

Potential predation of *Cyclops fuscus* Jurine, 1820 (Crustacea Copepoda) against two mosquito species *Culex pipiens* Linnaeus, 1758 and *Culiseta longiareolata* Macquart, 1838 (Diptera Culicidae)

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ABSTRACT

Due to the increasing pesticide-resistance by vectors, predation of mosquito larvae is one of the most important ecosystems' services contributing to public health and safety from mosquito-borne diseases. In order to contribute in developing newer and safer biological control tools, the evaluation of the predatory potential of natural mosquito enemies is required. In this regard, our study aimed to evaluate the predatory potential of *Cyclops fuscus* Jurine, 1820 (Crustacea Copepoda) against two mosquito species, *Culex pipiens* Linnaeus, 1758 and *Culiseta longiareolata* Macquart, 1838 (Diptera Culicidae), at four different larval stages (L1, L2, L3, L4) under laboratory conditions. During the 3 days of the experiment, *C. fuscus* showed an efficiency in the predation of mosquito larvae at some development stages. However, this efficacy followed decreasing rates after the first day. The larvae from the two first instars were almost predated in the first 24 hours with an average mortality of $L_1=16.0 \pm 2.64$ (80%); $L_2=7.33 \pm 2.08$ (95%) for *Cx. pipiens* and $L_1=19.0 \pm 1.0$ (36.65%); $L_2=9.0 \pm 1.0$ (45%) for *Cs. longiareolata*. Then, the predation rate decreased to negligible. By the end of the experiment, the total consumed larvae of L3 and L4 stages was only 20% for both instars in the case of *Cx. pipiens* against 25% and 40% of L3 and L4 for *Cs. longiareolata* larvae. Therefore, our results revealed that developmental stage of the culicidae is an important factor to determine their trophic position ($p < 0.001$). On the other hand, *C. fuscus* seems to be having a restrictive role to pre-imaginal Culicidae populations, and thus can be considered to regulate the mosquito population dynamics.

KEY WORDS

Mosquitoes; Biological control; Crustacea; *Cyclops fuscus*; *Culex pipiens*; *Culiseta longiareolata*; predatory potential.

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INTRODUCTION

Insects, particularly mosquitoes, play an important epidemiological role in human and veterinary

health (Rodhain & Perez, 1985; Mansouri et al., 2013; Hamaidia & Soltani, 2021). Mosquito females are hematophagous, mostly responsible for the transmission of multiple pathogens (protozoa,

arboviruses, and microfilariae) (Benmalek et al., 2018). *Culex pipens* Linnaeus, 1758 and *Culiseta longiareolata* Macquart, 1838 (Diptera Culicidae) are known as the most ubiquitous mosquito species in the world (Farajollahi et al., 2011) especially in North Africa and Algeria (Brunhes et al., 2000; Bouabida et al., 2012; Boudemagh et al., 2013; Dahchar et al., 2017; Hafsi et al., 2021).

For long, neurotoxic insecticides have been used to control mosquito population spread. However, these products widely used caused several environmental problems such as ecosystems contamination and natural resistance of mosquito to chemical products (Carlson et al., 2014; Eba et al., 2021). Therefore, great efforts have been made to develop biological control methods of same efficiency but with less collateral damage (Zaidi & Soltani, 2011). As part of ecological rebalancing strategies, natural predators have been proposed as suitable tools to control pathogens vectors particularly mosquito populations. Therefore, it is necessary to broaden the knowledge on the trophic habits of the different mosquitos' predators (Quiroz-Martínez & Rodríguez-Castro, 2007; Mahmoudi et al., 2022). Selection of biological control organisms should be based on their capacity for unintended impact, and their ability to respond, adapt to climate, and maintain very close interactions with target prey populations (Saha et al., 2020).

Predation plays a central role in the composition and functioning of ecosystems (Buxton et al., 2020). Although copepods are mostly known as marine zooplankton, they are also well present in lentic ecosystems with three orders Harpactoida, Calanoida, and Cyclopoida that can inhabit lakes, ponds and even temporary waters such as tree holes and puddles (Dussart & Defaye, 2006; Früh et al., 2019). These tiny organisms can be either omnivorous, herbivorous or even carnivorous (Albushabaa et al., 2019). With the increasing need for mosquito control, copepods have been suggested as antagonists of first instar mosquito larvae (Hurlbut, 1938). Since the mid-1990, several species have already shown interesting predatory potential against the mosquito (Quiroz-Martínez & Rodríguez-Castro, 2007). This study aimed to evaluate the predator potential of *Cyclops fuscus* Jurine, 1820 (Copepoda) towards the larvae of two mosquito species *Cx. pipiens* and *Cs. longiareolata* according to exposure time and development stage of the prey.

