

GENETIC POLYMORPHISM BASED ON β -NAPHTHYL ACETATE IN DIFFERENT AGE GROUP OF *GAMBUSIA AFFINIS* IN BANGLADESH

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ABSTRACT

Genetic polymorphism of esterase isozymes was examined in different developmental stages of *Gambusia affinis* after staining with β -naphthyl acetate as substrate by 7.5% polyacrylamide gel electrophoresis. Samples were just after birth, seven, 14 days old fry, 21, 28, 35, 42, 49 days old male and female. Altogether three esterase bands named as Est-1, Est-2 and Est-3 were observed. Est-1 was absent in seven samples out of 13, Est-2 was absent only in just after birth, Est-3 was absent in seven different samples out of 13 samples. This result indicates that Est-1 and Est-3 were less active and Est-2 was very active at β naphthyl acetate.

Key words: Esterase, Isozyme, *Gambusia affinis*, Age group, β -naphthyl acetate

INTRODUCTION

Western mosquitofish are small viviparous fish that feed primarily on zooplankton and invertebrate prey at the top of the water column. Adults are known to feed on their young opportunistically (Benoit *et al.* 2000). This species is also well-known for its high feeding capacity. Chips (2004) observed maximum consumption rates of 42 - 167% of their body weight per day. These organisms also require a high density of refuge to maintain populations at or near their asymptotic density (Benoit *et al.* 2000). Because of their reputation as mosquito-control agents, both *G. holbrooki* and *G. affinis* have been stocked routinely and indiscriminately in temperate and tropical areas around the world. In the United States the first known introduction of mosquitofish took place in the early 1900s (Krumholz 1948). In 1905 about 150 *G. affinis* were introduced into Hawaii from Texas to test their effectiveness in preying on mosquito larvae (Seale 1905), and by 1910 their descendants had been released into parts of Oahu, Hawaii, Maui, Kauai, and Molokai (Van Dine 1907, Stearns 1983). Also, in 1905 *Gambusia*, reportedly from North Carolina, were released into New Jersey waters for the purpose of controlling mosquitoes (Seal 1910, Krumholz 1948). In 1922 mosquitofish from Texas (900 from Austin and 300 from Hearne) were introduced into a lily pond at Sutter's Fort. That lily pond served as a

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hatchery used to spread *G. affinis* across California and Nevada during 1920s and 1930s (Stockwell *et al.* 1996). Mosquitofish were commonly and widely introduced during the following decades by such organizations as the former U.S. Public Health Service, in large part because they were thought of as an effective and inexpensive means of combating malaria (Krumholz 1948). In recent years, employees of many state and local health departments apparently view the use of mosquitofish to control mosquito larvae as an attractive alternative to the use of insecticides. Many mosquito species has developed resistance to a variety of insecticides (WHO 1986). It is necessary to know the genetic make-up of a species for any successful management. Moreover, knowledge about the genetic variability in different age group of the fish is a fundamental necessity for biological control of mosquitoes. So far a few genetical or biochemical researches have been done on fisheries in Bangladesh and the present study is the first. There is no measure to determine the extent of genetic abnormality due to the application of different insecticides with a view to controlling mosquitoes. Esterase isozymes are one of the lipid-hydrolyzing enzymes, possess high significance in genetics and toxicology (Market and Moller 1959, Callaghan *et al.* 1994). Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. The residual effect of pesticide in aquaculture specifically in fish is high which in turn cause death of fish particularly, after the Rainy season (Debnath 1978, Sahib and Rao 1980). Esterase isozyme appears to play a critical role in offering resistance to insecticides (Karunaratne *et al.* 1999). Organophosphate acts as an inhibitor of certain esterases. When organisms are treated with insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase causes them to be shaky and have writhing movements. This may result sudden death of an organism. The condition occurs due to low production of esterase or lack of gene that produce this isozyme. If any animal tissue bears sufficient esterase, the organism will be more resistant against poisonous agent. As guppy are used for mosquito control in different aquatic habitat where insecticides are applied for the same purpose, it is a requirement to investigate how much the guppy are resistant and its various stages of fry to insecticide. An organism may develop resistance to insecticides by producing large amount of specific esterase enzymes which either break down the insecticide molecules or bind to it so tightly that it cannot function. Considering the above facts, it is essential to understand the genetic status in terms of esterase variability. The paper deals with polymorphic pattern of esterase isozymes in different age groups of *G. affinis* with β -naphthyl acetate.

