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CHANGES IN BIOMODAL OXYGEN UPTAKE OF OBLIGATE AIR BREATHING FISH- ANABAS TESTUDINEUS (BLOCH)

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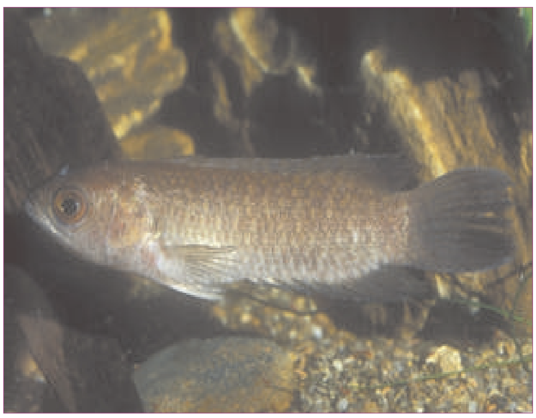
ABSTRACT: -
Several studies on water-breathing species of fish show that the toxicity of waterborne substances increases at low levels of dissolved oxygen (Southgate et al. 1933, Downing 1954, Lloyd 1961, Pickering 1968, Hicks and Dewitt 1971, Smith and Oseid 1972, Voyer et al. 1975, Thurston et al. 1981, Gupta et al. 983 and Verma et al. 1985). Gupta et al. (1983) and Verma et al. 1985 also showed such an effect using bimodally respiring species at 5 mg O2.L-1. These findings in respiratory physiology and behaviour suggest that in a toxic medium, by resorting to surface use (ASR or air breathing), fish can prolong their survival by reducing the rate of gill ventilation to lower the toxin uptake through the respiratory water current.

KEYWORDS: *Several studies , water-breathing ,respiratory physiology .*

INTRODUCTION :

All these studies on the effect of oxygen on toxicity have failed to consider the impact of surface access and its interaction with dissolved oxygen on toxicity. This appears to be a serious lapse because it is well established that surface access under hypoxia is important for the survival and activity of fish. Under hypoxia, water-breathing species rise to the water surface to respire at the well oxygenated thin surface film (Lewis 1970), a response termed as aquatic surface respiration (ASR) by Kramer and Mehegan (1981). During ASR, fish can reduce the frequency of gill ventilation significantly over what would be required if using the water column (Gee et al. 1978). ASR in hypoxic water improves oxygen levels in blood (Burggren 1982) and increases survival and activity (Kramer and Mehegan 1981, Kramer and McLure 1982). Fish with bimodal respiration also depend on surface access. These species are capable of respiring both dissolved and atmospheric oxygen, and during air breathing, gill ventilation may be reduced (Johansen 1970). Bimodal respiration characterizes a diverse array of species from the tropics (Jhingran 1975, p. 790-800) which are notable for their resistance to environmental hypoxis and other stresses (Dehadrai and Tripathi 1976).



Support for such a change in respiratory mode to reduce toxin uptake comes from anecdotal evidence for the voluntary surfacing of fish when exposed to different kinds of toxins, including rotenone (Bhuyan 1968, Konar 1970, Hickling 1971, p. 95, Chakraborty et al. 1972, Davies and Shelton 1983, Tiexeira et al. 1984, Hegen 1985) and copper sulphate (kulakkattolickal personal observations on grass carp, Ctenopharyngodon idella, 1983.

There is experimental evidence that bimodal species of fish also adjust to the increased oxygen demand on exposure to toxins, by depending more on air breathing than on water breathing. Bakthavathsalam and Reddy (1983) showed that on exposure to lindane, Anabas-testudineus showed a significant increase in the rate of oxygen uptake from air with relatively little increase in the rate of oxygen uptake from water. Natarajan (1981) found a similar effect for Channa Stristus exposed to metaystox. Although both these works failed to relate the survival time of fish to oxygen uptake from air, It seems that such an increased dependency on air breathing rather than on water breathing to meet the increased oxygen demand produced by exposure to toxin, was to limit the toxin uptake as much as possible, through the respiratory water current. In addition to the studies on air-breathing fish already cited, Smatresk and Cameron (1982) have demonstrated that the gar (Lepisosteus oculatus) increased the use of atmosphere and decreased the use of dissolved oxygen on exposure to hyperosmotic solutions, and Burggren (1978) reported similar responses to dissolved carbon dioxide. So far no study has been conducted to establish the survival value of either air breathing or ASR in fish exposed to toxins in hypoxic water.

