

## PREFACE

This book presents a comprehensive review of various studies in animal science. Topics discussed herein include the developmental expression profile of cockroach genes; zooplankton composition in an estuarine area; noninvasive methods of DNA sampling; morphology and distribution of four pill millipedes; and immunotoxicity of azadirachtin in freshwater mussle.

Chapter 1 - The population of *Neritina virginea* (Linnaeus, 1758) in Barra Grande estuary, Ilha Grande, state of Rio de Janeiro in southern Brazil (23° S) was observed from February 1999 through December 2000. The sandflat where the neritids live was divided into 5 sectors, each 30 m long, and each sector was sampled separately. Densities were higher in spring and lower in winter. Reproduction was continuous year-round, but with increased activity in early spring. Fecundity was about 80 eggs/embryos per capsule, and the sex-ratio was 1 males: 2.89 females. This population of *N. virginea* attained higher densities and shell size in the inner part of the estuary. A total of 40,596 individuals was sampled, with shell length varying between 2.50 and 18.80 mm. Life span varied between 1.78 and 2 years for the first and second years respectively. Mortality rates did not differ significantly between years. Production varied from 0.099 g (AFDW) m<sup>-2</sup> year<sup>-1</sup> to 0.135 g m<sup>-2</sup> year<sup>-1</sup> while the ratio between production and biomass (P/B) ranged from 1.75 to 1.55. The larger body size of *N. virginea* may account for its low P/B compared to hydrobiids and *Bittium* spp.

Chapter 2 - Mating, oviposition, post-oviposition period, development, and generation period of the two land snail species *Monacha cartusiana* (Müller) and *Eobania vermiculata* (Müller) were studied in the laboratory in Egypt during the two seasons of 2003/4 and 2004/5. Data revealed that mating is essential for both snail species to lay eggs, thus it occurs when snails reached their sexual maturity (from adulthood till mating) during a period averaged 190.3 and 228.6 days for *M. cartusiana* and 231.6 and 568.3 days for *E. vermiculata* after adulthood in the two different seasons respectively. Pre-oviposition period (from mating till laying first egg) lasted an average of 2.7 and 8.2 days for *M. cartusiana* and 3.5 and 9.2 days for *E. vermiculata* during the two seasons respectively.

Oviposition period (from first laying egg till last one) averaged 22.5 and 64.1 days during which the snail of *M. cartusiana* deposited an average of 63.0 and 177.5 eggs during the two seasons respectively. On the other hand this period averaged 15.2 and 24.9 days for *E. vermiculata* during which the snail individual deposited and total average of 89.6 and 162.0 eggs during 2003/4 and 2004/5 respectively. While, post-oviposition period (from last egg till death) lasted 142.4 and 71.9 days for first snail species and 372.5 and 660.1 days for the

second one during the same seasons respectively. The generation period (from egg to egg) extended an average of 369.8 and 402.6 and 481.7 and 868.7 days for both species during the same seasons respectively.

