salt)³, but it became delayed in high salinity with increasing mortality. Similarly, all the immatures of *Culicoides molestus* (Skuse) were died at three to four times the salinity of seawater³⁵. They opined that seawater salinity could be an essential factor of habitat suitability for *C. molestus* immatures. The higher saline concentrations of seawater inhibit the survivability and maturation of immatures of *C. molestus*, whereas lower concentrations of natural seawater are more suitable for survivability. A correlation between larval habitat and salinity has been established for several *Culicoides* species^{36,37}. *Culicoides circumscriptus* and *C. furens* could survive in hypersaline water, i.e., 1.5 times and three times that of seawater, respectively³⁸. The increased larval mortality in higher

salinity indicates that salt concentration may be responsible for disrupting the larval osmotic homeostasis by gain of ions and loss of water.

CONCLUSION

Substrate parameters and environmental conditions play a vital role in habitat selection and survivability of *Culicoides* spp. The survival of *Culicoides* spp. was maximum during rearing, with temperatures ranging from 20-26°C. During inconducive conditions, the mortality rate increased with an altered sex ratio. In the laboratory alterations in sex ratios affect mating experiments. Likewise, temperature ranges affect the vector species' distribution, ultimately linked to the outbreak of diseases worldwide. Most species adapted to low salinity with increased salinity but very few species tolerated >2 ppt salinity. As larval mortality increased, their survivability was significantly affected in the higher salinity range. Our understanding of these parameters will help establish a laboratory colony, ultimately generating information about disease epidemiology and the vectorial capacity of these vectors. The emergence of bluetongue virus and other *Culicoides*-borne arboviruses are dependent on environmental drivers³⁹. Previous studies reported the pattern of *Culicoides*-borne disease in tropical endemic areas related to climatic factors, primarily temperature and rainfall⁴⁰. Besides several other factors like vegetation, physiochemical parameters of habitat and hosts greatly influence the abundance of vector species which are related to the appearance of disease outbreak and vectorial capacity³⁹.

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