Based on the anecdotal and experimental studies already cited, I felt the need to establish the importance of surface access on survival time of fish exposed to toxins. Therefore, the main goals of this investigation were to quantify: (1) the effect of dissolved oxygen levels on toxicity of a waterborne toxin (Croton tiglium seed extract) to a water breathing species Brachydanio rerio, the zebrafish, and a bimodal species Clarias macrocephalus, (2) the role of surface access as a modifying factor of toxicity at different levels of dissolved oxygen for both species, and (3) the effect of toxin on the rate of air breathing in Clarias macrocephalus.

The original contributions of this study are as follows:

1) This study provides the first experimental evidence for the effect of surface access on toxicity for both air-breathing and a water-breathing species of fish.

2) This study gives the first experimental evidence that toxicity under hypoxia is magnified for a water-breathing species of fish when surface access is denied.

3) As an extension of previous studies showing that toxicity is increased at lower levels of dissolved oxygen, this study gives the first evidence of such as effect for a proteinaceous plant toxin on a water-breathing species of fish.

4) This study provides the first evidence for a decrease in toxicity at very low levels of dissolved oxygen for an air-breathing fish with surface access.

I believe that these results have applications in aquaculture and sampling of wild fish populations. When applying toxins to eradicate unwanted fish from aquacultural ponds (Bhuyan 1968), selecting a time of day with high or low dissolved oxygen levels as required and preventing surface access can increase the rate of action of toxins on fish. This should reduce the quantity of toxin applied and hence should result in increased cost-effectiveness and reduced environmental contamination.

The surfacing behaviour of fish in natural water bodies in response to rotenone has been used for sampling wildfish populations (Shireman et al. 1981). On the basis of the results of this study it seems that such surfacing behaviour is the toxin-induced aquatic surface respiration (for water-breathing species of fish) and perhaps air breathing (for air-breathing species of fish). If this is established through further research, this might result in better ways of manipulating the toxin-induced respiratory behaviour of fish for sampling wild fish populations.

The labyrinth fishes, belonging to the suborder Anabantoidei, derive their name for having a labyrinth-like accessory breathing organ on either side of the head. Two widely known Asian members of the group are climbing perch (Anabas) and gouramy (Osphronemus). The climbing perch, Anabas testudineus (Bloch), also popularly known as 'kabai' is a well known air breathing edible fish, inhabiting fresh waters and brackish waters of Southeast Asia. The common name, climbing perch, originated from the Asian legend that Anabas climbs palm trees to suck juice. Probably the origin of this myth is that birds pick Anabas when they travel overland and place it on palm trees (Norman, 1975).

Wakiama et al., (1997) studied the genetical relationship of the 17-anabantoid fishes, and based on similarity of their alleles, divided them into three groups comprising: 1) Anabas testudineus, Ctenopoma acutirostre, Osphronemus goramy, Ctenops nobilis and Luciocephalus pulcher; 2) Trichogaster leeri, T.

trichopterus, T. microlepis, T. pectoralis, Colisa lalia, Sphaerichthys osphromenoides and Helostoma temmincki; 3) Betta splendens, Macropodus opercularis, Parosphromenus deissneri, Trichopis vittatus and Pseudosphromenus dayi. Based on these studies, the Anabantidae, Macropodinae (excluding Ctenops) and Trichogastrinae are considered to be suitable taxonomic units.

