

BIOLOGICAL SCIENCES

1. BIRDS OF CENTRAL SIERRA MADRE, AURORA AND NORTHERN QUEZON: A PRELIMINARY REPORT

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The Sierra Madre mountain range is one of the world's biodiversity hotspots. Its continued survival for the future generations is constantly threatened by habitat destruction, particularly through logging. Yet, its biodiversity remains largely unexplored. This study was conducted to document the biodiversity of the least known areas in the Central Sierra Madre, in Aurora and Northern Quezon. Birds are the most studied group of vertebrates and are highly correlated with the health of ecosystems and thus a good indicator of habitat quality. Three watershed areas of the Central Sierra Madre, namely: along the Umiray River in Dingalan, Aurora and General Nakar, Quezon, in San Luis, and Dilasag, Aurora were surveyed and their biodiversity assessed. A combination of several survey techniques was used. These include: a) a two-kilometer line transect over 40 hours of observation; b) mist netting; and c) general audio visual observations. A total of 130 species of birds were identified, representing 23% of the total number of birds in the Philippines, 57% of which are Philippine endemic. There are 25 species of Luzon endemics, 13 species of which were recorded in the survey. In the lowland forest of Dingalan, Aurora and General Nakar, Quezon, 60 species of all Philippine bird species were recorded, 32 of which are Philippine endemic and 5 species are Luzon endemics. In San Luis, Aurora, 87 species were recorded, 46 of which are Philippine endemic and 10 species are Luzon endemic. In Dilasag, Aurora, 83 species of birds were recorded, 49 are Philippine endemic and 12 are Luzon endemic. Threatened species of hornbills, *Buceros hydrocorax* and *Penelopides manillae*, and the Luzon Bleeding heart pigeon, *Gallicolumba luzonica* were recorded in the area. A restricted range forest species of Pitta, *Pitta kochi*, thought to have been wiped out from its former range, was caught and released. It appeared to thrive in a lowland residual forest in the Amro watershed in Dilasag,

Aurora. The results of the avifaunal survey indicate that the Central Sierra Madre is home to an important group of wildlife, both endemic and threatened. This information warrants that the area should be included as one of the priorities for biodiversity conservation.

Keywords: birds, Sierra Madre, Aurora and Northern Quezon, vertebrate

2. BRACKISHWATER SHELLS ALONG THE EASTERN PART OF CATANDUANES: AN INVENTORY

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Brackishwater shells in the eastern part of Catanduanes, Bicol region were collected using quadrat techniques- by shoveling and hand picking- from April to December 1996. The shells were identified based on the Treatise of Invertebrate Paleontology adopted by malacologists and guide to Philippine Flora & Fauna by Garcia (1986). Population density and some physicochemical parameters were determined by instrumentation. These shells are very important source of protein. The study, a part of the project Inventory of Brackishwater Shells in Catanduanes, aimed to identify the economically important and available edible species found in this island province. Results of the study showed that the presence of the mollusks was dependent on the soil characteristics, i.e. sandy-silt -clayey; pH which ranged from 6.5 to 7.5 and salinity values, from 1 ppt to 30 ppt. The mollusks found in Eastern Part of Catanduanes belonged to class *Gastropoda* and *Bivalvia*.

Gastropoda had three orders; namely *Archeogastropoda*, *Mesogastropoda* and *Neogastropoda*. *Archeogastropoda* had one superfamily, *Trochacea* and one family *Trochidae* with two genera. *Mesogastropoda* had four superfamily: *Littorinaceae*, *Cerithiacea*, *Strombiacea* and *Cypraeaceae*. The three superfamily had one family and one genus each except for *Cerithiacea* which had two families *Cerithridae* and *Polaminadadea* which has six genera. *Order Neogastropoda* had two superfamilies *Buccinacea* and *Murizacea* both containing one genera.

Class Bivalvia had three orders: *Taxodonta*, *Isodonta* and *Heterodonta*. *Taxodonta* had one superfamily *Archacea* on family *Archidae* with two genera.

Isodonta had two superfamily: *Ptericea* and *Ostreacea*. *Ptericea* has three families: *Pteriidae*, *Isognomonidae* and *Placimidae*. *Pteriidae* had one genus *Isognomonidae* and *Placunidae* had two genera. *Ostreacea* had one family, *Crassostrea* and one genus.

Heterodonta had five superfamilies: *Dreissensiacea*, *Cardiacea*, *Veneracea*, *Mastracea* and *Telenacea*. Each of these superfamilies had one family with several genera.

Keywords: brakishwater shell, *Gastropoda*, *Bivalvia archeogastropoda*, *Mesogastropoda*, *Neogastropoda*, *Taxodonta*, *Isodonta*, *Heterodonta*

3. FOOD COMPOSITION OF SOME FISHES FROM LAKE TAAL, BATANGAS PROVINCE

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An analysis of the stomach content of Lake Taal fishes (obtained from the fish landing in Talisay wet market, Batangas, from January 1995 to March 1996) indicated that both plant and animal food are habitually eaten by *Therapon plumbeus* and *Ophiocara aporos*. Only animal food items were identified from the stomach of *Apogon thermalis*, *Glossogobius giurus*, and *Atherina forskali*. Many stomachs were found empty or contained unidentifiable digested matter. The most common recognizable food items based on the mean values of monthly percentages of occurrence for the five fishes were: *T. plumbeus*, plants (macrophytes); *O. aporos*, gastropods (*Vivipara anguillar*); *A. thermalis*, fish (*Atherina forskali*); and *A. forskali*, chironomid larvae.

