

BIOLOGICAL SCIENCES DIVISION

TERATOGENICITY OF ORGANOPHOSPHATE PESTICIDE ON *Tilapia nilotica*

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Since the ban on organochlorines, organophosphates have been extensively used in agriculture as pesticides in order to increase crop production. However, their indiscriminate use has resulted in the presence of their residues in rivers and lakes. This study evaluates the effect of commercial grade malathion [S-(1, 2-dicarbethoxyethyl)-O,O-dimethyldithiophosphate] EC 57 on *Tilapia nilotica* embryos. Results of this study would provide (1) baseline data on pesticide effects on nonpests, particularly on fishes, and (2) information for government agencies that will guide them in the formulation of policies on pesticide use.

Day-10 post hatching embryos that were exposed for 60 days to sublethal doses of 0.3 mg/L malathion exhibited various aberrations. Brain defects included nuclear blebs, swollen short-stranded endoplast, and degranulated endoplasmic reticulum. The cytoplasm exhibited an electron-light background and extensive vacuolations. The gonads of pesticide-treated embryos showed delay in ovarian differentiation and cavitation in older embryos. The notochord lost its secondary sheath and was significantly larger than that in the control. The gills showed epithelial lifting and mucus secretions.

**HEMATOLOGICAL RESPONSE OF TILAPIA
(*Oreochromis niloticus*) TO ZINC
AND MERCURY EXPOSURE**

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Tilapia fingerlings of 4-5 cm total length were exposed to 0, 2, 5, and 10 ppm zinc sulfate for 30, 60, and 90 days while adult fish of 15-20 cm total length were exposed to 0.05 mg/L mercuric acetate for 7 days. Anisocytosis and poikilocytosis which are frequently observed in most anemias were noted in blood smears of zinc-treated fish. Hemoglobin level was reduced in fish reared at 10 ppm for 90 days while hematocrit was increased in fish reared at 2, 5, and 10 ppm in all treatment periods. An increase in hematocrit was correlated with fish response to hypoxemia to compensate the oxygen-carrying capacity of the circulating blood. Anemia is attributed to reduced hemoglobin content of erythrocytes. In mercury-treated fish, hemoglobin, RBC count, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin content decreased significantly while a significant increase in number of immature erythrocytes was observed. Abnormalities in erythrocyte morphology such as heart-shaped cells, triangular cells, and irregularly contracted cells were noted. Atomic absorption spectrophotometry revealed elevated mercury levels in the head and kidney.

**THE ECOLOGY OF THE MIGRATORY LOCUSTS,
Locusta migratoria manilensis MEYEN**

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The migratory locust is an endemic pest of the Philippines. Infestations have been recorded as early as the 1500s by Augustinian friars. However, until now there is a dearth of information as far as the ecology of this pest in the Philippines is concerned.

Recent findings regarding flora and fauna associated with locusts in breeding areas; updated morphometrics of solitary and gregarious phases; new behavioral

observations; correlates of soil properties and egg-laying; and recent factors about migration of locusts in Central Luzon and other parts of Luzon are discussed.

INCIDENCE OF GILL AND INTESTINAL PARASITES FROM THREE SPECIES OF SCOMBRID FISHES

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Eleven species of parasites belonging to different genera were recovered from the gills and intestinal tracts of three species of scombrid fishes obtained from a local wet market. The parasites were 2 Digenea, 5 Monogenea, 2 Copepoda, 2 Nematoda, and 1 Acanthocephala. The digenetic trematode, *Lecithocladium* sp. Luhe, 1901 was the most prevalent parasite recovered from *Rastrelliger brachysomus* (Bleeker) (100%) and *R. chrysozonus* (Rupell) (96.7%). In *Scomberomorus commerson* (Lacepede), the highest incidence of infection was due to the gill fluke, *Scomberocotyle* sp. (100%). Mean intensity of the infection in *Rastrelliger* spp. was the highest for the same parasite (*Lecithocladium* sp.); in *S. commerson*, the highest mean intensity of infection was shown by the nematode *Spinitectus* sp. Fourment, 1883.

