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CHIRONOMID (DIPTERA: CHIRONOMIDAE) DEFORMITIES AS INDICATOR OF POLLUTION STRESS IN RICE FIELDS OF HOOGHLY DISTRICT, WEST BENGAL.

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Abstract

Morphological deformities of Chironomid (Diptera: Chironomidae) larvae, particularly in the teeth of the mentum, have been proposed as a bioindicator of sediment quality and environmental stress. Larvae of Chironomus sp were collected from rice fields located at Rishra, Serampore and Khanakul (District- Hooghly, West Bengal, India). At each site physico-chemical parameters of water and sediment were recorded. Field data exhibit high incidence of deformity in Rishra compared with Serampore and Khanakul. The agronomic practices revealed an excessive application of pesticide in the rice field for better yield. The rice field ecosystems thus contaminated by pesticides and cause toxic effects in Chironomus sp. Despite that another main contaminating agent i.e. industrial effluents in the adjoining rice fields.

Key words. *Chironomus* sp., Deformity, Pollution, Pesticides, sediment.

Introduction

Deformities of Chironomid larvae represents more traditional and successful methods for biological assessment of water quality. The relationship between morphological deformities and the occurrence of heavy metals and pesticides within the sediment was reported by Hamilton and Sæther (1971). Veroli et al. (2012a) suggested that the incidence of mouthparts deformities reflect the potential toxicity of contaminated sediment. Bhattacharya et al. (1999) recorded high incidence of deformity of mouthparts of Chironomid larvae occurring within the river Damodar flowing through the industrial zone of West Bengal. Arambourou et al. (2012) suggest that the use of mentum variation as an indicator of toxic stress in *Chironomus riparius*. Chironomids were considered as one of the most useful groups in assessing the quality of running waters because of their abundance, diversity and colonizing ability (Sæther, 1980). The application of chironomid deformities as bioindicators of pollution stress has been reviewed and shown primarily for bioassessment purposes (Rosenberg, 1992). Efforts to show the relationship of deformity incidence in chironomid larvae with water and sediment quality have been made (Vermeulen, 1995). Deformities could provide a useful tool for assessing aquatic pollution, specifically that relates to industrial wastes and agricultural runoff (Wiederholm, 1984; Warwick, 1985; Warwick and Tisdale, 1988; Janssens de Bisthoven et al. 1992; Vermeulen, 1995; Bhattacharya et al. 2005, MacDonald and Taylor 2006; Al-Shami et al. 2010a, c). Veroli et al. (2012b) reported that the application of chironomid mouthpart deformities as an endpoint in biomonitoring programs. In this context, many researchers have developed several deformity indices based on different types of chironomid larval head capsule deformities to better understand the causes (Hamalainen, 1999). Chironomidae have been recorded in rice fields throughout the world including India, Australia and the USA (Stevens et al. 2006). The rice field is a unique man-made environment with huge diversity of aquatic organisms and this diversity are closely related to the environmental changes of rice agro-ecosystem (Al-Shami et al. 2008, Ali, 1996). The rice fields are recognized as a very important component in the economic life of the rural people. The ecosystem of rice field is a dynamic but now a day it was highly disturbed ecosystem by anthropogenic activity. Traditional agricultural practices including ploughing, draining, fertilizer and pesticide applications, harvesting and desiccation brought about the wet and dry climate cycles influenced the diversity the aquatic community. The *Chironomus* is considered ecologically versatile in nature because it colonizing ability in most varied conditions and increase response to organic enrichment (Stevens et al. 2006). Generally, rice fields show a wide variation of water parameters such as temperature, pH, dissolved oxygen, conductivity, nitrate and phosphate. Rice fields are dynamic systems of particular environmental concern because they exhibit a high capacity to concentrate and deliver pollutants. The use of pesticides in rice field can change community structure and alter trophic cascade and also damage adjacent water bodies and ultimately affected the aquatic environment (Faria et al. 2007). Al-Shami et al. (2010a) reported that Carbofuran application on rice agro-ecosystem had a negative effect of chironomids and it was more toxic than Fipronil. Madden et al. 1992 experimentally proved that DDT and Dacthal pesticide induced deformities in *Chironomus* sp. and also proved the dose related mentum deformity. In fact, they receive large pesticide applications, they are also extensively irrigated with waters which may contain significant amounts of pollutants, and their sediments are periodically re-suspended, enhancing water–biota exchanges. This study investigate that the incidence of deformities in *Chironomus* sp. Larvae collected from three sites of rice fields that differed in their contamination and larval mouthparts deformities as bio-indicators of pollution stress.