Keywords: food composition, Lake Taal, Batangas, Taal fishes

4. COMPLETE LIFE CYCLE OF PHILIPPINE KING CRAB (*Scylla oceanica* DANA) UNDER CONTROLLED CONDITIONS

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Scylla oceanica (Dana), known as king crab ("alimango"), is the largest and fast growing portunid mud crab or mangrove crab in the Indo-West Pacific region. The demand for this species in international markets is very high. However, the production supply is limited due to low availability of seedstock from the wild.

To support the needs of the developing crab grow-out aquaculture in the Philippines, as potential replacement for declining prawn industry, a research to

understand the reproductive and developmental biology including the ecological requirements of the king crab, *Scylla oceanica*, in connection with the development of a less costly and efficient mudcrab hatchery technology in Southeast Asia was undertaken. Gravid spawners were bought live from the provinces of Iloilo, Aklan, Capiz and Antique and were reared in the broodstock tanks for the natural spawning. The development of eggs and larvae of *S. oceanica* was monitored periodically using Nikon microscope interfaced with video camera using Global Lab Image analysis computer software. The fecundity, duration of embryonic and larval development, distinguishing characteristics of larvae, and ecological behavior were closely documented. The larvae hatched within 9-12 dys of incubation depending upon the water temperature and salinity. It took the larvae 21-25 days to metamorphose into the first crab instar in culture medium with recorded physico-chemical parameters of pH 8.0-8.3, 32-34 ppt salinity, and 28-30°C. The early larval stages of mudcrabs were composed of five zoeae and one megalopa. The onset of sexual maturity (berried crabs) was observed after four months of culturing the hatchery produced crab larvae in the ponds. This is the first time that the life cycle of *S. oceanica* has been completed under controlled condition. The data obtained in incubating the eggs and culturing the larvae until the crablet stage became the bases in the development of the first commercial crab hatchery in the Philippines.

Keywords: *Scylla oceanica*, crab hatchery, crab larvae, developmental stages, life cycle

5. USE OF CERTAIN BACTERIA IN THE CONTROL OF *Vibrio harveyi* INFECTIONS IN PRAWNS

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Luminescent vibriosis caused by *Vibrio harveyi* has recently become a serious problem in the prawn industry. The use of antibiotics has been unsuccessful in controlling the disease mainly because of the limited efficacy of existing and readily available drugs, the possible development of resistant bacterial strains, and the limited tolerance of the shrimp larvae to the drugs. Lately, however, there were reports abroad on the occurrence in natural and rearing waters of certain beneficial bacteria capable of repressing the growth of pathogenic *Vibrio* species. Most often, these so-called 'probiotics' or 'biocontrol agents' increase the survival rates of cultured fish

and crustaceans. In this study, five of the 34 bacterial isolates from marine and freshwater environments were found to inhibit the growth of *Vibrio harveyi* when screened using *in vitro* assays. Results of biochemical tests run to initially characterize and identify these potential biocontrol agents suggested that the five strains belong to the genus *Pseudomonas* with the two of them identified positively as *Pseudomonas aeruginosa* using the API 20 NE test kit (bioMerieux). *In vivo* trials are underway to determine their effects on the survival rates of *Penaeus monodon* larvae, the culture of which is currently beset with luminiscent vibriosis. Tests to elucidate the vibriostatic factor produced by these strains are likewise being worked out. The results of this study are expected to generate relevant data on the possible application of these vibriostatic bacteria in controlling luminiscent vibriosis in hatcheries and in grow-out ponds.

Keywords: bacteria, *Vibrio harveyi*, *Pseudomonas* species, *Pseudomonas aeruginosa* probiotics, biocontrol, vibriosis, vibriostatic factor, prawns, *Penaeus monodon*

6. UTILIZATION OF A MARINE ALGA FOR HANDMADE PAPER MAKING

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A marine alga known in Bicol as “Mariwbariw” was tested for use in handmade paper making. Results of the study showed effective utilization of this marine alga as a raw material for paper making. Short fibers were greenish, but tending to slight crispness, tight and somewhat dense surface and had moderate absorbency. A high volume of fiber equivalent to 40% could be obtained per kilo of cooked dry leaf. The fiber imparted a distinct sheen to any fiber which it was mixed. The strength was weak due to fiber shortness. With these results, the use of “mariwbariw” seems practical to use due to its high yield and nonlaborious harvesting and cooking. The unusual appearance of the paper does seem to recommend for art uses. Fiber of this alga may be added to any pulp for decorative effects, thus the handmate paper produced in such combinations could be converted into various novelty items.

Keywords: Marine alga, handmade paper making, Bicol, “Mariwbariw”

7. VARIATIONS IN THE PHYCOBILIPROTEIN PROFILES OF SOME STRAINS OF CYANOBACTERIA ISOLATED FROM PHILIPPINE WATERS

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Taxonomic studies on cyanobacteria had been previously dominated by traditional descriptive analysis. An active field of experimental analysis involves the elucidation of the phycobiliprotein profiles of cyanobacteria. This information can add to the possible characters that can be used for algal classification, physiological, biochemical and ecological studies (Siegelman 1978). The phycobiliproteins were estimated spectrophotometrically using the method of Shibata *et al.* (1954) as modified by Shibata (1958). Results confirmed cyanobacteria absorb maximally at 620 nm. The type of phycobiliprotein present was believed to be phycocyanin in which the chromophore is phycocyanobilin, an open-chain tetrapyrrole (Glazer 1984).