GENERAL CHARACTERISTICS OF ANABAS TESTUDINEUS

The fish has a posteriorly compressed oblong body with a rather broad head and an anterior part. The mouth is not protractile and has small conical teeth on jaws and vomer. The gill covers are serrated and, unlike other teleosts, the opercular and subopercular bones are not fused into a single operculum. Instead, a thin, flexible membrane binds them, so that the fish has two sections of the gill cover hinged separately: the opercular on the suspensorium and the subopercular on the rear part of lower jaw. The gill covers open very widely and the subopercular rotates ventrally as well as laterally.

Dorsal and anal fins are long and composed of spines and soft fin rays. Dorsal and anal spines are strong with a larger soft ray portion. The caudal fin is rounded, scales are ctenoid. Fish are grayish black to dark brown in colour. Young fish have transverse dark strips on hind part of the body and tail and a large dark spot at the base of caudal fin and a small one at hind border of the operculum.

Anabas testudineus shows variability ill morphological characters and body colouration. In body proportion, the Bengal forms are rather elongated than the Madras forms (Day, 1889). Based on morphometric characters, Das (1964) identified three ecological sub-species, viz; Anabas testudineus riveri (river Anabas), A. testudineus lacustri (lake and tank Anabas) and A. testudineus ricei (swamp Anabas). Rao (1968) revealed for the first time the presence of two species of Anabas in India, A. testudineus Bloch, 1792 that is distinguishable from A. oligolepis Bleeker, 1855 by less body depth,

Ramaseshaiah and Dutt (1984) compared electrophoretically both these species, which are found in a single habitat lake Kolleru of Andhra Pradesh, and found them to be closely related. A. oligolepis has 46 chromosomes, while A. testudineus has 48 chromosomes (Dutt and Ramaseshaiah, 1980). The diploid number of chromosomes is 48 in either sex of A. testudineus (Kaur and rivastava, 1965; Nayyar, 1966; Natarajan and Subramanian, 1974).

A. Testudineus is an obligatory air-breathing fish. The fish gets asphyxiated, if denied access to water surface for longer periods.

in addition to four pairs of gills, it bears special structures called accessory air-breathing organs consisting of a pair of labyrinthine organs and the respiratory membranes covering the suprabranchial chamber. Each of the accessory respiratory organs situated on either side of suprabranchial freely with bucco-pharyngeal cavity on one side and opercular cavity on the other. The detailed morphology and anatomy of the accessory respiratory organs of Anabas have been dealt by many authors (Misra and Munshi, 1958; Sexena, 1964; Munshi, 1968; Reddy and Natarajan, 1970, 1971; Hughes and Munshi, 1973).

Habitat, food and feeding habits

A . testudineus is found in all types of waters of tropical and subtropical areas. They are more prevalent in derelict and swampy waters which are regarded as their habitual abode. Even though it is primarily a fresh water form, it shows a high salinity tolerance. A. testudineus fry measuring 14.0 mm can survive up to 11.5% salinity (Khan et al., 1976). Though the optimum tolerance of water temperature of the fish is 20-30 O C, it can resist very low temperatures (Hora and Pillay, 1962). A. testudineus is quite hardy and is able to aestivate during the dry season, by burying in mud, like the African Lungfish (Thiraphan, 1984).

The natural food spectrum of A. testudineus is very wide and it may vary from a diet of filamentous algae to that of pure canvorous nature. Larvae and Young fry feed on phytoplankton and zooplankton, large fry and adults feed on crustaceans, worms, molluscs, algae, soft higher plants and organic debris (Potongkam, 1972). Anabas has been described as a predator, carnivore (Pandey et al., 1992) or an insectivore (Ahyaudin, 1992). However, gastric contents analysis of 204 specimens of Anabas showed that the stomach contained 19%

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crustaceans, 3.5% insects, 6% molluscs, 9.5% fishes, 47% plant debris and 16% semidigested matter (Nargis and Hossain, 1987).

Major food items in the gut were found to be more or less consistent irrespective of spatial and seasonal distribution in Bangladesh (Nargis and Hossain, 1987), indicating that Anabas is an omnivore. The gastric pH was in the range of 5.96-6.58 indicating that Anabas is a stomached fish (Pandey et al., 1992). During the larval period, 3-17 days from the inception of feeding, they can be fed only on live food (Doolgindachabapom, 1994).