Methodology

Study area

The study was conducted at three locations (Fig-1) of Hooghly district, West Bengal - (i) Rishra (22°42'N and 88°20'E) is an industrial town. This was polluted area and various types of industrial effluents discharge into the rice field periodically. Beside industrial effluents the farmer uses large amount of pesticide in the rice field. (ii) Serampore (22°75'N and 88°34'E) is a pre-colonial town on the right bank of Hooghly River. (iii) Khanakul (22°71'N and 87°83'E) is predominantly non industrialized agricultural area.

Test organism

The larvae of non biting midges belonging to the Genus *Chironomus sp* (Diptera: Chironomidae) are widely used in bio-monitoring and assessment of fresh water environment (Hamilton and Sæther, 1971). The *Chironomus sp* larvae are abundant in close contact with the sediment of rice agro-ecosystem and develop in fourth insters with three moulting processes in between them (Chaudhuri and Chattopadhyay, 1990 and Al-Shami et al. 2010a).

Field Collection of Chironomid Midges

Samples were randomly collected monthly from three sites from July 2009 to September 2012. Adult were collected by sweeping in the rice fields with a long handled insect net. Larvae were collected from mud bottom in the rice fields with the help of mud scrapers and a scoop sampler. The sediment was taken in a bucket, washed with tap water and sieved (300-µm pore). The residue on the sieve was transferred into a white plastic tray and sufficient tap water was added. The larvae were collected with a dropper and transferred to small vials. Egg masses were collected from water surface mostly adhered to twigs either by the application of brush or needle but in case of larvae, brush was used to avoid larval injury.

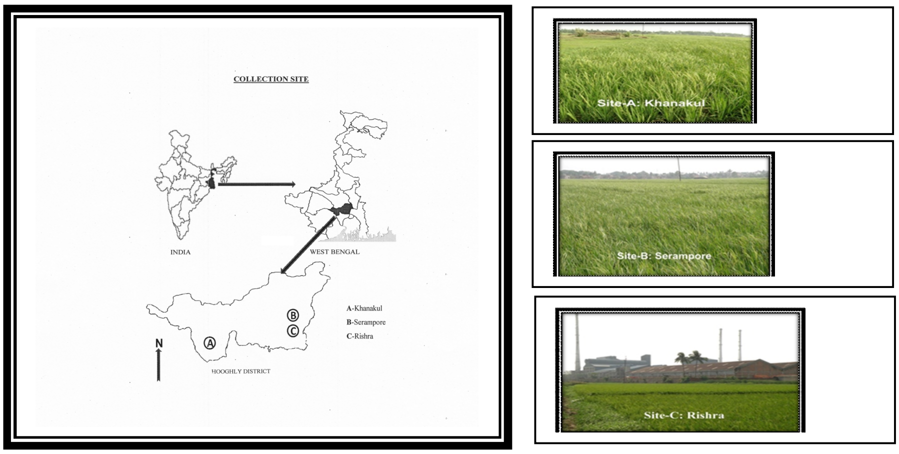

Fig.1. Location of sampling sites.

Culture of chironomids

This unit comprised 30-40 small sized petridishes (8-12 cm in diameter) covered with long tube like transparent plastic having a mesh lid at the top (20-25 cm tall and 8-12 cm in diameter). 8-10 third or fourth instar larvae collected from rice field, reared in laboratory in order to obtain life stages i.e., pupae, pupal exuviae and adults. Each petridish was filled with water medium of particular collecting sites and was regularly changed with few amount of food i.e., dust of special fish food TOKYU-Spirulina was added. The autoclaved soil from the collected site was added to the petridishes. The culture experiment was conducted in the laboratory at 26.3 °C ±0.949with 68.5%±2.1 humidity and light and dark cycle of 14:10h in the Programmable Environmental Test Chamber (REMI). The pH, DO, and salinity (recorded from field data) were also maintained and pre aerated water was added. The adult were allowed to move freely, mate and oviposit in order to repeat for confirmation of the life cycle and life stages of each species. The newly emerged male and female were collected from each of the units with hand held aspirator. The rearing technique such as Pinder et al (1983), Chaudhuri et al. (1990), and Epler (1992) were followed in this study.