Keywords: cyanobacteria, phycobiliprotein, spectrophotometry, phycocyanin, chromophore, phycocyanobilin, algal classification

8. SOME ECOLOGICAL, EXPLOITATION AND MANAGERIAL ASPECTS OF BATO RIVER

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Basic surveys to obtain preliminary information on ecological aspects of Bato River were conducted from March to October 1996. Bato River is the longest river of the province of Catanduanes, Bicol Region. The biotic factors examined were benthic animals and fish fauna; the abiotic factors include water, temperature, dissolved oxygen, pH, conductivity, and transparency.

Keywords: Bato River, Catanduanes, Bicol Region

9. ECOTOXICOLOGICAL ASSESSMENT OF SELECTED SITE IN THE MANILA BAY AREA

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The indiscriminate discharging of wastes into our rivers and oceans has led to a growing concern about the state of our aquatic ecosystems. Unfortunately, the monitoring of these habitats relies heavily on conventional procedures which are time-consuming, costly and require specialized training on the part of the test operators. In particular, only a few biological procedures (mainly microbiological) suited to Philippine conditions, had been developed or adopted. The study, therefore, aimed to determine the potential of the bioassay, the *Artemia Acute Toxicity Test* (Crustacea: Anostraca), for the assessment of the quality of waters in selected sites along Manila Bay. *Artemia* commonly known as the brine shrimp is a popular source of fish food in aquaculture. The bioassay with this organism is based on the determination of the concentration of the sample which kills 50% of the nauplii after 24-and/or 48 hours of exposure known as the 24-and/or 48-h LC⁵⁰. Given the climatic conditions prevailing in the country, the results of toxicity tests with the reference chemical (K₂Cr₂O₇) showed that the sensitivity of *Artemia* was within the same range as those obtained in other studies abroad (24h LC⁵⁰ = 26.63 - 30.99 mg/L; 95% C.L. = 22.29 - 31.81 and 26.50 - 36.24, respectively). The results of the monitoring study conducted in the various test sites from October to December (1996) showed that the 48-h toxicity was found to be site specific. Samples obtained from sites associated with waste generating activities exerted toxic effects on the nauplii resulting in high mortalities. The study showed that *Artemia* can be useful and sensitive test organism in basic and applied aquatic toxicology. The study, therefore, proposes the use of the *Artemia* Toxicity Test as a useful component in the battery of tests used in the monitoring of our marine and brackishwater habitats.

Keywords: brine shrimp, ecotoxicology, Manila Bay, *Artemia* Acute Toxicity Test, *Artemia*

10. SALT-INFLUENCED GROWTH, PROTEIN PROFILES AND NITROGENASE ACTIVITY OF A MICROALGA ISOLATED FROM PHILIPPINE WATERS

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Microalgae isolates, collected from various Philippine waters were screened for salt tolerance. This study attempted to establish cultures of microalgae that will be useful as biofertilizers in salt-intruded agricultural lands in the coastal regions. One of these isolates, viz., IB-Btgs O1 was observed to be heterocystous and was grown for 14 days in BG-11 medium modified variably with 60, 120, 180 mM NaCl. Growth response was determined through the cell count method and analysis of chlorophyll a levels. Electrophoretic analysis was adopted for soluble and membrane proteins. Nitrogenase activity was monitored using the acetylene reduction test. Results showed that the plotted growth response mean values derived through the cell count method were not significantly different. Growth responses based on chlorophyll a levels, however, revealed a significant reduction in growth for cultures growth in 180 mM NaCl. Electrophoretic profiles of the soluble proteins extracted from treated cells reflected decrease in intensity of a 35 kDa band at low salt concentrations and its disappearance at high salt concentrations. Profiles for membrane proteins revealed the disappearance of a 46 kDa band in lanes containing extracts from all the salt-treated cultures. Nitrogenase activity of cells grown in medium contain 60 mM Na Cl produced the highest amount of ethylene.

Keywords: microalgae, biofertilizers, salt-intruded agricultural lands, salt tolerance, growth, protein, nitrogenase

11. TERATISMS IN MILKFISH *Chanos chanos* REARED IN NAVOTAS-MALABON-TENEJEROS-TULLAHAN RIVER, MALABON, PHILIPPINES

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A two-year study on the Malabon ponds showed that water had acidic pH (6-3-6.6), low salinity (3.0-11.5 ppt) and high water temperature (17-29°C). Copper and

zinc levels of 0.065 mg/L and 0.083 mg/L respectively were higher than those set by the National Pollution Control Commission for class C water-water fir for aquaculture. Fish samples (19-30 cm length) showed high copper and zinc content in gills, kidney and liver. Metal content in these tissues were much higher than those in water. Copper values ranged from 0.55-479.8 mg/L; those of zinc were 11.5-22.3 mg/L. Lamellar fusion, clavate lamellae and epithelial lifting were the pervasive gill hispatologies. Other aberrations found were hyperplasia of the gill epithelium at the bases of the gill arches and increases mucus secretions. Hepatocytes exhibited lipid type vacuolations, dilated bile duct and sinusoids and presence of blood in the blood in the blood vessels. Nephropathology included dilation of the Bowman's space, presence of dense bodies and epithelial stratification in the tubules.