MATERIAL AND METHODS

Biological material sampling

Copepods were collected from the Lake Tonga, located in northeastern Algeria (36°51'511 N, 8°30'100 E) (Loucif et al., 2020), by the dipping method during March 2021. The dip net (0.1 mm mesh) was immersed in water and moved in a uniform motion to avoid any eddies. Then, the collected fauna was placed in containers filled with the lake water, hermetically sealed and labeled. At laboratory, based on specific taxonomic descriptors and keys (Perrier, 1979), specimens of *C. fuscus* (adults) were identified and isolated from other copepods using microscopic observation.

In order to obtain mosquito larvae, Culicidae (adults) were collected from buildings cellars of Sidi Amar town (northeastern Algeria). The mosquitoes were captured using a test tube placed gently on insects at rest, which fly then through it right to the trap. The insects were fed with dates suspended in breeding cages equipped with containers of dechlorinated water where females laid their eggs clusters in the form of a floating pod on the water surface. After hatching, the larvae were fed with a mixture of cookies (75%) and dry yeast (25%), as described in Bendali (2006), until the experiment. Finally, all mosquito larvae were sorted into cohorts (individuals at same developmental stages) L1, L2, L3 and L4 based on the size of the head capsules (Shinkarenko et al., 1986). Using microscopic observation, the larvae of *Cx. pipiens* and *Cs. longiareolata* were identified and isolated from other species using a specific dichotomous identification keys (Himmi, 2007) and data processing (Schaffner et al., 2001).

Predation assays

The predation tests were carried out in plastic boxes containing 250 ml of filtered water in order to eliminate the presence of any other nutrient sources. Five predatory individuals adult females (body size 1.6–1.9 mm). In each box, twenty larvae (n = 20) of each stage (L₁, L₂, L₃ and L₄) were exposed to five predators (females with eggs) of 1.6–1.9 mm that were deprived of food 24 hours before each test. The test lasted three consecutive days divided on 3 periods of 24 h, 48 h and 72 hours within

an ambient temperature 24 °C and 76% of humidity. The experiment included a control step and three replicates in order to establish the predation rate.

Statistics

The statistical analyses were carried using R software (R Core Team, 2019) version R 3.3.0. The results were expressed by averages \pm standard deviation (mean \pm SD). The effects of species, larval developmental stage and time of exposure on the predation rates were verified through a one-way ANOVA test, while the combined effects between these variables were verified through a two-way ANOVA test.

RESULTS

The results of the predatory activity (Table 1, Fig. 1) showed that most of L1 and L2 larvae of the two preys species *Cx. pipiens* and *Cs. longiareolata* were consumed in the first 24 hours with an average mortality of 16.00 ± 2.64 larvae (80% of preys) and 19.00 ± 1.00 larvae (95% of preys) respectively, while L3 and L4 larvae were barely attacked (L3 = 2.00 ± 1.00 ; L4 = 1.00 ± 0.00 larvae and L3 = 5.00 ± 1.00 ; L4 = 2.00 ± 1.00 larvae respectively).

After 48 hours, the larvae of both species at stage L1 and L2 have been totally consumed (100% of preys). Even so, the consumption of the larvae at

Times (H)	Instars	<i>Culex pipiens</i>		<i>Culiseta longiareolata</i>	
		Predation (%)	Mean \pm SD	Predation (%)	Mean \pm SD
24	L ₁	80.00	16.00 ± 2.64	95.00	19.00 ± 1.00
	L ₂	36.65	7.33 ± 2.08	45.00	9.00 ± 1.00
	L ₃	10.00	2.00 ± 1.00	25.00	5.00 ± 1.00
	L ₄	5.00	1.00 ± 0.00	10.00	2.00 ± 1.00
48	L ₁	20.00	4.00 ± 2.64	5.00	1.00 ± 1.00
	L ₂	36.65	7.33 ± 2.08	40.00	8.00 ± 2.00
	L ₃	5.00	1.00 ± 1.00	10.00	2.00 ± 1.00
	L ₄	5.00	1.00 ± 1.00	6.65	1.33 ± 1.57
72	L ₁	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
	L ₂	23.30	4.66 ± 1.52	15.00	3.00 ± 1.00
	L ₃	5.00	1.00 ± 0.00	5.00	1.00 ± 0.00
	L ₄	10.00	2.00 ± 1.00	8.30	1.66 ± 0.57
Total predation	L ₁	100	20.00 ± 0.00	100	20.00 ± 0.00
	L ₂	100	20.00 ± 0.00	100	20.00 ± 0.00
	L ₃	20	4.00 ± 2.64	40	8.00 ± 2.00
	L ₄	20	4.00 ± 2.64	25	5.00 ± 1.00

Table 1. Results of predatory activity of *Cyclops fuscus* towards the larvae of two Culicidae species *C. pipiens* and *C. longiareolata* at four larval instars (L1, L2, L3 and L4).