Collection of Physico-Chemical data

The physic-chemical parameters were recorded at random from the rice field and at regular monthly intervals during 2009 and 2012 according to Bhattacharya et al. 2006 and Chaudhuri et al. 1990. Parameter like pH, water and air temperature, dissolved oxygen (DO), humidity and BOD were recorded at the sampling sites with the pocket pH tester (pH-ep, HI-98107, HANNA), thermometer, digital-DO-meter (LT-Lutron-DO-5509) and digital humidity meter respectively. BOD was calculated by standard method of APHA (1998).

Preservation and storage

The egg masses, larvae, larval exuviae, pupae, pupal exuviae and adults collected both from field and from experimental unit were most conveniently preserved and stored in 70–90% ethyl alcohol. Since alcohol preserved specimens poised certain drawbacks such as distortion of original colour and difficulty in mounting and a prolonged preservation of specimens, Kahle's solution (30 pt. 95% ethanol, 12 pt. formaldehyde, 4 pt. glacial acetic acid and 60 pt. distilled water) was sometimes used as preservative for both larvae and adults in order to retain normal colour of the specimen.

Mounting and slide preparation

The phenol–balsam technique of Wirth & Marston (1968) was mainly applied for the microslide preparation of larvae, pupae and adults. The larvae, pupae and adults were put in liquid phenol-ethanol mixture (3:1) for a minimum of three hours and were mounted after dissecting required parts in freshly prepared phenol balsam (a homogeneous mixture of one part of liquid phenol and two parts of canada–balsam). In case of larva, the head capsule removed from the body were treated with a warm 10% KOH solution. Thereafter, these were washed in distilled water and put into 70% alcohol and transferred to alcoholic phenol. The larval head capsules were dissected and spread on the slide as per the recommendation of Warwick (1985). The microslide-mounts of specimens were put in a hot plate or hot oven (35°C–45°C) for drying.

Identification

Larvae, pupae and adults were collected from the field were identified upto Genus and confirmed after comparison with other specimen loaned from different depositors. In identifying the material assistance of the work of Townes (1945), Freeman (1961), Sæther (1971 and '80), Oliver & Roussel (1983); Langton (1991), Epler (1992, and '95) Pinder (1978, '83, '86 and 89); Pinder et al. (1983 and 86); and Cranston et al. (1989) were taken. Morphological deformities of chironomid larvae were evaluated on the basis of Warwick and Tisdale (1988), Warwick (1985, '88, '89, '90a, '90b, '90c, '91, and '92). Mouthparts of each larva were examined for deformities of mentum during taxonomic identification. Deformed larvae were then separated and calculated percentage of deformity.

Measurements, Photograph and Data analysis

Measurements of different parts of larvae and adults were taken with the help of a compound microscope and micrometer and are expressed in micrometer (μm). Photograph of various parts of larvae were taken for deformity analysis by the help of MLX-TR (Magnus) Trinocular Microscope. The collected data were determined adopting statistical methods such as Mean and Standard Deviation (S.D.) by using the statistical software SPSS programme, version 17.

Result and Discussion

Physico-chemical parameters measured during July 2009-September 2012 are summarized in the Table-1. The Physico-chemical parameters were more variable in three sites. The mean water temperature was (1-2°C) higher in Rishra compared with the Khanakul and Serampore. The mean value of water pH in three sites ranges from 6.9-7.7. The Biological Oxygen Demand was higher in Rishra in compare to other two sites. This indicates that Rishra site were highly polluted than other two sites. The temperature, dissolve oxygen and pH were higher in contaminated site than other (Faria et al. 2007).