Keywords: teratism, milkfish, *Chanos chanos*, Malabon

12. BIOCHEMICAL AND PATHOLOGICAL CHANGES IN VERTEBRATE ORGANS EXPOSED TO SYNTHETIC AND NATURAL TOXICANTS

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Synthetic pyrethroids, considered as the newest major group of broad spectrum organic pesticides are now manufactured as alternatives to organochlorides, organophosphates and carbamates. A natural product, the neem kernel extract is widely used in some countries like India for antifertility purposes. Any damaging effects of the two toxicants on vertebrate tissues were analyzed separately. Changes in the levels of total lipids and total proteins of the fish brain were determined quantitatively by spectrophotometric assay every seventh day during the 28-day period. An electron microscope study was done on the mouse testes. Brain total lipids and total protein showed general decline. Ultrastructure analysis of the testes showed cellular damage. Synthetic pyrethroids and neem kernel extract have deleterious effects on vertebrate tissues.

Keywords: vertebrate organs, synthetic and natural toxicants, synthetic pyrethroids

13. EFFECTS OF SUBLETHAL LEVELS OF CADMIUM CHLORIDE ON THE BLOOD VALUES OF NILE TILAPIA

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The effects of sublethal levels of cadmium chloride on the blood values of Nile tilapia were studied. The 96 h LCD cadmium chloride to young *Oreochromis niloticus* (TL =60-115 mm, 4.0-17.7 g) was 53 mg/L. The toxicity of cadmium chloride was affected by the concentration of the metal and the pH of the water. Acute exposure of fish to 37 mg/L and 26.5 mg/L cadmium chloride at pH 7.1-7.8 and 5.0-5.8 induced changes in RBC count, Ht, and Hb concentration and leucocyte counts. Leucopenia mainly due to lymphopenia, and a tendency toward neutrophilia were observed in treated fish.

Keywords: Cadmium chloride, Nile Tilapia, sublethal levels, blood values

14. EMBRYOTOXICITY IN TILAPIA NILOTICA EXPOSED TO SUBLETHAL DOSES OF ORGANOTIN MOLLUSCICIDE

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Snail infestation in ricefields since 1989 has caused destruction of thousands of hectares of riceland. The use of organotins has successfully reduced golden snail infestation. However, reports on the adverse effects on farmers and freshwater organisms have led to the government ban on the use of these chemicals. This study identified tissue aberrations in the homeostatic organs in embryos. Ninety-day exposure of 21-day old embryos to sublethal doses of 0.2, 0.05 and 1.25 ug/L of trimethyl tin resulted to numerous aberrations. Kiney tubules exhibited cytoplasmic vacuolations, cellular debris and stratified epithelia that resulted to the occlusion of the lumina. The glomerulus showed hypertrophy and thick Bowman's capsule. Hepatocytes showed cytoplasmic vacuolation. Gills exhibited epithelial lifting and lamellar fusion.

Keywords: embryotoxicity, Tilapia nilotica, organotin molluscicide

**15. SELENIUM AS AQUATIC POLLUTANT: TOXICITIES
INDUCED IN THE MOUTH LINING AND PHARYNX OF
*Arius manilensis***

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Selenium is an essential trace element but is toxic in high concentrations to aquatic organisms as well as humans. It is available in the environment from volcanic sources, combustion of fossil fuels and effluents discharged from industrial processes. Waterborne selenium of 12 mg/L or greater has been considered hazardous to health and long-term survival of fish and wildlife. The effect of selenium on the mouth lining and pharynx of fish was assessed in this study since these areas are in contact with toxicants as water passes from the mouth to the gills. Representative areas from the mouth lining and pharynx of adult fish exposed to 11, 19, and 57 ppm (LD50-96hr) sodium selenate for 96 hours were processed for paraffin sectioning and examined under the light microscope. Thickening of the epidermal layer brought about by proliferation in the number of goblet cells, mucous cells, and epithelial cells were seen in the mouth lining of treated fish. Pharynx of selenium-treated fish exhibited sloughing of epithelial lining the tunica mucosa, epithelial lifting, and localization of mucous cells at the mucosal fold area, epithelial lifting and proliferation of mucous cells have been regraded as mechanisms to remove the metal stressor.

Keywords: Selenium, aquatic pollutant, *Arius manilensis*

**16. BIOMAKERS OF EXPOSURE TO NEEM SEED EXTRACT
IN THE TESTIS OF *Mus musculus***

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The neem tree (*Azadirachta indica*) has been reported to induce sterility. The antispermatogenic activity of neem extract on ICR mice (*Mus musculus*) was investigated after treatment with various doses for 35 days. Potential histological biomarkers of exposure to the extract are: cytoplasmic vacuoles, loosened peritubular tissues, defective cell-to-cell interconnections and multinucleated spermatophyte and spermatids.

