https://doi.org/10.33451/florafauna.v31i1pp153-159
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2025 Vol. 31 No.1 PP 153-159 ISSN 0971 - 6920 (Print)

Impact of predatory fish kairomones on morphology and life history traits of *Cladocera species: Macrothrix spinosa*

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Received **:** 18.03.2025; ***Accepted*** : 18.04.2025

How to cite : Bhusanale RK, Tambe DS. Impact of predatory fish kairomones on morphology and life history traits of *Cladocera species*: *Macrothrix spinosa. Flora and Fauna* 2025. 31(1) : 153-159.

ABSTRACT

The study was carried out on a locally common cladoceran, *Macrothrix spinosa*.In present study, the effects of exposure to predator kairomones on some morphological (body length, carapace length) and life history (clutch size, age at first reproduction) characters in *M. spinosa* was investigated. The *G. affinis*kairomones significantly altered both morphological and life history characteres of the cladoceran *M. spinosa*.

Figures : 03 References : 37 Table : 01

KEY WORDS : Chemical cues, Cladocera, Daphina, Life history, Predator.

Introduction

Evolutionary mechanisms produce life history primarily to increase fitness. Life history traits include survivorship, growth rate, fecundity *etc.* Interaction with predators also influences life histories, as it poses a direct threat to survival and reproduction, and to offspring. Chemical communication is a well-known ecological phenomenon mediating interactions between organisms *via* info-chemicals. The ability of prey to detect predators using kairomones or alarm cues may be innate or acquired through experience.Some prey makes use of chemicals originating from predators, using these cues as an indicator of the level of predation risk and changing their morphology. Predation-induced polyphenism occurs across a variety of animals including insects, crustaceans, fishes and amphibians.

Life history is a product of evolutionary processes primarily to maximize fitness. This manifests through morphological, developmental (traits) or through behavioral adaptations (strategies), influenced by both biotic and abiotic environment, often resulting in local adaptations in populations[10]. Important life history traits include survivorship, growth rate, fecundity *etc.* Interaction with predators also influences life histories, as it poses a direct threat to survival and reproduction, and to offsprings[29]. Hence, adaptations favouring these characters and other morphological traits (*e.g.*, antipredator defenses) which tend to maximize the Lifetime Reproductive Success (LRS) of the organism are inherited. The morphological characters are also important which are indirectly interacting with the life history traits[4].

According to the evolutionary theory of senescence (ETS), populations exposed to high extrinsic mortality (predation pressure) are expected to evolve shorter life span and larger investments in early-life

ACKNOWLEDGEMENTS : This work is a part of Ph.D. Work of Bhusanale R.K. and carried out at the research centre, Department of Zoology, Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, Loni, Maharashtra, India. Special thanks to Dr. Pawar B. A., Head of research centre. All the authors thank the funding agencies for their support and the reviewers for their constructive comments.

reproduction, as the contribution of later ages to overall fitness in such populations is reduced[4.12]. In contrast, low extrinsic mortality would select for longer lifespan and delayed reproduction[4,12,35]. These phenomena mostly occur in r-selected species, characterized by high fecundity, small body size, early maturity onset, short generation time, and the ability to disperse offspring widely[10]. Organisms that exhibit r-selected traits can range from bacteria and diatoms, to insects and grasses, to various semelparous cephalopods and mammals, particularly small rodents[14]. The organisms of these group show certain adaptation when they exposed under risk of predation. *e.g.*, in aquatic habitats, adaptations shown by zooplankton in morphological characters and life history traits under fish predation such as formation of "Helmets" and/or spines in *Daphnia* sp. and *Brachionussp*[18,32], size at birth or size/age at the first reproduction, have been reported earlier[20,31].

Chemical communication is a well-known ecological phenomenon mediating interactions between organisms *via* infochemicals[5]. In any ecosystem, Chemical cues play an important role in life activities of organisms, *e.g.,* for the behavior (namely aggressive and sexual behavior of turtles), social behaviour of aquatic animals[7,22,27]. The relevance of species-specific odours for species recognition, mate searching and mate choice *i.e.,* mate quality recognition - in reptiles. Chemical cues of diverse origin may serve as the signals for predation risk assessment[8,36]. Predators use them to find prey. A kairomone is a chemical substance, produced by an organism, which mediates interspecific interactions in such a way that benefits an individual of another species which receives it, without benefiting the emitter. The kairomones or chemical cues have different aspects in biological interactions. There are two main components of kairomones: a) the predator cue (released by predator itself) and b) the "alarm" cue (release by injured conspecifics and recognized by other conspecific individuals)[10]. In natural conditions, different predators can represent different selective forces or predation pressure; both direct and indirect, and affect life history traits such as reproduced earlier, at a smaller size[30].