Chapter 3 - Sundarbans biosphere reserve is a unique ecosystem and characteristic of diverse types of habitats ranging from mudflat to sandy beaches. Nutritionally, economically and ecologically important estuarine edible crab, *Scylla serrata* (Arthropoda: Crustacea: Decapoda) occupy diverse habitats and their survival is dependent on multidimensional immune responses mostly offered by hemocytes – the chief immunoeffector cells of hemolymph. The circulating hemocyte number is a stress indicator and hemocyte counts may be a valuable tool in monitoring the health status in biounsafe environment in *S. serrata*. The enumeration of total and differential hemocyte has been enumerated under the exposure of 1, 2 and 3 ppm of sodium arsenite for 1, 2, 3, 4, 7, 15 and 30 days in controlled laboratory condition. In *S. serrata* diverse subpopulations of hemocytes were identified as hyalinocytes, semigranulocytes and granulocytes. Alteration in hemocyte counts and density of hemocytes were recorded under 1, 2 and 3 ppm of arsenic in static water environment. Shift in THCs and DHCs recovered partially in arsenic exposed crabs maintained in arsenic free water up to 30 days. Exposure to 1 ppm of sodium arsenite for 30 days yielded a highest THC as  $312.9 \pm 3.6 \times 10^5$  cells / ml against control value of  $47.319 \pm 4.8 \times 10^5$  cells / ml. Relatively high THC in the crab maintained in toxin free water for 30 days of span indicates a persistence of cellular stress induced by arsenic exposure. A highest depression in hyalinocyte density was recorded under the exposure of 1 ppm of arsenite for 30 days as 1.17% against control value as 7.47%. Similarly decrease of granulocyte was recorded as 16.21% in 30 days of exposure against control value of 27.24%. Elevation of semigranulocyte was recorded as 82.62% against the control value of 65.29%. Study of possible restoration of DHC was carried out by maintaining the animals in toxin free water for 30 days. All groups of treated animals excepting the groups exposed to 2 ppm for 30 days and 3 ppm for 30 days recovered the DHC of the control. Depression in hyalinocyte and semigranulocyte density was recorded under the exposure of 3 ppm of arsenite for 30 days against control value. Arsenic induced alteration in hemocyte density indicated a state of immunological stress in estuarine crab. Data is suggestive of cellular stress which may lead to decline in endemic population of *S. serrata* in arsenic affected districts of West Bengal.

Chapter 4 - *Lamellidens marginalis* (Mollusca: Eulamellibranchiata) is a freshwater edible mussel distributed in the wetland of different districts of West Bengal of India. Hemocytes – the blood cells of this species perform diverse immunological functions including nonself recognition and phagocytosis of nonself particles. Assay of nonself surface (glass) adhesion of hemocytes was determined under the exposure of 0.006, 0.03, 0.06 and 0.09 ppm of a biopesticide azadirachtin in diverse span of exposure i.e. 1, 2, 3, 4 and 7 days in controlled laboratory condition. Shifts in percentage of adhesion were recorded against all the concentration screened. However, the patterns of shift were not uniform.

Azadirachtin induced shift in surface adhesion of hemocytes indicated a state of immunological toxicity and stress in the species. Phagocytosis of nonself particulates is considered as a classical immunological response in bivalve. We have examined phagocytosis of yeast (*Saccharomyces cerevisiae*) by hemocytes of mussel exposed to 0.006, 0.03, 0.06 and 0.09 ppm of azadirachtin for 1, 2, 3, 4 and 7 days *in vitro*.

Alteration in phagocytic response and adhesion of hemocytes is suggestive of cellular stress which may lead to decline in population of *L. maginalis* in xenobiotic affected districts of West Bengal of India.

Chapter 5 - Sundarbans biosphere reserve of India is an ecologically important zone which shelters diverse forms of animals and plants. *Scylla serrata* (Arthropoda: Crustacea: Decapoda) or estuarine mudcrab is an important consumer of this mangrove ecosystem. Blood or hemolymph of *S. serrata* contains hemocytes, capable of performing diverse physiological functions. Hemocytes the circulating blood cells are considered as immunoreactive agents capable of performing phagocytosis, nonself adhesion and aggregation. Arsenic is a major environmental threat in the specific districts of the state of West Bengal of India. Estuarine water of Sundarbans mangrove bears the risk of arsenic contamination due to anthropogenic and geological consequences. Magnitude of hemocyte aggregation was studied in depth under the exposure of 1, 2 and 3 ppm of sodium arsenite for 1, 2, 3, 4, 7, 15 and 30 days of exposure. Arsenic exposure yields a dose responsive decrease of hemocyte aggregation against a control level of aggregation of 67.77%. Estuarine ecosystem of Sundarbans mangrove is characteristics of severe intra and interspecific struggle. In this dynamic ecosystem, the inhabitants participate in the struggle of niche occupation for survival and existence. Situation often leads to a state of acute predation and fight among animals. As a result, the animals experience physical wounding and loss of body fluid. Aggregation of hemocyte at wound site prevents the loss of blood and entry of microorganism and considered as an immunological response. Magnitude of aggregation was screened under the experimental exposure of sodium citrate, EDTA, heparin, fructose and mannose at different concentrations. Study was aimed to screen the effect of chelating agent, sugars and anticoagulants on aggregation. For all the chemicals screened, a drastic decrease in the occurrence of free cells were reported which is suggestive to role of these agents in the physiological process of hemocyte aggregation. Moreover exposure to arsenic may lead to gradual loss of blood cell homeostasis of mudcrab distributed in its natural habitat. Continuous exposure to toxic arsenic may lead to a population decline of mud crab and loss of biodiversity in this region.