Keywords: neem tree, *Azadirachta indica*, *Mus musculus*, testis

17. GILL LAMELLAR RESPONSES IN *Oreochromis niloticus* LARVAE AND FRY ACCLIMATIZED IN ARTIFICIAL SEAWATER

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Tilapias are essentially freshwater fish, although saltwater living species had been reported. A problem in tilapia aquaculture is the lack of suitable natural freshwater venues for the culture. The prospect of successful culture in seawater is one way of addressing this problem. Survival and growth of fish in seawater have been positively linked to the osmoregulatory mechanisms; and gills play a crucial role in osmoregulatory mechanism. This study evaluated the gill lamellar responses of *Oreochromis niloticus* of various ages (1, 11, 21, 31, and 41 days posthatching) that had been acclimated from freshwater to saltwater of 37 ppt. Lamellar samples obtained were processed for paraffin sectioning and light microscopy. Lamellar sections showed significant enlargement of chloride cells for seawater-adapted groups. Epithelial lifting and hyperplasia of interlamellar epithelium were also observed. Damage to the epithelium caused the loss of cellular integrity of the lamella. Significant correlation between chloride cell enlargement were noted indicating the major role of chloride cell modification in seawater adaptation.

Keywords: Gill lamellar responses, *Oreochromis niloticus* larvae, artificial seawater

18. PROTECTION OF FISH BY LIMING AGENT AGAINST COPPER-INDUCED TOXICITY

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The extent of lipid peroxidation in the hepatocytes of the African tilapia, *Oreochromis mossambicus* during short-term exposures of the fish to copper sulfate (4 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with and without the addition of liming agent, calcium carbonate (50 mg/L CaCO_3), was determined by the thiobarbituric acid reaction test (TBA Method). Hepatic Malondialdehyde levels were assayed at 24, 48, 72, 96 and 168 hours of exposure to the chemicals as well as to CaCO_3 alone and carbon tetrachloride (0.25 ml/L CCl_4), a known hepatotoxicant provided by CaCO_3 . The findings also presented implications and the use of calcium carbonate for improvement of metal-contaminated waters for fishery purposes.

Keywords: liming agent, copper-induced toxicity, African tilapia, *Oreochromis mossambicus*

**19. IN VITRO STUDIES ON THE ACTIVITY OF
COMMERCIAL PROBIOTICS AGAINST
Vibrio harveyi and *Vibrio anguillarum***

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The effectiveness of four commercially available probiotics against *Vibrio harveyi* and *Vibrio anguillarum* was examined under three *in vitro* procedures. In the parallel-streak plat technique, only Probiotic A exhibited dominant growth over *Vibrio harveyi* and *Vibrio anguillarum*. In the cross-streaking procedure, there was no apparent macroscopic inhibition or overgrowth of the two *Vibrio* species by any of the probiotics. In the second procedure where equal volumes of a probiotic and a *Vibrio* species were mixed, there were changes in the viable counts of the *Vibrio* species when monitored for 72 hours. At dilution 10^{-6} , *V. harveyi* became undetectable after 48 hours when mixed with Probiotic D and after 72 hours when mixed with Probiotic A or with Probiotic C. On the other hand, only Probiotic A and Probiotic C were found to be effective in repressing the growth of *V. anguillarum*. Results of the experiments suggest that Probiotic A and Probiotic C are effective in repressing the growth of both species while Probiotic D is effective for *V. harveyi* only. Probiotic B was found to be ineffective for both species. This study provides local prawn farmers with relevant information that will guide them in choosing the appropriate probiotics used to control prawn diseases.

Keywords: probiotics, biocontrol, *Vibrio harveyi*, *Vibrio anguillarum*, prawns

20. INSECT ANTIFEEDANT ACTIVITIES OF SOME PHILIPPINE PLANTS

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The ethanolic extracts of 10 Philippine plants were tested for their antifeedant activities against ants, cockroaches, and houseflies. Using a 10% sucrose solution as food base, the extracts of *Annona reticula*, *Caesalpinia pulcherrina*, *Curcuma longa*, *Mangifera indica*, *Odontonema stricta*, *Pandanus odoratissimus*, *Premna odorata*, *Tinospora rumphii* and *Tithonia diversifolia* were active against ants. On the other hand, the extracts of *Odontonema stricta*, *Pandanus odoratissimus* and *Tithonia diversifolia* exhibited antifeedant activities against cockroaches using a starchy food base (pre-weighted pieces of cake). The extracts of *Mangifera indica* and *Odontonema stricta* prevented houseflies from feeding on a 10% brown sugar solution.

Keywords: insect antifeedants, *Annona reticulata*, *Caesalpinia pulcherrina*, *Curcuma longa*, *Mangifera indica*, *Momordica charantia*, *Odontonema stricta*, *Pandanus odoratissimus*, *Premna odorata*, *Tinospora rumphii*, *Tithonia diversifolia*

21. PRELIMINARY STUDIES ON LECTINS FROM *Dolichos lablab* (BATAO)

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Crude extracts from *Dolichos lablab* seeds tested showed positive for lectin activity using hemagglutination assay with human blood types A, B, AB and O. Strongest activity was observed with blood type AB. Sugar inhibition assays demonstrated that the activity was inhibited by mannose and galNAc. The extracts were passed through mannose and galNAc Sepharose. Native PAGE and gel filtration of affinity purified extracts showed the lectin to have a molecular weight slightly above 30 kD. Affinity purified extracts were observed to be stable up to 60°C. The study also demonstrated that agglutination activity was lowest at pH 4.0 and highest at pH 8.0 and 9.0 when dialyzed against EDTA, agglutinating activity was abrogated. Upon dialyzing against buffer with Ca ++, Mg++, Mn++ and Zu++ activity was

restored indicating a nonspecific dependence on cations. Presently, the test for lectin's ability to induce respiratory burst in human neutrophil is yielding promising results. Respiratory burst is characterized by the release of superoxide anions, an important aspect during infections and in antitumor activities. Other plant lectins have been found to induce respiratory burst. Thus, an investigation on similar potentials in lectins from *Dolichos lablab* offers interesting possibilities.