The ability of prey to detect predators using kairomones or alarm cues may be innate or acquired through experience[8,36]. *e.g.,* Mice are instinctively afraid of the smell of their natural predators, including cats and snakes. This occurs even in laboratory mice that have been isolated from predators for hundreds of generations. Some prey makes use of chemicals originating from predators, using these cues as an indicator of the level of predation risk and changing their morphology[32] (*Daphnia* sp. and *Brachionus* sp.) or behavior (Diel vertical migration) if need be. In aquatic ecosystems, a most commonly used sensory mode of predator detection and discrimination is chemoreception[8,9,16]. Several aquatic prey species including insects, crustaceans, fishes and amphibians have been shown to possess innate mechanisms of predator detection, which include chemical cues[8,16,36]. Changes in morphology caused by predator presence is known as predator-induced polyphenism[23], and occurs across a variety of animals. In freshwater habitats, fish are known predators of zooplankton. In such conditions fish not only directly influence Daphnia populations through predation, they also produce chemicals, kairomones, which induce changes in life-history traits as well as behaviour and morphology of their prey in such a way that the predation risk decreases[20].

Daphnia species show formation of helmetlike structures when exposed to predators *i.e.*, planktivorous fish or the water they have lived in. They respond to these kairomones by doubling the size of their helmets and develops spine like protective structure. These changes in morphology are thought to provide protection against predation[18,32]. Effects on life history traits such as changes in clutch size, age at first reproduction have been reported earlier for *Daphnia* sp.[34]. Most of the work on life history characteristics of cladocerans is derived from detailed studies on species belonging to the genus *Daphnia*[17,18,20]. Most of these have been carried out in species distributed in the temperate region. Species distributed in tropics differ in their ecology, morphology and also in life histories owing to the great difference in climatic conditions to which they are adapted. Hence, this study was carried out on a locally common cladoceran, *M. spinosa*[25] due to its easy availability and stability in laboratory culture. Also, unlike the case in most studies[8], animals in this habitat have not been exposed to fish predation earlier; hence there was this possibility of observing changes different from those reported earlier. The predator fish (*Gambusia affinis*) was used as it is known to feed on many aquatic species including plankton[24]. Mosquito fish are aggressive foragers, feeding on a variety of prey, including the eggs, fry and larvae of native biota. This is presumably the reason why they were widely introduced as a biocontrol agent against mosquitoes (and mosquito borne diseases) in the early and mid- 1900[s]. Presently, they are considered among the 100 worst invasive species worldwide.

Materials and Methods

For the experiment of effect of predator kairomone on life history traits of *Macrothrix spinosa*, the predator and alarm cue were prepared in lab. To get the chemical cue *i.e.,* kairomone, the predator fish *Gambusia affinis* (obtained from a nearby garden pond), were maintained in lab in rectangular glass aquarium with aged tap water and fed with M. spinosa twice in day.

5 adult fish were taken in 500 ml aged tap water, fed with *M. spinosa* (approx. 100 individuals) and kept for 2 hours to prepare the kairomone containing both the predator and conspecific alarm cues[3]. After 2 hours this water was filtered through 40 ìm mesh to remove any particulate matter and solid free filtrate was taken. This cue was added to the experimental 24 well plate. Same procedure was repeated up to the end of the experiment.

24 adult females of *M. spinosa* (having embryos in their brood chamber) were isolated from the clonal culture, that was established earlier. Morphometric measurements (head length, carapace length, carapace width, number of eggs in brood chamber etc.) were taken for all these animals under stereo microscope (Magnus MS24) at 40X zoom. These were kept in 24 well plate (volume of each well = 3ml) in aged tap water and fed with 100 ìL of algae (cell density approx. 10^6 cells/ml).