Chapter 6 - The fat body of female *Periplaneta americana* is the main site of vitellogenins (Vgs) synthesis. The Vgs are large molecules synthesized in the fat body and encoded by two different *Vg* genes (*Vg1* and *Vg2*). The present study indicates that both *Vg* genes are represented in the genome by a single copy as revealed by Southern blot analysis.

The *Vg2* gene is predominantly expressed in the fat body cells and its expression preceded that of *Vg1* gene. The expression profile and level of *Vg* genes are found to be synchronized and up-regulated by the hemolymph JH titer and suppressed by the ecdysteroid titer during vitellogenesis, as revealed by RT-PCR and real time PCR analysis.

The differences in the expression levels of *Vg* genes, suggest the possible differences between the two genes in their responsiveness to the hemolymph JH titer and will pave the way toward further investigation on the JH responsive elements that regulate the expression of these two genes.

Chapter 7 - The carbohydrate expression of goblet cells in the intestine of pigs, rabbits and horses was characterized using lectin histochemistry. Paraffin embedded intestinal slices were incubated with the following biotinylated lectins: Con-A, WGA, DBA, SBA, PNA, RCA-1 and UEA-1. Except for some rectal goblet cells present in pigs and horses, no reactivity was found to Con-A. On the contrary, reactivity to DBA, WGA and RCA-1 was

heterogeneous in all the intestinal sections of the studied species. PNA reactivity was absent in the small intestine with the exception of few positive cells present in the horse. DBA did not bind to colonic goblet cells in the pig, while SBA did not bind the same cells in pigs and horses. RCA-1 staining was negative in the large intestine of the horse. The lectin binding with UEA-1 differed among different intestinal sections and species, remaining unstained in the small intestine of rabbits, colon of horses and rectum of pigs.

Our results led us to conclude that the lectin binding pattern of the intestinal goblet cells is characteristic of each animal species and shows variations related with the intestinal segment analyzed.

Chapter 8 - The relationship between ciliated fibroblast found in the periodontal ligament of rodent incisor teeth and eruption rate was studied under different eruption conditions, using an estimation of eruption rate and ultra structural aspects of primary cilia. Inferior left incisor teeth were cut for 24 days to increase the rate of eruption. During this period, the rate of eruption doubled; however, after this period, the number and frequency of ciliated fibroblasts was not altered. This lack of change does not necessarily signify that ciliated fibroblast do not have a direct or indirect role in the process of eruption, as suggested by the characteristics of the periodontal ligament structure and emerging functions for the primary cilium found in other tissues.

Chapter 9 - The Gulf of Maranhão is the largest and more important estuarine complex in the state of Maranhão, Brazil. São Marcos Bay located in the northeast region of the gulf, contains many small estuaries. The water circulation is due mainly to the tidal currents. This report describes the structure of the zooplankton community in relation to hydrographic conditions in the area, during the dry season/2000. The zooplankton samples were obtained in horizontal tows using a net with 200  $\mu\text{m}$  mesh size. Temperature, salinity, dissolved oxygen and chlorophyll-*a* were measured in water samples. Temperature and salinity were homogeneous throughout the water column. The highest zooplankton densities were observed in São Marcos Bay (up to 19,500 ind. $\text{m}^{-3}$ ), and the lowest were recorded in the Cachorros River and Coqueiros Strait (less than 8,000 ind. $\text{m}^{-3}$ ). Fifty zooplankton taxa were identified. The copepods, decapods and chaetognaths represented 99% of the total density. *Parvocalanus crassirostris*, *Acartia tonsa*, *Subeucalanus pileatus* and *Oithona hebes* were the most abundant species. *Parasagitta tenuis* and *Ferosagitta hispida* were also abundant. Zooplankton diversity varied from 1.33 to 1.71 bits.ind $^{-1}$ . The diversity index followed the variations in the density, and was similar to those found in other coastal systems of northeast Brazil.