MATERIALS AND METHODS

Gambusia affinis was collected from the drains of Curzon Hall Campus of Dhaka University and were transferred to the brood-rearing aquariums. Experiments were

conducted in Genetics and Molecular Biology Laboratory. Esterases were identified in the gels following basically the technique described by Johnson *et al.* (1964) by using the β -naphthyl acetates. Entire technique for polyacrylamide gel electrophoresis (PAGE) Shahjahan *et al.* (2008) was followed.

RESULTS AND DISCUSSION

Est-1, Est-2 and Est-3 could be revealed from different stages of guppy development. Est-1, Est-2 and Est-3 were designed from the highest to lowest relative mobilities, that is, from the lower molecular weight to the higher molecular weight. All of the 3 bands were not observed in all the developmental stages. Each of the gel plate, figures shows the intensity variation of esterase isozymes bands in different developmental stages of guppy. The intensity variation of observed bands could be in three categories. The intensity of observed bands positively co-related with the esterase activity in different stages (Alam 2000). Est-1, the band of highest relative mobility was found all of the stages and deeply stained in most of the stages except males (Fig.1 and Table 1). In embryo just after birth and at day seven the bands was absent (Figs 1, 2) in different observations. After that up to 14 days the intensity increased explaining the fact of higher esterase activity. It was noticed that the entire female esterase band was deeply stained but in male it was medium or faint. Therefore, higher esterase activity was observed at later developmental stages. Est-2 was also found deeply stained in most of the stages except males with β naphthyl acetate. Like Est-1, Est-2 showed similar results except at later developmental stages where esterase activity was comparatively lower both in male and female from where esterase activity again rises up. Est-3, the band of lowest relative mobility was found medium or faintly stained at different developmental stages or was absent in some cases. Est-3 was absent at seven, 21, 24, 35, 42 and 49 days male. Each band represents a single allele. Harris *et al.* (1990) observed three polymorphic loci in shrimp, *Penaeus vannamei* which is similar with the present study. The esterase activity was not same in all the developmental stages. In certain stages it was highly active whereas in other it was less active or absent. In 14 days old fry a new esterase band (Est-3) was observed medium stained and at the same time higher esterase activity was observed in other two bands (Est-2 and Est-3). Differences in esterase synthesis among stages are probably due to regulatory mechanisms acting in agreement with the requirements of a variable number of processes in which esterases are involved (Lima-Catelani *et al.* 2004). After 21 days, male and female were clearly distinguished and was separated to observe male-female esterase variability with aging. Est-3 switched off after 35 days in male but remain less active in female. Higher esterase activity was found in female than male. The ontogeny of the esterase isozymes of the teleost,

Fundulus heteroclitus also noted that one group of esterase isozymes is present at all stages of development, whereas others gradually appeared at later stages of development, or abruptly appeared at such dramatic developmental events as hatching (Holmes and Whitt 1970). In the lake chubsucker (*Erimoizon sucetta*) and the green sunfish (*Lepomis cyanellus*), some isozymes of esterases have been reported to be present at high levels in the earliest embryonic stages due to maternal synthesis, but declined during subsequent development toward hatching (Champion *et al.* 1975). Stage specific expression patterns have also been observed in studies of esterase bands or isozymes in *Drosophila* sp. by Brady and Richmond (1990) and Sergeev *et al.* (1995).