Keywords: lectins, "batao", *Dolichos lablab*

22. THE USE OF RECOMBINANT DNA TECHNOLOGY IN RECOVERING A NATURAL PRODUCT FROM A LUMINOUS BACTERIUM

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A lot of gram-negative polarly flagellated bacteria possess the ability to emit light. They are called luminiscent/luminous bacteria. Most of them are marine forms living freely or symbiotically. Luminescence is brought about by the enzyme luciferase, a long chain aliphatic aldehyde flavin mononucleotide (FMN) and oxygen. Recombinant DNA technology plays a tremendous role in the recovery of natural products. This technique was employed in *Xenorhabdus luminescens*, a strain Hm primary, a luminous bacterium that produces a red pigment. This offers the possible exploration of its pigment as an alternative food dye in the industry. This paper presents studies about the genetics of pigment formation. The pigment genes were cloned and expressed in *Escherichia coli*; the recombinant plasmid (pUC 18+ the pigment genes) named pCGLS 100 was screened and isolated and a partial restriction map was constructed. This fragment was translated and subsequently used as a probe for other species.

Keywords: recombinant DNA, Natural products, *Xenorhabdus*. luminous dye, genetics cloning, plasmid, nick translation

**23. AMPLIFICATION AND MOLECULAR CLONING OF
TENEBROSIN GENES FROM THE SEA ANEMONE,
*Actinia tenebrosa***

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Using degenerate oligonucleotide PCR primers TNC-2 and TNC-3, and genomic DNA as template, a 306 bp amplified band was cloned and sequenced by means of standard protocols. Sequencing of this amplicon yielded two very similar sequences for tenebrosin. The first one was confirmed to be that of tenebrosin-C from the known primary structure of the protein while the second sequence was designated as tenebrosin-X. The sequences, when translated, were found to represent amino acid residues 38 to 139 of tenebrosin. There were no introns, no cysteine residues and sequence comparison revealed changes in some codons leading to both replaced and silent amino acid substitutions. Analysis of the compared sequences disclosed that ten codon changes led to amino acid substitutions most of which were conservative. Five codon changes did not alter the corresponding amino acid. Any of the two-cloned sequences was a good probe to search for and clone other tenebrosins from the same organism or from other marine organisms which produced related toxins or cytolytins. Toxins are important from a biotechnological point of view because they are potential sources of new biopesticides, pharmacological drugs or immunotoxins as cancer chemotherapeutic agents.

Keywords: cloning, molecular cloning, tenebrosin genes, sea anemone, *Actinia tenebrosa*

**24. PHYLOGENY OF CONE SNAILS BY NONRADIOACTIVE
DNA SEQUENCING**

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Venoms from *Conus* snails contain a wide variety of small peptides which are currently used as pharmacological tools for neuroscience and drug development. The *Conus* phylogeny served as a guide for choosing the particular snail to be investigated for a specific peptide. In this study, the DNA was extracted from hepatopancreas of *Conus* species using a rapid technique, a 168 technique using

silver staining for DNA detection. The resulting sequences together with previously known sequences for other species, were used to construct phylogenetic trees.

Keywords: cone snails, DNA, DNA sequencing, PCR peptides, phylogeny and silver staining

25. MITOCHODRIAL DNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM AS GENETIC MARKERS IN YELLOWFIN TUNA (*Thunnus albacares*)

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The tuna industry is one of the major dollar earners for the Philippine economy. This resource, however, is threatened from exploitation due to inadequate specific management and conservation measures. Genetic population structure studies of Philippine tuna can serve as guiding reference in establishing fishery management policies for sustainable development. This research aims to develop restriction fragment length polymorphism (RFLPs) from the 16S rRNA segment of mitochondrial DNA as genetic markers to analyze yellowfin tuna (*Thunnus albacares*) populations in the Philippines. The total DNA of yellowfin tuna was extracted from liver and muscle tissue through a rapid one-step extraction (ROSE). The 16S rRNA segments were amplified using the polymerase chain reaction (PCR). Preliminary data indicated monomorphism results for 11 samples from five Luzon provinces as shown by three out of six restriction enzymes tested.

Keywords: yellowfin tuna, ROSE, PCR, mitochondrial DNA, 16S rRNA, RFLP

26. PCR-RFLPs OF THE mtDNA D-LOOP and 16s rRNA GENE SEGMENTS IN MILKFISH, *Chanos chanos*

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The Polymerase Chain Reaction (PCR) is a rapid and simple method of amplifying specific genes or DNA sequences, which can be applied in the study of genetic

variability of marine species such as milkfish. PCR amplification conveniently provides enough copies of the mt DNA D-loop region 16s rRNA gene segment for subsequent restriction with enzymes for the detection of variability in the form of restriction fragment length polymorphisms (RFLPs). RFLPs which characterize certain populations or species can be used as markers in the study of genetic population structure. In this study, a 2Kb fragment of the mtDNA D-loop region and a 600 bp fragment of the 16s rRNA gene was amplified from milkfish DNA. Results of restriction digestion of both PCR products using 12 enzymes showed polymorphic RFLPs in the D-loop region but no variation in the 16s rRNA segment. The data affirm the utility of D-loop RFLPs as genetic markers for milkfish population studies.