Chapter 10 - Pill millipedes, belonging to the genus *Arthrosphaera* (Family: Sphaerotheriidae) are endemic giant millipedes found mainly in the upper moist soil horizons of the forests of Southern India and Sri Lanka. They are also known as rollers as they roll into sphere or pill on conglobation as defense behavior. The bulldozing shape of body of pill millipedes helps to till and mix the soil with litter fragments, transfer organic matter into soil through burrows and uplift organic matter from underneath to generate moder humus. They hibernate into the subsoil surface on the onset of summer and attain quiescent state. Their large body size confers several advantages like tolerance to elevated moisture stress and increase the burrowing ability. The large body size is also known to affect energy acquisition and utilization, and in turn the rates of organic matter decomposition. The order, Sphaerotheriida characterized by 13 body segments (tergites) and 21 pairs of walking legs. The tergites are strongly curved and horny. Each tergite (except for pygidium) is overlapped

slightly by the preceding tergite. In the process of conglobation, the head and ventral structures are completely covered by the pygidium, which helps in protection of the head and sense organs. Further, special closing ledges (locking carinae) are located on the inner side of the tergites. Stridulation (rubbing the last legs against the sides of last tergite generate sound) is an interesting phenomenon in *Arthrosphaera*. Stridulation behavior was seen usually in females and occasionally in male *Arthrosphaera* of the Western Ghats. The male stridulatory organ is the harp and in female it is washboard located at the anterior telopod and subanal plate respectively. Systematic descriptions of the order Sphaerotheriida are lacking, the current classification scheme lacks sufficient description and demands more morphological features to define monophyletic groups in *Arthrosphaera*. The current study, in addition to available morphological descriptions, emphasizes other essential features to revise the description of four species of *Arthrosphaera* (*Arthrosphaera dalyi*, *A. davisoni*, *A. fumosa* and *A. magna*) found in the Western Ghats along with their distribution pattern. The morphological description such as color, head, antennae, mouthparts, gnathochilarium, collum, thoracic shield, tergites, endotergum, anal shield, legs, female sexual features and male sexual features have been furnished.

Chapter 11 - The molecular phylogeny using mitochondrial DNA sequences provides valuable insights for wildlife conservation. However, the inference of phylogenies may be affected by the marker type as well as by the statistical model applied for data analysis. This study reports a comparative evaluation of neighbor-joining (NJ) and maximum-parsimony (MP) methods for phylogenetic inference among three *Oryx* species (*Oryx leucoryx*, *Oryx dammah* and *Oryx gazella*) as well as two closely related (same subfamily but different genera) outgroups including Addax and Roan, using 12S rRNA and 16S rRNA genes. We observed that the 12S rRNA based phylogenetic inference is unable to differentiate between the genus *Oryx* and Addax, regardless of the method used. However, the 16S rRNA gene segment accurately grouped all the five taxa using MP but not the NJ suggesting the possible superiority of the former method for phylogenetic analysis using similar data structure. This report also describes a stepwise protocol that can easily be followed for constructing the trees using PHYLIP software.

Chapter 12 - An organophosphate pesticide (Methyl parathion) was presented singly and also in combination with two different strains of aeromonads, once at a time, to *Channa punctatus* (Bloch). In case of combined treatments, adult fish were exposed to a sublethal dose (0.001ml/l) of methyl parathion coupled with challenge of sublethal dose ( $4 \times 10^7$  cfu/ml) of either *Aeromonas hydrophila* (MTCC 646) or *Aeromonas salmonicida* (MTCC 1522) strain injected intramuscularly. The bacterial challenge was initiated 5 days after the pesticidal exposure. The experiment comprised measurement of nine haematological parameters at 3, 7, 14, 21 and 28 days of intervals respectively. A steep increase in the total leucocytic population and corresponding leucocrit values were observed initially in combined treatments compared to fish groups exposed only with pesticides. However, all the treated groups generally showed a drop in lymphocyte population (lymphocytopenia) in the blood. Most of the erythrocytic parameters were depressed in fish groups treated only with pesticide while erythrocytic depression was less pronounced in combined treated groups.