MOLECULAR CHARACTERIZATION OF *Bacillus thuringiensis* ISOLATES HIGHLY TOXIC TO YELLOW STEM BORER

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Bacillus thuringiensis (*Bt*) is a rod-shaped, Gram-positive bacterium that produces insecticidal crystal proteins during sporulation. The toxin proteins CryI_{Ac}, CryI_C, and CryII_A have been found to be most effective against the rice yellow stem borer (YSB), *Scirpophaga incertulas*. Close to a thousand representative *Bt* isolates from the collection of the International Rice Research Institute (IRRI) have been bioassayed for toxicity to YSB. Eighteen *Bt* isolates that showed consistently high

toxicity, comparable to the standard *Bt* strain, *Bt* strain HD-73 which produces only CryIAC in repeated bioassays, were chosen for more detailed evaluation. A series of PCR primers specific to ten different *cry* genes were used to determine the group/subgroup of the toxin present in each isolate. Three *Bt* isolates harbored only one *cry* gene each while the other fifteen contained at least three *cry* genes. Immunological analysis using an antibody to the CryIIA toxin indicated that the *cryIIA* gene may be "silent" in some isolates. From the combined PCR and immunological analyses, *Bt* isolates 182, 222, 271, and 734 stand out as particularly interesting. They are of high toxicity to YSB yet do not have *cryIAC*, *cryIC*, or *cryIIA*. It is possible that these four isolates contain novel *cry* genes of potential use in rice and our initial cloning efforts will be focused on them.

DNA FINGERPRINTING OF THE BACTERIAL BLIGHT PATHOGEN OF RICE USING PCR-BASED METHODS

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Repetitive DNA sequences are pervasive in the genomes of most organisms. Their conserved nature and variable distribution in the genome allows the detection of many polymorphisms that are useful in differentiating individual strains. IS112 is a high-copy number of repetitive elements isolated from the bacterial blight pathogen. A primer pair complementary to IS112 was used to fingerprint a set of 71 *x oryzae* pv. *oryzae* strains using PCR and ligation-mediated PCR (LMPCR). To allow amplification of long DNA fragments, we amended standard amplification conditions by increasing the pH, adding dimethylsulfoxide, decreasing denaturation time, and increasing extension time. Both PCR and LMPCR revealed useful polymorphism among individual strains and allowed their genetic relationships to be efficiently deduced. Good correlation was found between the major clusters obtained by the two methods. The bootstrap values, which indicate the strength of the groupings, are generally high for the major clusters produced, with PCR giving the most robust clusters. Overall, the PCR method was most efficient in terms of simplicity and economy. The PCR and LMPCR methods were used to characterize field isolates of the bacterial blight pathogen. Similar groupings of the isolates were obtained. Further, these groupings corresponded well to those derived using restriction fragment length polymorphism.

LITTORAL BENTHIC FAUNA OF LAKE TAAL, BATANGAS

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Core samples were collected from six stations in the shore areas of Lake Taal from August 1994 to January, 1996. The animals recovered from the samples and their average densities per liter of sample were: gastropod molluscs (39.1), pelecypod molluscs (4.1), annelids (8.9), crustaceans (18.3), and insect larvae (1.7). The predominant species of gastropods, annelids, and crustaceans found were *Thiara granifera*, *Dero* sp., and *Sphaeroma* sp., respectively. The pelecypods belonged to one species, *Corbicula manilensis*. The insect larvae were chironomids. Variations were observed in the spatial and temporal distribution of the benthic animals. Higher densities were observed in sampling sites with moderate to abundant aquatic vegetation and during the months of April to July.

THE DIVERSITY AND ABUNDANCE OF THE ZOOPLANKTON IN LA MESA DAM, METRO MANILA

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The zooplankton community of La Mesa Dam, the storage reservoir for pre-treated drinking water in Metro Manila, exhibits (i) remarkable species diversity in spite of its small volume and surface area and (ii) little or no variation in the composite densities of the Cladocera and Rotifera.

Samples collected in the littoral and limnetic zones of La Mesa Dam beginning in 1975 yielded 32 species of Rotifera, 22 spp. of Cladocera, and 8 spp. of Copepoda. In 1990-1991, the numbers of Cladocera and Rotifera varied very little in spite of the seasonal variations in the level of the water of the dam. Plankton densities showed no correlation with some water quality parameters that were monitored.

**THE DEVELOPMENTAL BIOLOGY OF THE
ANGEL WING CLAM (*Pholas orientalis*), AN ENDANGERED
SPECIES OF MOLLUSK NATIVE TO PANAY AND
NEGROS ISLANDS***

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The angel wing clam (*Pholas orientalis*), or more popularly known as “diwal” in the Hiligaynon dialect, is a large mud-burrowing bivalve native to the tidal mud flats of Panay and Negros islands. The name “diwal” is derived from the habit of the clam in extending its long siphon out of the mud when feeding on minute plankton in the seawater column. Because of its large size, juiciness, and sweet taste, “diwal” has been one of the most expensive and most sought after edible bivalves in the Philippines. Unfortunately, such exceptional characteristics make it not only a local delicacy but also a much sought after export commodity.