The neonates released by these adult females were taken and transferred to two other 24 well plates-one for control (aged tap water) and other for treatment (fish kairomones). The protocol for treatment was identical to that mentioned earlier for aged tap water, except the water (control) and cue (kairomone) change was done twice for a day, at early in the morning and at evening. Experimental set up was kept in incubator at temperature 20-22°C, with ambient photoperiod maintained using a CFL bulb. The experiment was continued up to the maturation of these individuals (control and treatment) *i.e.,* till embryos could be observed in their brood chambers. This observation was taken as Age at First Reproduction (AFR). The animals were fixed in 4 % of Formalin. Morphometric measurements (mentioned earlier) were taken for both control and experimental animals after fixation.

Statistical analyses : All data were checked for normality using Shapiro-Wilk test, and analyzed using appropriate methods[37]. Distribution-free methods such as box-plots and jitter-diagrams were used to visualize the variation in the data. Normally distributed variables were analysed using t-test, while non-normal variables were analysed using Mann-Whitney test.

Results

The morphometric variables were observed to follow a normal distribution, while the life-history traits did not (Shapiro-Wilk test, p (normal) >0.05 and <0.05 respectively). In order to visualize the normal range of morphological characters and life history traits of *M. spinosa,* the morphometric measurements and life history traits of 48 animals were plotted as box-plots and jitter plots.

Following results were obtained for the morphomeric measurements of the control and treated animals, where significant reduction in all the measured traits was observed. The head length: carapace length ratio was also significantly reduced in treated individuals (t-test, $p < 0.001$).

The clutch sizes for animals of both groups remained similar, however an extreme value (8) was observed for one of the treated individuals (Fig. 3A).This difference was not significant (Tab. 1). In the experiment, about 95% animals exposed to kairomones matured earlier than the controls (Fig. 3B), showing a significant reduction in AFR (Table-1)

Discussion

Predator-induced morphological changes have

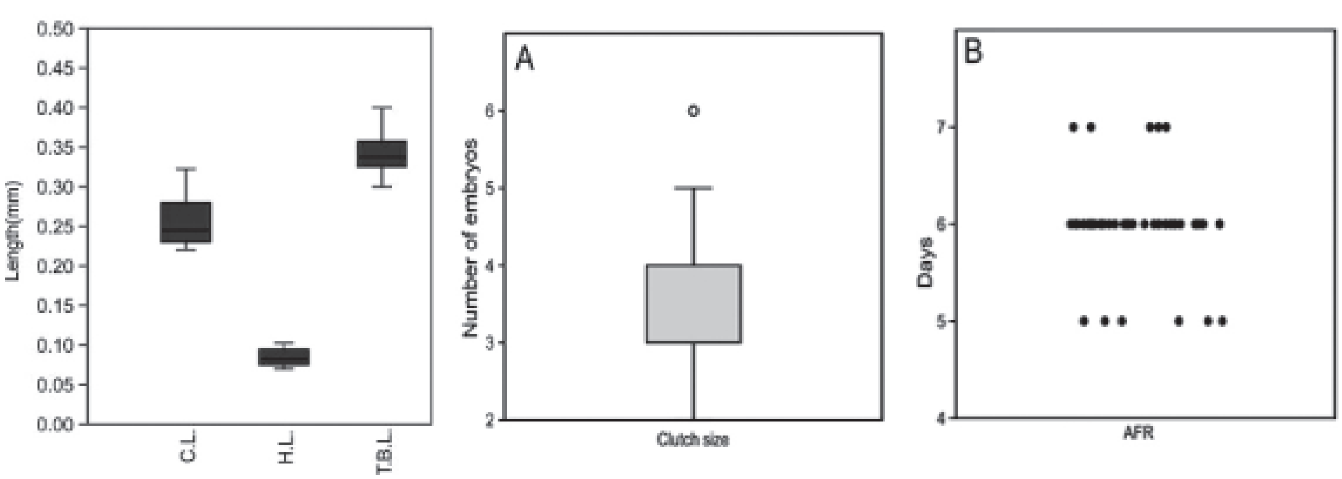

Fig. 1 : Normal ranges for morphometric variables (C.L.- carapace length, H.L.- head length & T.B.L.- Total body length – Fig. A) and life history traits (Fig. B &C) of *Macrothrix spinosa*

TABLE-1 : Comparative observations of life history traits of control and treated animals.