Chapter 13 - Two new species each under the genus *Scutascirus* Shiba (Family: Cunaxidae) and *Gamasellodes* Atheis-Henriot (Family: Ascidae) collected from dried mushroom *Pleurotus ostreatus* in West Bengal, India are described and illustrated.

Chapter 14 - The dependency on noninvasive sources of DNA such as feces, hair and feathers for genetic analyses is particularly important in studies where conventional sampling is hard to practice, as in the case of wildlife animals. In view of earlier reports, we hereby address some important issues on merits, demerits and precautions related to the use of noninvasive methods of DNA sampling for molecular markers analysis.

Chapter 15 - Thyroid hormones promote normal growth and development and regulate a number of homeostatic functions in the body (Fujita, 1988). We hypothesized thyroid hormone may have an important role in maintaining the glucose homeostasis and morphology of cardiac muscle and skin in rat. We used methimazole 20 mg/kg and thyroxine 600 µg/kg body weight/day for 14 days intraperitoneally to the experimental groups of rat to induce hypo- and hyperthyroid like conditions respectively. Serum T<sub>3</sub>, T<sub>4</sub> hormones were measured by radioimmunoassay (RIA). Serum Glutamic Oxaloacetate Transaminase (SGOT) or Aspartate Amino Transferase (AST) measured by Spectrophotometric method. T<sub>3</sub> and T<sub>4</sub> levels were significantly reduced in hypothyroid rat but increased in hyperthyroid rat as expected. Thyroxine induced hyperthyroid rat exhibited hypertrophy in heart muscle and elevation of serum SGOT level.

Both hypo- and hyperthyroid induction changes the glucose homeostasis as reflected in Insulin Tolerance Test (ITT). The elevated blood glucose level returned to the basal level after 120 minutes of insulin challenge in control rat. But the same does not happened in hypo- and hyperthyroid rat. Consequently methimazole induced thyroid hormone deprivation also changes the skin architecture.

Chapter 16 - The Oncotic cytology appeared from a study developed by George Papanicolaou, with the objective to analyze the cervical squamous cells in all stages of the menstrual cycle periods, becoming a basic mean of prophylaxis for the cervical cancer. The technique consists of the analysis of the slides with cervical smear and of the detection of cellular alterations with pre-malignant or malignant characteristics. During the cytological analysis fungus, parasites, signals of inflammation, among others alterations can be found. The results are formatted following to Bethesda System, to Report of Papanicolaou or Brazilian Nomenclature for Report of Cervical Pathologies. This article had the goal to introduce a review about the characteristics of the cervical cancer and the techniques that are used to diagnostic it.

Chapter 17 - Haemocyte mediated non-self phagocytosis and generation of nitric oxide (NO) as reactive nitrogen intermediate were examined to investigate the acceptability of the parameters as biomarkers of sodium arsenite induced stress on *Lamellidens marginalis* (Mollusca; Bivalvia; Eulamellibranchiata), at sublethal concentrations. The animals suffered a remarkable immune compromise by the natural toxicant. Partial recovery was observed in the phagocytic potential and NO generation by the haemocytes of the animals over a period of thirty days. Quantitation of phagocytic efficiency and intrahaemocyte NO generation suggests reliability of the parameters as cellular biomarkers of sodium arsenite induced stress on freshwater molluscs.