L3 and L4 stages remained negligible until the end of the experiment (72 hours) for both species with a total consumption of L3 = L4 = 20% of *Cx. pipiens* larvae and L3 = 25% ; L4 = 40% of *Cs. longiareolata* larvae.

The ANOVA results (Table 2) showed that there was no effect of the species on the trophic tendency of *C. fuscus* ($P > 0.05$). *C. fuscus* had the same trophic behaviour towards both prey species *Cx. pipiens* and *Cs. longiareolata*. However, this predator was more attracted by insects at early larval stages (L1, L2) than those at more advanced stages with very highly significant difference ($P \leq 0.001$). On the other hand, the time of exposure of preys to the predator seems also a factor of a very highly significant effect ($P \leq 0.001$) where most preys were consumed in the first 24 hours.

Two-way ANOVA results revealed that the interaction between species and the time of exposure on one hand and between the larval stages and time of exposure on the other hand had a considerable effect on the predatory activity of *C. fuscus*.

DISCUSSION

The appropriate use of mosquito control methods is to prevent or control mosquito-borne diseases (WHO, 1982). Predation is an important component of vector population control either as an applied strategy or as a service to the existing ecosystem (Valter et al., 2016). Most studies have been conducted on fish like *Gambusia affinis affinis* Baird et Girard, 1853 (Meisch 1985; Soltani et al., 1999, Bendali et al., 2001), *Poecilia* Bloch et Schneider, 1801 (Sabatinelli et al., 1990), *Panchax* Valenciennes, 1846 (now accepted as *Aplocheilichthys* McClelland, 1839), *Cyprinus* Linnaeus, 1758, *Tilapia* Smith, 1840, *Umbra* W.H. Kramer, 1777 (Bay, 1985), *Pseudophoxinus callensis* (Guichenot, 1850) and *P. guichenoti* (Guichenot, 1850) (Bendali et al., 2001; 2006). Other researchers have performed predation tests on carnivorous mosquito larvae of the genus *Toxorynchites* Theobald, 1901 (Sherratt & Tikasingh, 1989) and on aquatic hemipterans of the family Notonectidae (Hazelrigg, 1976, Collins & Washino, 1985).

In order to contribute in the development of biological control methods, our study aimed to evaluate the predatory potential of hydracarians

(Hydrachnellae) on two Culicidae species *Cx. pipiens* and *Cs. longiareolata*. Our research team is interested in the development of different biological control methods. Thus, we have tested the predatory potential of hydracarians (Hydrachnellae) on many species of Culicidae (Bendali-Saoudi et al., 2014; Boudemagh et al., 2018). Hydracarian can ingest up to 8 mosquito larvae per day but the predation rate is negatively correlated to larvae development (Laird, 1947). Similarly, Murugan et al. (2013; 2015) reported that *Mesocyclops aspericornis* (Daday, 1906) (Copepoda) showed more interest in the predation of *Aedes aegypti* Linnaeus, 1762 at early instars and aggressivity against larvae at the 4th stage larvae which is similar to our results. Furthermore, many species of Coleoptera, Heteroptera, and Odonata of have also showed interesting predatory potential to mosquito larvae. It has been described that 29 mosquito larvae can be devoured by one dragonfly larva in 5 days (Twinn, 1931), 15 by one *Enallagma civile* (Hagen, 1861) in 24 hours (Miura & Takahashi, 1988), 434 by a single *Dytiscus* Linnaeus, 1758 (Coleoptera Dytiscidae) in 48 hours (Chidester, 1917), 35 (anopheles) by two tadpoles in 36 hours (Derivaux, 1916). Copepods and Rana-trinae were also reported as good predators of mosquito larvae (Darriet & Hougard, 1993). The predatory performance of three selected odonatan nymphs - *Pantala flavescens* (Fabricius, 1798), *Trithemis aurora* (Burmeister, 1839), *Libellula fulva* Muller, 1764 - was investigated against mosquitoes' 3rd instar larvae by Khan et al. (2022), the study concluded that odonatan species play a vital role in control of mosquito larvae. Cuthbert et al. (2019) suggest that increasing the diversity of copepod predators in Australian temporary wetland ecosystems additively increases the risk to prey across different water depths and may help regulate disease-carrying mosquito populations. Additionally, Nunes-Silva et al. (2020) concluded that the copepod *Mesocyclops longisetus* (Thiébaud, 1912) can be successfully transported to target areas for the biological control of *Aedes aegypti* mosquitoes with insignificant mortality.