Life history trait	Median value for		Mann-Whitney (at á = 0.05)
	Control	Treatment	
Clutch size	4	4	p = 0.95708
Age at first reproduction (AFR)	6	5	p < 0.001

especially addressed the costs and benefits of morphological anti-predator changes for prey organisms[20]. No changes such as helmet or spine formation were observed in the present study, both of which have been reported earlier for members of the Daphnidae[32]. However, the reduction in size was observed, as reported[21]. Reduction in body size can have a protective effect for the animals, especially from visual predators such as fish[21]. This is also observed in natural conditions, where larger sized species are not observed in habitats where fish are present (*eg.* Calanoid copepods). Hence in absence of deterrent characters such as spines, reduction in body size can be beneficial. Predation affects not only the life history of cladocerans but also the morphology and behaviour of cladocerans[29]. Despite the considerable phenotypic plasticity of habitat choice patterns in *Daphnia*[26]. Fish, through the release of kairomones in the surrounding water strongly influence the Diel vertical migration (DVM) of zooplankton[29,34].

Effects of predator kairomones on life history have been reported[21,29], *Daphnia sp.*1993. In this experiment, the clutch size remained unchanged in treated individuals, but the early maturity was observed, which has also been observed in cladocerans[6]. Under the risk of predation, animals can show early maturity; but the increase in fecundity seemed to be constrained by the reduction in body size[29]. Such changes in life history traits, especially in AFR and clutch size have been observed in different *Daphnia* species as reactions to kairomones exuded by different predators[30,33]. There is very limited information on the few *Daphnia* species found in the tropics with respect to the life history experiments[29], being true for the Cladocera as well.This makes the current study important.

Gambusia affinis (mosquito fish) is presently considered one of the most widely distributed freshwater fish species around the world, and is believed to be the most widely disseminated natural predator in the history of biological control[2]. *Gambusia* is a voracious feeder species, and has been considered a pest when it introduced into new habitats. These fishes are well adapted to feed near the water surface, having a flattened head and an upward-directed mouth. Also, the position of the head during feeding is flexible, so the

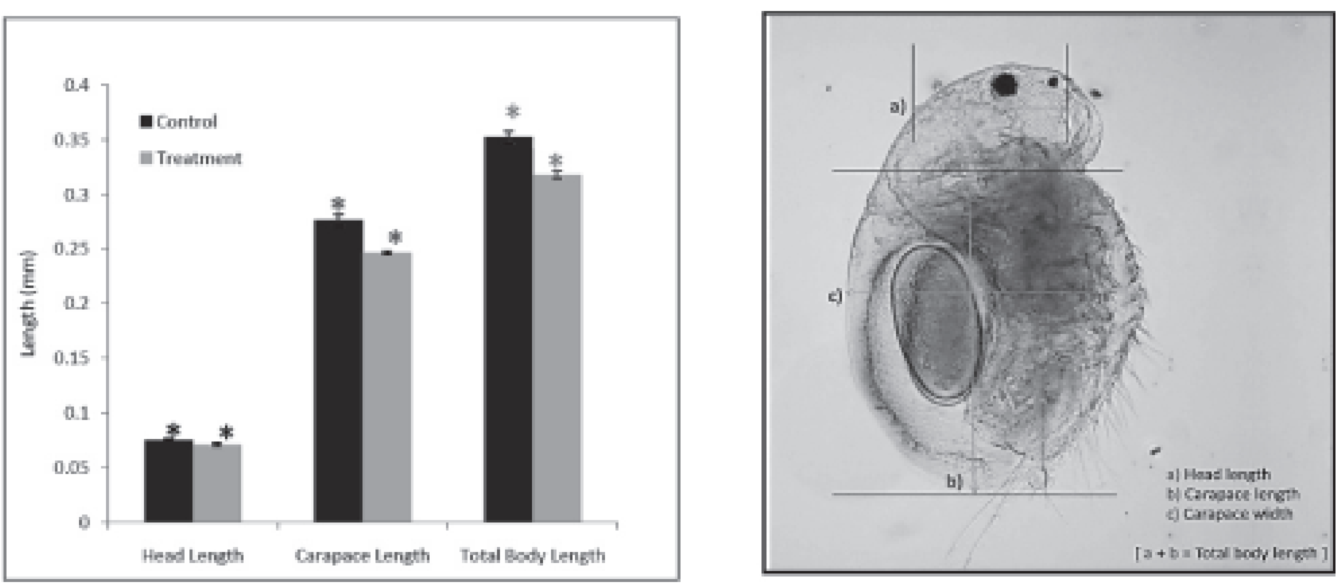

Fig. 2 : A) Comparison of normal and treatment animals morphometry, B) Morphometry of *M. spinosa*