Chapter 18 - Spawning of the killifish, *Aphanius dispar dispar*, have been studied in freshwater under controlled laboratory conditions. Two breeding systems, with cages and without cages, were used to study the fish spawning. Five replicates for each system were used. One male and two females were stocked in each tank for ten weeks. The total production of eggs in the tanks with cage system was 144 eggs and the average egg production per tank per week was 2.88 eggs. 59 fries were hatched and the hatchability rate

was 41%. No eggs were found in the tanks without cages probably because of predation by their parents. There was a highly significant ( $P < 0.0001$ ) difference between the two systems in egg production. It was also observed that the eggs were infected with fungus in the breeding and incubation tanks which reduced the eggs production and hatching rate, and caused fry mortality. The present study revealed that the tanks with cage system was the best method to prevent predation of fish on eggs and fry, and to increase fry production. The Ultra Violet (UV) system is recommended to disinfect water of breeding tanks and incubation jars to eliminate fungal infection of eggs. This will help in mass production of *A. dispar dispar* eggs and fry for restocking of freshwater bodies for biological conservation of the fish to be used for biological control of mosquito larvae and ornamental purpose.

Chapter 19 - Polysaccharides from *Phaffia rhodozyma* SDS-treated cell walls were successively obtained via cold water, hot water, and dimethylsulfoxide extractions. For this, processed yeast cells were grown under a physiological condition of active production of astaxanthin, its characteristic carotenoid metabolite. Afterwards, the extracted fractions were fractionated via freeze-thawing and precipitation with either Fehling reagent or Cetavlon. An  $\alpha$ -D-glucan and a heteropolysaccharide were isolated and structurally investigated using monosaccharide composition determination, methylation analysis, and COSY, TOCSY and HMQC techniques of  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR. The DMSO-extracted and Fehling-purified  $\alpha$ -D-glucan was determined to be a linear chain of  $\alpha$ -D-(1 $\rightarrow$ 3)-glucopyranosyl units. The hot water-extracted and Cetavlon-precipitated heteropolysaccharide showed a complex structure containing  $\alpha$ -D-(1 $\rightarrow$ 4)-mannopyranosyl units-based main chain with side chains containing  $\beta$ -D-(1 $\rightarrow$ 4)-xylopyranosyl units along with D-mannose, D-galactose, and D-glucose units. In addition, these monosaccharides are found to be non-reducing ends of the heteropolysaccharide as well. The cell wall extraction schedule also yielded several minor glucan fractions displaying a positive iodophilic reaction which was abolished after pretreatment with crystalline *Bacillus subtilis*  $\alpha$ -amylase. The knowledge of these cell wall features is of importance for the application of whole *P. rhodozyma* cells as single cell protein, improved digestibility, and astaxanthin release.

Chapter 20 - Zoonotic visceral leishmaniasis is a re-emerging disease caused by *L. infantum/L. chagasi*. The disease is transmitted by phlebotominae sand flies and dogs are the main urban reservoir of the parasite. In the natural history of *L. chagasi* infection in dog, named canine visceral leishmaniasis (CVL), following transmission, the parasites multiply in macrophages in the skin at the site of infection. From this localized cutaneous infection, the parasite can be disseminated via lymphatic or blood vessels, infecting macrophages of other organs such as the bone marrow, lymph node, liver and spleen, as well as the kidneys and gastrointestinal tract of the dog. In these naturally infected dogs, the outcome of CVL can vary considerably and probably correlates with the capacity of local skin cells to control parasite infection. CVL clinical manifestations are associated with distinct patterns of immune responses to *Leishmania* parasites. After infection, some dogs develop an impaired cell-mediated immune response that permits parasite dissemination and tissue lesion formation (symptomatic dogs), whereas others control parasite proliferation and dissemination to the different tissues (asymptomatic dogs). These infected dogs present positive lymphoproliferative assay *in vitro* or/and a positive skin test early in infection. However, as the disease progresses in susceptible dogs, these responses diminish. The cellular basis and mechanisms for the development of T-cell unresponsiveness in CVL are not

understood fully. In the present review it will be discussed the local immune response in skin, other affected organs, and cellular compartments as well as the possible mechanisms involved in dissemination of the *L. chagasi* infection in the dog model of VL.

Chapters 21 and 22 feature abstracts regarding animal science and issues by several different authors.