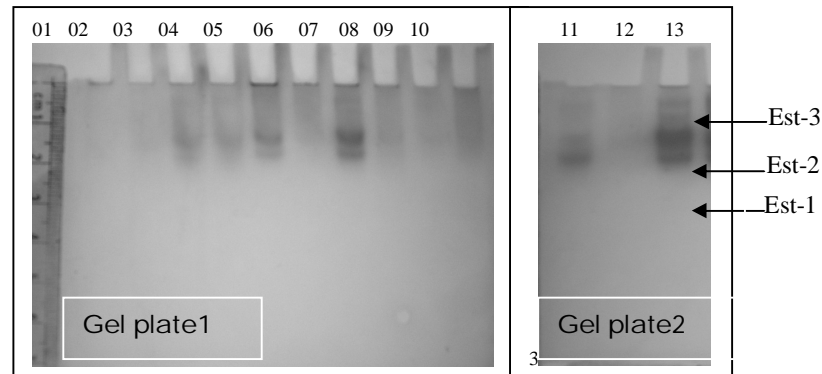


Fig. 1. Esterase banding pattern of different developmental stages of *G. affinis*.

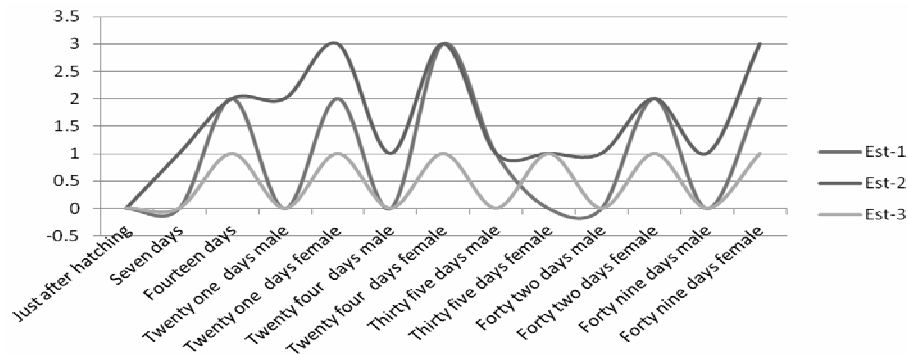


Fig. 2. Flow of stage specific staining intensity variation of esterase isozyme bands observed in *G. affinis* during development. Three point hedonic scale ranges: 0 to 1 = Faint stained, 1.01 to 2 = Medium stained, 2.01 to 3 = Deep stained.

Table 1. Electrophoretic banding pattern showing the real and relative mobilities of esterase isozymes in different developmental stages of *G. affinis* (scored from β naphthyl acetate stained gel). Numerical number indicates mobility and followed by letter indicates staining intensity.

Age of samples	Est-1 (1.25 \pm 0.02)		Est-2 (0.97 \pm 0.03)		Est-3 (0.25 \pm 0.02)	
	Real	Relative	Real	Relative	Real	Relative
Just after hatching	-	-	-	-	-	-
Seven days	-	-	1.5F	1	-	-
Fourteen days	1.9M	1.26	1.5M	1	0.4F	0.26
Twenty one days male	-	-	1.5M	1	-	-
Twenty one days female	1.9M	1.26	1.4D	0.93	0.3F	0.2
Twenty four days male	-	-	1.4F	0.93	-	-
Twenty four days female	1.9D	1.26	1.5D	1	0.4F	0.26
Thirty five days male	1.8F	1.2	1.4F	0.93	-	-
Thirty five days female	-	-	1.4F	0.93	0.4F	0.26
Forty two days male	-	-	1.5F	1	-	-
Forty two days female	1.9M	1.26	1.5M	1	0.4F	0.26
Forty nine days male	-	-	1.4F	0.93	-	-
Forty nine days female	1.9M	1.26	1.5D	1	0.4F	0.26

D =Deep stained, M =Medium stained, F = Faint stained

CONCLUSION

Esterase bands showed a great deal of variation in their number and staining intensity. Est-1, Est-2 and Est-3 were absent in just after hatching and Est-2 was present in all the later developmental stages. Est-1 was absent in seven, 21 and 24 days males, 35 days female, 42 days male and 49 days male and Est-3 was totally absent in seven, 21, 24, 35, 42 and 49 days male.

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