All these studies were in line with our results which showed that *C. fuscus* has an important predatory potential towards mosquito larvae at early developmental stage. This could be due to the physiological state of the predator and its trophic preferences, the size of the prey, an opportunistic choice

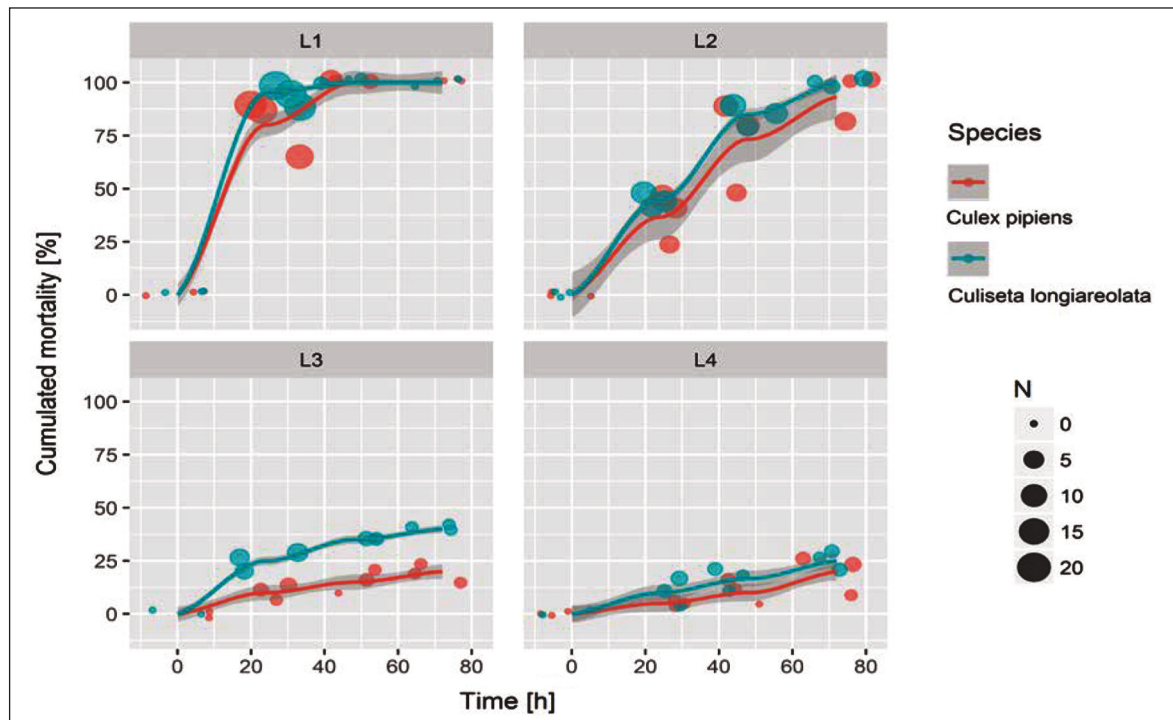


Figure 1. Temporal variation of cumulative predation rate (%) of *C. fuscus* against *Cx. pipiens* and *Cs. longiareolata* at four larval instars. The size of the scattered point is fitted to the number of consumed larvae.

Analysis	Source	SS	MS	F	P	Signification
One-way ANOVA	Species	3.8	3.8	2.84	0.097	ns
	Larval stages	313.8	104.6	79.06	<0.001	***
	Time	786.6	262.2	198.20	<0.001	***
Two-way ANOVA	Species × Stage	3.3	1.1	0.83	0.484	ns
	Species × Times	25.4	8.5	6.41	<0.001	***
	Stages × Time	804.5	89.4	67.57	<0.001	***
Three-way	Species × Stages × Time	17.7	2.0	1.48	0.173	ns
* : $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns: $P > 0.05$						

Table 2. ANOVA results of the predation rates of *C. fuscus* towards two culicidae species (*Cx. pipiens*; *Cs. longiareolata*) at four different larval stages (L₁, L₂, L₃, and L₄) and three different exposure time (24, 48 and 72h) (SS=Sum-of-squares; MS=Mean squares; F=F-statistics; P=P-value).

or even the evolution of the taxon. Abiotic factors, particularly temperature, may affect predatory efficiency of copepods (Tuno et al., 2020). Furthermore, the performance of copepod offspring fell

when the density of mosquito larvae was high, probably because mosquito larvae had adverse effects on copepod growth through competition for food resources.

CONCLUSIONS

Mosquitoes are vectors of many diseases and viruses throughout the world. Many chemical control methods have been used for a long time to control the populations of these vectors. However, these methods have become the source of enormous environmental problems such as pollution, and pesticide-resistance. The results of our study showed that the crustacean *Cyclops fuscus* has an interesting predatory potential against larvae of *Cx. pipiens* and *Cs. longiareolata* at early instar stages. Therefore, some copepods may be useful and suitable tools to control population vectors as part of ecosystems services or even specific strategy based on biological approach.

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