Keywords: *Chanos chanos*, milkfish, polymerase chain reaction, restriction fragment length

27. DNA VARIATION AND GENETIC RELATIONSHIP AMONG FILIPINO TRIBES AND MAJOR/MINOR GROUPS

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The Filipino population is so diverse consisting of more than 100 morphologically and/or linguistically distinct groups. This diversity may be reflected as DNA variation. Data on DNA variation would provide us insights into the genetic relationships among Filipino groups and help resolve issues about our origin. But a more practical application of these data is in constructing the genetic databases needed in DNA typing for forensics. The country's crime agencies, the National Bureau of Investigation, and the Philippine National Police are developing their capability to use DNA typing for forensics. The genetic databases are needed to properly interpret DNA typing results. Human blood/saliva was collected from volunteers with informed consent and hospitals/blood banks. DNA was extracted and analysed for variation in known, discrete regions. The patterns of differences found among population groups were compared statistically. Initial results from Metro Manila population sample showed that the locus D1S80 can be used for forensic DNA typing as well as for analysing genetic variation.

Keywords: DNA variation, genetic relationship, forensic DNA typing

28. MOLECULAR MARKERS FOR CLONAL FINGERPRINTING IN BANANA AND MANGO

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The more recent development in DNA markers has produced abundant and highly variable new markers that may overcome the limitations of traditional markers used in varietal identification in banana and mango. The study classified banana and mango cultivars using PCR-based random amplified polymorphic DNA (RAPD) and microsatellite markers. Genomic DNA from immature leaf samples of banana and mango were amplified by PCR using a set of single primers of 10 nucleotides or primer pairs of *Musa* sequence-tagged microsatellite sites. Protocols for RAPD analysis for mango and banana were optimized. Preliminary results in both crops indicate that RAPD analysis can reveal significant differences among cultivars tested.

Keywords: RAPD, DNA markers, microsatellites, mango, banana, *Musa*, *Mangifera indica*

29. OPTIMIZATION OF PCR CONDITIONS FOR THE RAPD ANALYSIS OF SEVEN TREE SPECIES

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The ability to differentiate between cultivars is necessary for breeding work and is just a step towards the eventual genetic engineering of the coconut (*Cocos nucifera* L.) durian (*Durio zibethinus* Murr.) ilang-ilang (*Cananga odorata* Hook.f.) narra (*Pterocarpus indicus*), chico (*Manikara indica* (L.) van Royen), and mango (*Mangifera indica* L.). One method of differentiating cultivars is via RAPD (Random Amplified Polymorphic DNA). But before RAPDs can be used in these three species, it is necessary to optimize the PCR conditions.

High-MW genomic DNA was extracted from the leaves of said tree species using a modified Doyle & Doyle (1990) CTAB Method. Eighteen arbitrary decamers

were then screened and used in the optimization of the PCR conditions. DNA, primer and MgCl₂ concentrations, and annealing temperature were varied to obtain optimum results. Clear and distinct RAPD bands were generated using about 0.8 ng/μl DNA, 0.3 μM primer, and 3mM MgCl₂ in the PCR reaction mixture. The PCR program which gave optimum results (with Amplitron I thermal cycler) were as follows: a 5 min initial dwell at 94°C, followed by 45 cycles of 1 min at 92°C, 1 min at 34-35°C (depending upon primer) and 2 min at 72°C, and a final dwell of 10 min at 72°C.

Primers that gave positive results can then be considered potential RAPD markers for the said tree species. Preliminary tests of said primers generated polymorphism with chico.

Keywords: PCR, RAPD, *Cocos nucifera*, *Durio zibethinus*, *Cananga odorata*, *Pterocarpus indicus*, *Manikara indica*, *Mangifera indica*, DNA polymorphism

30. NOVEL SOMACLONAL VARIANTS REGENERATED FROM CALLUS OF RICE *Oryza sativa* L.

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Genotypic variability in the occurrence of somaclonal variation in regenerated plants and progenies of regenerated plants of some Philippine rice varieties was evaluated. Mature seeds of varieties Elon-elon, C-4, PARC-3 and Dinorado were induced to undergo callus formation and plant regeneration via somatic embryogenesis *in vitro* on Murashige and Skoog's basal medium supplemented with auxins and cytokinins. In the first generation of regenerated plants, significant variations for plant height, lengths of internode, flag leaf and panicle, number of spikelets, and percentage fertility were observed. Although negative changes in some agronomic traits appeared, two novel somaclonal variants in variety Dinorado having significantly increased panicle length, number of spikelets, increased fertility and reduced plant height due to increase in internode length were isolated and found to breed through for three generations. This improvement in character expression in two isolated lines of Dinorado showed that somaclonal variation is a promising biotechnological technique for crop improvement.