The foregoing review of literature on A. testudineus indicates that the species has been studied well only in North India. Further, the knowledge on the reproductive biology of the species is fragmentary, whereas information on the development and maturation of the gonad at cellular level are scant. The species has not been considered for detailed investigations in Kerala. The present study therefore, is an attempt to learn more about the reproductive biology and the related aspects of reproduction of Anabas testudineus (Bloch).

Classification

Order	:	Perciformes
Suborder	:	Anabantoidei
Family	:	Anabantidae
Genus	:	Anabas Cuvier, 1817
Species	:	Anabas testudineus Bloch, 1792

Anterior part of the body and head are broad while the posterior part is compressed. Mouth is terminal and relatively large. Jaws bear villiform teeth. Dorsal fin has 16-18 strong spines and 8-10 soft rays. Pectoral fins are blunt and rounded. Pelvic fins are with one spine and five soft rays and Caudal fin is rounded. Live fish is dark to pale yellowish/greenish in cvolour; posterior margin of opercle bears a dark spot; base of caudal fin also possess a dark spot. Iris is golden reddish in colour; while, scales are large and ctenoid.

Extremely efficient food conversion ratio (FeR) values, air-breathing ability and tolerance to adverse environmental conditions make A. testudineus, an excellent candidate for aquaculture, perhaps more so than any other tropical fish. However, slow growth rate may be a constraint for commercial viability.

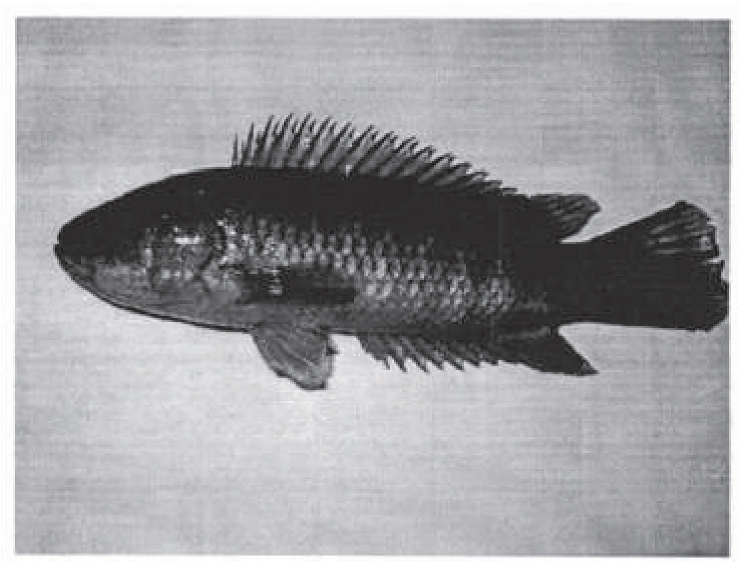

Plate 1. Anabas testudineus (Bloch)

DISCUSSION

The birth of the modern pesticide era in the late 1940's was hailed as a major break-through for mankind. The philosophy that these new chemicals would stop the innumerable pests in their track thereby eradicating disease and eliminating crop reduction led to a progressive increase in their use. A belated second thought on the environmental persistence of organochlorines (new era pesticides) led to the invention of less persistent pesticides like organophosphate, carbamate and synthetic pyrethreoids.

The problem of pesticidal impact on the ecosystem has assumed considerable proportions owing to the modernisation of agricultural operations and the consequent widespread and indiscriminate permeation of the ecosystem with these pesticides. The effects of pesticides on ways, since the majority of them are non-selective and produce detrimental and sometimes fatal side effects on non-target species. Knowledge on pesticide toxicity levels, either by acute toxicity, residual or physiological studies, is essential to develop effective protective measures for the conservation of our already depleted freshwater fauna.