PARAMETERS	KHANAKUL			SERAMPORE			RISHRA		
	Jul	Aug	Sept	Jul	Aug	Sept	Jul	Aug	Sept
Water Temp. (°C)	24.7± 0.823	24.5± 0.707	23.6± 0.843	26.5± 1.080	25.9± 0.876	25.1± 0.994	26.3± 0.949	25.3± 0.823	24.9± 0.738
Water pH	6.9± 0.163	7.3± 0.235	7.5± 0.124	7.4± 0.362	7.3± 0.244	7.5± 0.133	6.9± 0.298	7.5± 0.419	7.7± 0.326

DO (mg/l)	7.3± 0.244	7.5± 0.133	7.4± 0.362	6.6± 0.368	7.6± 0.326	7.4± 0.182	7.7± 0.326	7.9± 0.133	8.1± 0.205
BOD (mg/l)	4.5± 0.326	4.0± 0.182	4.0± 0.200	5.8± 0.323	4.0± 0.226	3.9± 0.226	5.0± 0.194	4.0± 0.200	4.4± 0.323
Air Temp.(°C)	27.8± 0.789	27.6± 0.516	26.6± 0.699	28.3± 0.949	28.1± 0.738	27.3± 0.823	30.2± 0.789	29.3± 0.949	28.2± 1.033
Humidity	70.1± 1.449	68.7± 1.636	68.2± 1.476	67.4± 1.713	66.8± 1.398	66.6± 1.350	68.5± 2.121	67.6± 1.350	66.7± 1.160

Table: 1 Physico-chemical characteristics (Mean ± SD) of three sampling sites during 2009-2012.

The detailed description of different types of larval deformities in *Chironomus* have been postulated by Warwick (1985), Janssen de Bisthoven (1992), Bhattacharya et al.(1999), Bird (1994), Al-Shami et al.(2010d) and Veroli et al. (2012a,b). In our study morphological deformities were found in mentum, mandible and antennae, of which the mentum deformities were the most common deformity of the larvae. The mentum of *Chironomus sp.*bears median trifid and two pairs of mediolateral teeth and four pairs lateral teeth. The median trifid tooth comprises one median larger and two smaller teeth. The median teeth located in between the mediolateral teeth. We found that this tooth were either totally lost or broken at the tip and asymmetrical in appearance. The mediolateral teeth are found in both right and left side of the median teeth. We found that the mediolateral teeth were missing or broken and another cases there were also reduction in the size of some of them. Teeth loss or broken in various positions of the mentum and gap in between the lateral teeth was noticed. That means deformities in mentum range from the loss of inner lateral or median teeth to massive disorganization and loss of symmetry. Deformities in the head capsules of Chironomus sp larvae in the mentum and mandibles were describe by Koehn and Frank (1980), Warwick (1985, 88, 1990a and1990c), Warwick & Tisdale (1988), Janssen de Bisthoven et al. (1992), Al-Shami et al. (2010b) and Servia et al. (1998). Another several authors reported that to produce morphological deformities of various chironomid genera when they are exposed to contaminants (Hamilton and Saether, 1971; Wiederholm, 1984).

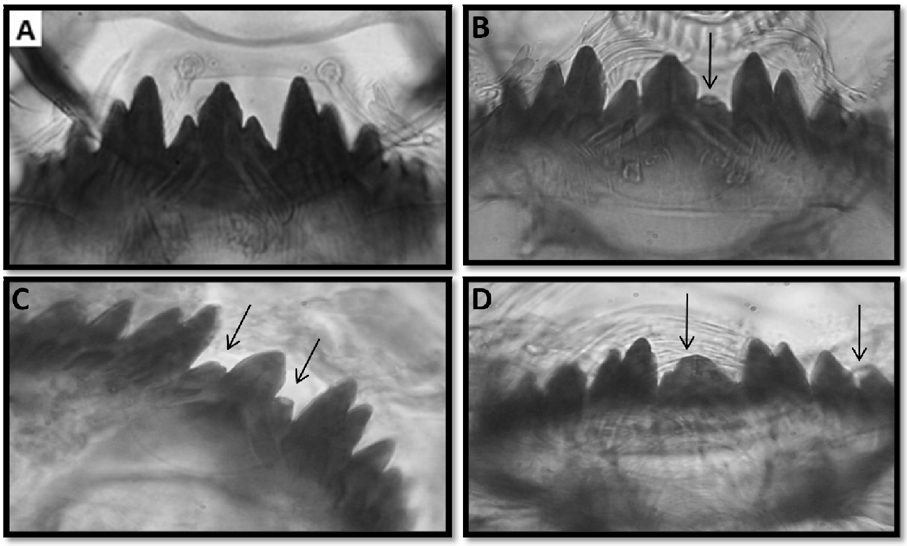

Fig: 2. A-normal mentum, B-D-deformed mentum.
Arrow (→) indicate deformity in *Chironomus* sp.