Keywords: auxins, cytokinins, callus, *in vitro* culture, plant regeneration, progeny, *Oryza sativa*, somaclonal variation, somatic embryogenesis, tissue culture

31. TRANSFORMATION OF *Zea mays* LINNAEUS (LINNAEUS) INBRED LINES IN THE PHILIPPINES VIA PARTICLE BOMBARDMENT: A STATUS REPORT

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Improving crop varieties through transformation process has been one of the most promising applications of biotechnology. While this technology has been successfully documented in developed countries, the Philippines has yet to produce its first transgenic plants. This study is part of an overall effort to improve maize production through genetic engineering of genes conferring effective resistance to the insect pest, Asiatic corn borer. This work is the first attempt to transform Philippine-bred maize lines under local conditions. The modest gains and problems encountered in the conduct of this type of research in the Philippines is presented. Recommendations to better improve the prospects of utilization of this technology are discussed.

Keywords: transformation, particle bombardment, genetic engineering, maize, *gus* gene, herbicide resistance, Asiatic cornborer

32. MOLECULAR CHARACTERIZATION OF LOCAL *Bacillus thuringiensis* ISOLATES EFFECTIVE AGAINST ASIATIC CORNBORER *Ostrinia furnacalis* (G.) AND DIAMONDBACK MOTH, *Plutella xylostella* LINN.

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Out of of 268 local isolates of *Bacillus thuringiensis* screened against asiatic cornborer (ACB), *Ostrinia furnacalis* and diamondback moth (DBM), *Plutella xylostella*, three were selected. LEP-20 and LEP-3(1) showed high toxicity to ACB but moderately toxic to DBM while LEP-#13 showed high toxicity to DBM but moderately toxic to ACB.

Analysis of solubilized insecticidal crystal protein revealed the presence of proposed protoxin and toxin subunits with molecular mass of about 130 and 65 kDA

proteins for LEP-20 and LEP-3(1) and an additional 140 kDa protein for LEP-#13. Plasmid DNA profile showed that only LEP#13 is different from LEP-20 and LEP3(1). This is possible since LEP-20 and LEP-3(1) was serotypically identified as *B. thuringiensis* subsp. *galleriae* while LEP-20 and LEP-3(1) were *B. thuringiensis* subsp. *kurstaki*. Scanning electron microscopy presented the different shapes of crystals within each isolate, PCR reveals the presence of cry1Aa, cry1Ab and cry1Ac genes in LEP-3(1) and LEP-20 while cry1Aa, cry1Ab and cry1B genes in LEP-#13.

33. DELTA-ENDOTOXINS OF LOCAL *Bacillus thuringiensis* STRAINS: BIOCHEMICAL AND TOXICITY STUDIES AGAINST THE ASIATIC CORN BORER *Ostrinia furnacalis*

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Awareness of the harmful environmental effects of chemical pesticides has led to the search for novel, pest-specific insecticides such as the crystal protein produced by a soil microorganism, *Bacillus thuringiensis* (Bt), against pests such as the Asiatic corn borer (ACB). The biochemical and toxicological properties of the crystal protein/s of the local BT strain LEP 20 in preparation to incorporating their respective Bt genes to corn were studied. The production and solubilization of the protoxin crystals fractionated the protoxin and bioassayed the fractions against the ACB. Sepharose 2B gel chromatography gave a broad band (139 and 140 kD by SDS-PAGE) with three peaks which showed low toxicity. Preparative SDS-PAGE generated major of 133 to 139 kD which had a lower toxicity (13.3%) compared with the crude sample (46.7%). Ultrafiltration yielded high-MW proteins (>100kD) possessing high toxicity (100%) while the low molecular weight fraction (>50kD>MW>10kD) had slight toxicity (13.3%). Specific staining (PAS) indicated that the protein is glycosylated. The ACB exhibited different susceptibilities to protoxins from other local Bt strains and one commercial strain. These results indicate that toxicity against the ACB can be attributed to more than one active fraction and their synergistic interactions.

Keywords: Crystal; endotoxin, *Bacillus thuringiensis*, *Ostrinia furnacalis*, corn, toxicity studies, gel chromatography, preparative electrophoresis, ultrafiltration.

**34. BIOMODIFICATION OF COCONUT (*Cocos nucifera*) Linn.
OIL AND NON-LAURIC OILS FOR THE PRODUCTION OF
SPECIALTY FATS AND OILS**

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With the great demand for vegetable fats and oils today, coconut oil, the most abundant lauric oil, is a much desired article because of its unusual properties compared to other cooking fats and oils. However, it is susceptible to hydrolysis and blends badly with nonlauric oil foam used in deep fat frying. The present knowledge on foaming during deep fat frying is limited. This research investigated the cause of foaming from the triglyceride distribution pattern of the oils, modified the triglyceride structures through lipase-catalyzed interesterification, developed a technology for the production of coconut and nonlauric blends through enzymatic interesterification, which could provide high value and better health benefits. Using three nonlauric oils namely: cashew, fish and sesame, the foaming activity of their straight blends (0 hr) with coconut oil in different ratios was observed to foam considerably. Lipase-catalyzed interesterification of the oils was carried out and foaming activity of the interesterified blends was decreased by 50%. Generally, modification by the enzyme of the oils decreased foaming. This was found to be accompanied by a significant change in the triglyceride profile which was determined by the equivalent carbon number.

Keywords: lipase-catalyzed, interesterification, triglycerides, foaming, fatty acids, lauric oils, non-lauric oils, enzyme, equivalent carbon number