It is apparent that human chemical additions have introduced or increased environmental stress for aquatic organisms and fishes in particular. Many of the effects of pesticides to fishes are subtle and insidious. Unlike direct eradication of populations (eg fish kills), the more serious long-term decline of stocks of fish are caused by indirect factors such as predation, disease and reproductive failure. Fish which are subjected to unnatural stresses in any part of their life history may be rendered less capable of performing those functions necessary ot fulfil their life cycle and if fish'es ability to defeat its natal stream is impaired by the presence of pollutants, then it may go unspawed and leave no natural means of perpetuating the species (Waldichuk, 1974).

Studies on the sublethal effects of pesticides have gained a great deal of impetus in the last decade, partly because of their practical importance and partly owing to academic interest. Quantitative assessment of the effects of pesticides on fishes has got cardinal importance in fishery management both from the biological and ecological points of view. Moreover, a sublethal both from biological and ecological points of view. Moreover, a sublethal effects of pollutants are now being recognized by regulatory agencies in establishing pollution controls. Rather than applying an arbitarary "application factor", as a safety factor, to the LC 50 data obtained in acute toxicity bioasays, poolution control is now being developed by using the sublethal threshold level, derived by chronic toxicity bioassays, as the limiting concentration. Even in administering the International Convention for the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (U.K. 1972), the term "harmlessness" of a particular substance is being defined by application of data from sublethal toxicity studies, among others (Waldichuk, 1979).

The investigations of the effects of pesticides, or any other pollutants, on aquatic organisms, especially fishes, aimed at delineating the pollution effects, mainly centre around two broad scientific approaches viz. ecological monitoring and laboratory investigations. Ecological monitoring and the efficacy of the approach mainly depend on the in situ effects of pollution, which in turn are controlled by the pattern of pollutant release in space and time. Negative ecological consequences of pollution manifest themselves in detrimental deviations from the normal state of individual investigations, mainly take into consideration the detrimental deviation from the normal state of individuals. Such impairments can be quantified. The philosophy of the present study holds true to the second approach.

Coastal zones are more prone to vulnerable to pollution, as this zone receives pollutants both from land and water sources. Further major industrial developments, transport and other activities causing pollution tend to take place in the vicinity of coastal zones.

Besides, coastal areas are densely populated and the coastal ecosystems are fragile by nature due to their high degree of variability in space and time. Conservation of this zone demands paramount importance as these are important areas for fisheries. Coastal area dumping grounds have much higher pollutant concentration not only because the material is being put into these shallow areas much more rapidly than it is being carried away by natural water motions, but also because of the normal structure of the oceans which tend to prevent the mixing of these inputs with the rest of the oceanic volume (Williams, 1979).

Among the various animal groups, fishes have been identified as being very sensitive to pollutants and have been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment. Fishes are one of the most important members of the aquatic food chain, and through them some toxicants may reach human beings as well. The selection of organisms for toxicity test is mainly based on certain criteria like its ecological status, position within the food chain, suitability for laboratory studies, genetically stable and uniform populations and adequate background data on the organism (Buikema et al., 1982). The species selected for the present study viz. Etroplus maculates satisfy most of the above protocols.

It is no longer sufficient to document aquatic pollution in terms of the chemical concentration of the contaminant. The use of bioassays as part of a comprehensive approach to pollution assessment is widely accepted nowadays. Toxicity is a biological response, which when quantified in terms of the concentration of the toxicant can constitute the basis for a bioassay procedure. Toxicity tests are defined here as estimation of the amount of biologically active substance by the level of their effect on test organism (Chapman and Long, 1983). The direct determination of the acute toxicity levels has been followed in the present study also in spite of limitations, as it provides the best and most practical methods of evaluatin the danger levels of pesticide contaminants commonly found in the aquatic environment and consequent risks to fish populations (Alabaster, 1969). Such as investigation is particularly essential with the fish E. maculates, as no such study has hitherto been undertaken.