A total of 1280 *Chironomus* sp. larvae were collected (Fig. 3) from three rice agro-ecosystems and the incidence of deformities were recorded. Out of the collected larvae, total deformed larvae were 26.63%. The result shows (Fig. 4) that out of the total deformities highest deformities obtained from Rishra rice field (55.10%) and other two Serampore (26.53%) and the Khanakul (18.37%). In our study the main sampling site are rice agro-ecosystem in an around Hooghly District in the state of West Bengal. The highest deformity was found in Rishra sampling site, this field was contaminated by various pesticides which was periodically used by the farmer for better production of crop. Despite that another main polluting agent receives from industrial effluents in the adjoining rice field. Other two sampling sites are Serampore and Khanakul, which shows relatively low deformity, these sites has no industry, mainly agricultural area. Morphological deformities in chironomid larvae prove that this was the main biomonitoring agent. Increasing frequencies of deformities may be caused by metals, agricultural runoff and pesticides (Bhattacharya et al. 2005 and Al-Shami et al. 2010c).

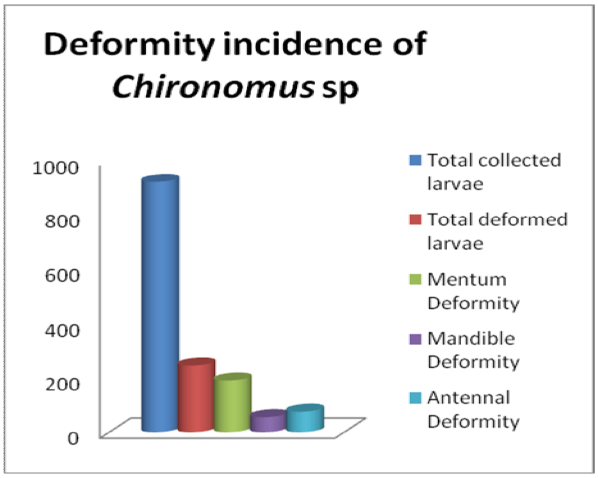

Fig. 3.Deformity incidence of *Chironomus* sp.

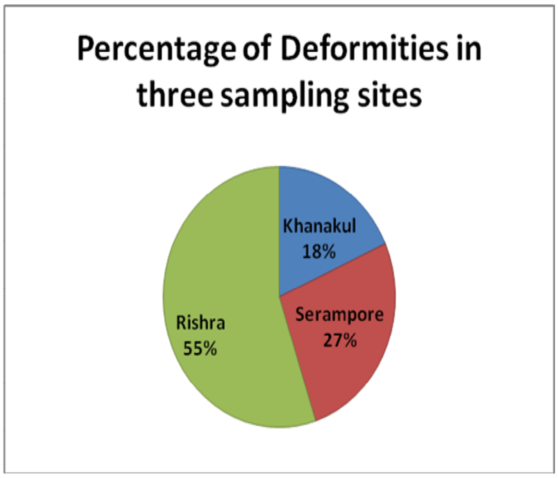

Fig. 4.Percentage of deformities in three sampling sites

Conclusion

In conclusion, this study showed the impact of industrial and anthropogenic contaminants in terms of deformities of various structures of head capsule of *Chironomus* sp. larvae inhabiting the rice fields of Hooghly, West Bengal. The incidence of deformities in larval mentum in the investigated rice field is relatively high compared with some similar earlier studies reported from temperate regions (Bird, 1994 and 1997). The identified deformities are indicative of certain environmental stresses on the studied habitats and could serve as an empirical tool for their assessment. The study also provides baseline data on some physico-chemical conditions prevailing in the rice field for future reference. It could serve as a useful early warning tool in assessment of pollutants prevalent in rice environment receiving anthropogenic, agricultural, and industrial discharges.

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