Quantitative assessment of larval desiccation tolerance in oriental *Chironomus* species

Fluctuations in environmental factors such as temperature and humidity lead to an imbalance between the influx of water and its loss through evaporation in living systems[1,2]. Under environmental dehydration bouts, few plants and animals possess the ability to persist in the desiccated form followed by resumption of metabolism upon rehydration[3]. The discovery of the phenomenon of desiccation tolerance dates back to the eighteenth century when Leeuwenhoek[4] reported the revival of 'dry animalcules'. This fascination subsequently led to the identification of newer desiccation-tolerant model systems. Research on desiccation tolerance has come a long way from purely inquiry-based studies to present day biomedical and biotechnological applications[5]. Previous reports have highlighted the role of biomolecules such as trehalose, HSP70, antioxidant machinery including SOD, CAT, glutathione and thioredoxin that serve as protectants to cope with the consequences of desiccation stress[5–9].

Complete or near complete desiccation followed by revival upon rehydration is a cardinal feature of desiccation-tolerant organisms. To date, desiccation tolerance is often described in purely qualitative terms[10] and hence there is a lack of quantitative measures that decide the degree of desiccation tolerance of organisms. The capacity of survival and reproduction upon rehydration remains the quintessential criterion of desiccation tolerance[3]. Although this criterion does not suggest a standard for consideration of a particular animal as desiccation tolerant, it has been taken as a useful guide to explore threshold desiccation tolerance in organisms. To the best of our knowledge, barring few attempts in prokaryotes[11], similar studies have not been undertaken for eukaryotic animals. In order to clarify this premise, we have attempted to design a simple mathematical tool, the 'desiccation tolerance index' (DTi) as a quantitative measure of the endurance to desiccation stress in animals.

In the purview of aquatic ecosystems, members of the dipteran family Chironomidae form an abundant group of insects inhabiting diverse ecosystems that include freshwater bodies, temporary rock pools, wetlands and Antarctic biomes[12]. *Chironomus* species, commonly referred to as non-biting midges are widely regarded as key components of freshwater food chains. Moreover, given their tolerance to environmental stressors, most *Chironomus* species are used as model systems for stress response studies including heat, radiation, hypoxic and desiccation stress[13] and also in aquatic biomonitoring programmes[14,15]. As water is one of the major limiting factors for aquatic habitats, organisms such as chironomid midges can be more prone to damages under physiological water deficits. In particular, larval desiccation tolerance of the oriental chironomids[7,16] has indicated the species-specific tolerance threshold in response to body water loss. In this study, we have specifically focused our attention to formulate an estimation tool termed as DTi for quantitative assessment of the degree of desiccation tolerance of nine *Chironomus* species, each representing a characteristic habitat.

Based on the existing literature[7,16] we gathered values of variables (namely water content and viability as listed in Table 1) that have been known to determine the ability to withstand desiccation exposure in insects. We start from a theoretical assumption to derive the DTi and study what this assumption predicts about the population. Consider W as the water lost by the organism at the end of desiccation exposure and V as viability post-rehydration. It has been noticed[3] that desiccation tolerance is directly proportional to V and inversely proportional to W. Hence DTi of the *Chironomus* species can be stated as

$$\mathrm{DTi} \propto (V/W)$$

$$\mathrm{DTi} = (V)\mathrm{A}/(W),$$

where A is the constant of proportionality.

Based on the data on the *Chironomus* species reported in this study, the values of DTi range between 0 and 1. Hence, without loss, this proportionality constant is taken as 1.

The expression for DTi can therefore be stated as

$$\mathrm{DTi} = (V/W).$$

This expression was used to fit the values of W and V of each of the nine *Chironomus* species (Table 1). DTi values thus generated, provided the threshold tolerance limits of these species.

As evident from this study, larvae of *C. salinarius* exhibited the highest desiccation tolerance index (0.76) whereas for *C. kiiensis* it was 0.32, the lowest index among all the species considered in this study. Indices of the remaining species, namely, *C. yoshimatsui*, *C. circumdatus*,

Table 1. Desiccation tolerance indices of the nine species of *Chironomus* larvae

Species (arranged as per descending DTi values)	Habitats of species	W (%)	V (%)	DTi
[16]*C. salinarius*	Brackish water	74.88	97.55	0.76
[16]*C. biwaprimus*	Eutrophic lakes	92.80	57.61	0.62
[16]*C. circumdatus*	Rivers, pond and ditches	90	55.61	0.61
[16]*C. crassiforceps*	Eutrophic ground pools	90.94	55	0.60
[16]*C. nippodorsalis*	Artificial reservoirs	100	60.71	0.60
[16]*C. yoshimatsui*	Eutrophic rivers	98.59	53.57	0.54
[16]*C. flaviplumus*	Artificial reservoirs and eutrophic lakes	100	51.57	0.51
[7]*C. ramosus*	Tropical lakes and rivers	85	45	0.52
[16]*C. kiiensis*	Paddy fields	94.40	30.38	0.32

C. flaviplumus, *C. biwaprimus*, *C. nippodorsalis*, *C. crassiforceps* and *C. ramosus* ranged between 0.5 and 1 (Table 1). In other words, *C. salinarius* has the highest tendency to sustain body water loss when compared with the remaining species. A thorough comparative study of all chironomid species using the expression derived in this study can pave the way to the understanding of mechanisms associated with differential sensitivity to desiccation stress among this taxonomic group. Each species is limited in time and space and exhibits a range of environmental tolerances and its occurrence and distribution is majorly dependent upon the limiting factor/s prevailing in its surroundings[17]. Our results are in agreement with previous interpretations[7,16] explained in the ecological context. The DTi values obtained using the quantitative tool proposed in this study also indicated that *C. salinarius* showed the highest ability to endure desiccation stress in comparison to the rest of the oriental chironomid species in question.

The numerical approach proposed in this study will be particularly important in the physiological categorization of desiccation tolerance limits regardless of the phylogenetic and taxonomic status of animals. However, it must be noted that desiccation tolerance is a rather complex phenomenon owing to the influence of abiotic elements (such as temperature, humidity, oxygen, etc.) on overall revival and survival upon rehydration. The base organisms considered in our study (nine oriental *Chironomus* species), may not form an exhaustive compilation; nonetheless, they serve as representatives for the generation of a numerical grading system for the categorization of other desiccation-tolerant animals as well. As the degree of water loss during desiccation and viability post-rehydration form the crux of desiccation tolerance, DTi can be extended not only to other chironomid species but also to other animals, thereby capable to serve as a general test for the estimation of endurance of an organism towards desiccation. Furthermore, DTi can be a useful measure for categorizing organisms based on their threshold limits of desiccation tolerance to understand the altered physiological responses exhibited by desiccation-tolerant organisms during seasonal and/or stochastic fluctuations of dehydration regimes. This quantifiable tool can be a useful prototype for ascertaining the hierarchical ranking of desiccation-tolerant animals in terms of their tolerance threshold.

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