In general, sublethal effects cover the effect of all those concentrations which are not lethal for individuals even after prolonged exposures, but increases the population mortality, decreases its size, or changes in composition. Thus, a group of effects that effect the growth, rate, metabolism, reproductive potential behaviour or which impair the defence mechanism of an organism are referred to as sublethal effects. In the present study sublethal effects of pesticides on a selected fish were looked into detail. Physiological responses like activation or inhibition of some selected enzymes, disturbances in haematology and histological changes are the parameters chosen for the assessment of the sublethal effects.

The present study involved investigation of the lethal and sublethal effects of three pesticides individually. The pesticides selected are the commercial formulations of DDT (organochlorine), Dimecron (organophosphate) and Gramoxone (paraquat dichloride). Synthetic pesticides, especially organochlorines and organophosphates have become increasingly important additions to chemical wastes polluting natural aquatic communities and many of these are considered hazardous because of their ability to kill or immobilize organisms even at very low concentrations. Generally the commercial formulations of pesticides are found to be more toxic to fishes than the respective active ingredient which seldom encounter with the aquatic communities.

Most toxic substances exert their effects on a basic level in the organism by reacting with enzymes or by affecting membranes and other functional components of the cells. Biochemical and Physiological techniques are commonly used in laboratories to measure such effects and together with histological, histochemical and haematological studies can contribute most fruitfully to reveal the toxic mechanism of a single or a group of substances (Bengtsson, 1979).

The impact of pollutants on an organism is initiated as disturbances at the subcellular and cellular levels. Since lysosomes are the subcellular units involved in the concentration, disintegration and elimination of toxicants, a knowledge on the concentration of important lysosomal marker enzymes is inevitable in monitoring the extent of pollution caused by biotic and abiotic factors. Cell membrane and the confluent endoplasmic reticulum are the first to confront pollutants. They are susceptible to the effect of pollutants as they bind to the lipoprotein layer of the membrane and induce variation in the permeability which upset the whole cellular systems. So a study of the activity of membrane bound enzymes becomes a useful index of the extent of pollution imposed (Annie, 1988). Investigations on the impact of pesticides on the activity of two phosphomonoesterases; Acid phosphatise, an enzyme bound to the cell membrane and endoplasmic reticulum (Ciro et al., 1975) is thought to be meaningful.

As stated by Meister (1955) “Transamination is a chemical reaction in which an amino group is transferred from one molecule to another without the intermediate participation of ammonia”. Transamination represents one of the principal metabolic pathways for the synthesis and deamination of amino acids. It allows an interplay between carbohydrate, fat and protein metabolism, an activity which can serve the changing demands of the organism (Cohen and Sallah, 1961). Transaminases are a group of enzymes that catalyze, the process of biological transamination. Of the many transaminasses, the most important and widely investigated are Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) which play an important role in the detoxification of ammonia. Their stability and relative easiness made them subject of analysis in a variety of animals. In fishes, investigation on these has gained only limited popularity, though tissue enzyme analysis is gaining increasing importance in the field of environmental toxicology. It is felt that the study of the activity response of these two enzymes, used by fishery biologists to diagnose sublethal insult of pollutants to animal as a whole or organ-wise, to pesticide exposure will further enlighten the knowledge of stress physiology.

In physiological studies of fish, haematology is often used as an index of the effect of xenobiotic compounds to these animals. The measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal concentrations of pollutants may provide a sensitive index in predicting the effects of chronic exposure on survival of the animal. Such analysis has considerable clinical importance in mammals. But in fishes such applications are only limited. A knowledge on the pathological effects of pesticides on the circulating blood elements and blood pigment can provide a frame work for simpler routine analysis of blood in fish toxicology. Pesticide-induced haematological changes may be of some value in assessing the impact of exposure to these chemicals and may serve as tools for biological monitoring (Murthy, 1986).

CONCLUSION

Several environmental contaminants have been found to induce histological changes in fish. Pesticides are no exception to this. However, histological effects of pesticides remain largely undefined and majority of the documented work are directed to acute toxicity. Most lesions have been extremely nons-specific and merely indicative of toxic insult. Since these subtle changes, that occur over long periods of exposure, are not grossly apparent, histopathological studies are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants (Meyers and Hendricks, 1988).

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