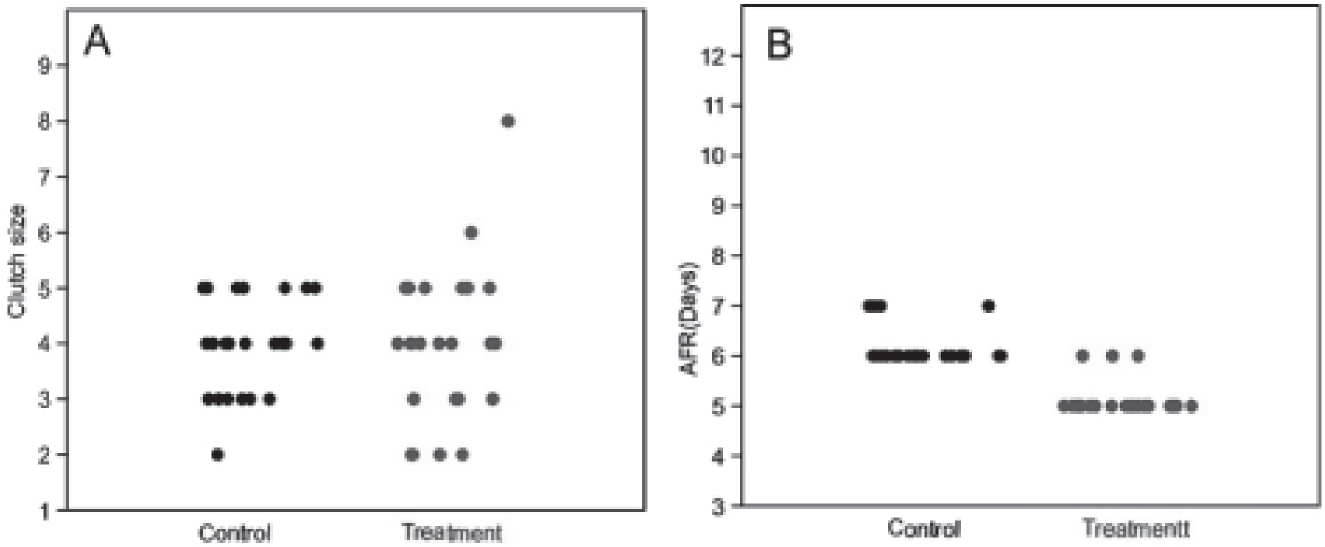

Fig. 3 : Jitter plot showing effect of kairomone treatment on clutch size and age at first reproduction (AFR) in *M. spinosa*

mouth can change from a dorso terminal to a ventral position. This flexibility allows mosquitofish to feed at the surface, on the benthos and in the water column[1].

Though the fish consumes mosquito larvae, it also largely feeds on other planktonic organisms, leading to destruction of entire food-webs. It also feeds largely on zooplankton, aquatic and surface insects, snails, amphibian tadpoles[25] and other fish species. The introduction of exotic fishes to naturally fishless waters is often associated with declines in species native to those waters[19].*G.affinis* has been reported to have essentially eliminated *Daphnia pulex* and *Ceriodaphnia sp.* populations, reduced *Diaptomus pallidus* and *Keratella quadrata* populations, had little impact on *Cyclops vernalis*, and caused large increases in *Cochlearis sp*., *Polyarthra sp*., *Synchaeta sp*., and *Trichocerca spp*. populations and in total phytoplankton[15]. In artificial pools *G. affinis* greatly reduced rotifer, crustacean, and insect populations and thus permitted extraordinary development of phytoplankton populations (2x10^8 cells per milliliter)[15] some[13] have documented the impact of recently established fish populations on invertebrate communities. In many freshwater bodies *G. affinis* has been introduced to control mosquito populations, but the hazards to the native fauna have been overlooked. Predation pressure by these fish cause both direct and indirect effect on the ecosystem in which they introduced. The uncontrolled growth and introduction of *G. affinis* in any fresh water ecosystem, has great potential to damage the ecosystem by large predation; which ultimately results in alteration of functions of that habitat.

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