Diwal was most abundant in Roxas City (notably in Barangay Bara) in the Capiz Province of Panay, and in the municipalities of Pontevedra and Hinigiran in Negros Occidental. However, there has been a dramatic decline in the population of diwal in both islands beginning in 1990. Once the pride of Roxas City, the sea food capital of the Philippines, diwal is now nearly extinct in Barangay Bara and rarely found elsewhere. The reason for this phenomenon remains unknown. The most likely causes are the same which caused destruction of most other marine life forms, namely over-harvesting, pollution, siltation, and destruction of the ecosystem. Understanding the biological conditions which affect the life cycle of diwal is difficult because there have been no published scientific data on its biology and ecology.

A research program was initiated to establish the development biology of diwal as part of the long term plan of rehabilitating the coastal environment. Adult diwal were successfully spawned naturally in the laboratory. The development of the embryos and larvae was monitored using a Nikon microscope interfaced with a

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video camera and using Global Lab Image analysis software. Newly spawned eggs measuring 42 μm were isolecithal with modified holoblastic cleavage. From the seventh cleavage stage the embryos became motile. The trochophore and veliger stages were active swimmers. It took the zygotes about 23 hours at 29 ppt salinity, 24.5-28.0°C, and pH 8.04-8.14 to reach post-set stage. Subsequently, the larvae were fed with "Akemi" diet up to 3 months old juveniles (390 μm). This is the first time that *Pholas orientalis* was naturally spawned and the hatchery technique for its larval production was developed.

The present technology is significant because it will pave the way for field introduction of hatchery-produced diwal for coastal rehabilitation in the very near future.

**DETECTION OF SEQUENCE POLYMORPHISMS
AMONG SELECTED CLONES OF ABACA
(*Musa textilis* NEES) BY RAPD ANALYSIS**

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The technique of random amplified polymorphic DNA (RAPD) was used to investigate DNA sequence variation in eight clones of abaca (*Musa textilis* Nees) obtained from existing germplasm collections. Conditions were optimized for the generation of RAPD markers for abaca. A total of 100 different random 10-mer primers from Operon Technologies were tested to reveal DNA polymorphisms among the different clones. Fifty-eight out of the 100 random primers tested yielded intense and well-resolved amplification products. Four primers (OPU-01, OPV-14, OPV-20, and OPX-04) used together enabled the discrimination of all abaca genotypes tested. However, primers OPU-01 and OPX-04 proved sufficient in discriminating genotypes collected from the Bicol region with collections from other places. This study shows that RAPD analysis can be employed to generate fingerprints of eight abaca genotypes and estimate their genetic relationships based on the analysis of polymorphisms identified.

NOVEL ANTIMICROBIAL SUBSTANCE FROM A STRAIN OF *Vibrio* sp.

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The structure of a novel antimicrobial substance isolated from a strain of the bacterium, *Vibrio* sp. was determined to be 7,7-bis(3-indolyl)-*p*-cresol on the basis of spectroscopic data. The substance showed specific inhibition against *Bacillus subtilis* with a minimum inhibitory concentration of 69 µg/mL but did not affect the growth of the other test microorganisms.

THE ANTIMICROBIAL ACTIVITY OF SOME MARINE SPONGES FROM KAUSWAGAN, LANAO DEL NORTE

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Sponges collected from Kauswagan, Lanao del Norte in September 1986 were investigated for their potential as sources of antimicrobials. Extracts of eleven

species of fresh and dried marine sponges belonging to eight families under Class Demospongia were tested for their activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida utilis*, and *Aspergillus niger*. The filter paper disc method of antimicrobial assay was done against solvent controls and the broad spectrum antibiotic, streptomycin. Ten of the sponge species namely *Adocia* sp., *Pellina* sp., *Cribrochalina* sp., *Xestospongia* sp., *Trachyopsis* sp., *Halichondria* sp., *Suberites* sp., *Phyllospongia* sp., *P. foliascens*, both juvenile and mature *Biemora fortis*, and *Plakortis* sp., demonstrated varying antimicrobial activity depending on the state of the material and the nature of the extract or the solvent used. Extracts from fresh sponges showed greater inhibitions of microbial growth compared with extracts from dried specimens. Ethanol showed greater efficiency in extracting active components from fresh sponge while methanol-toluene was efficient with dried sponge. Inhibition was greatest against Gram-positive bacteria: *Adocia* sp., *Pellina* sp., *Cribrochalina* sp., *Trachyopsis* sp., *Suberites* sp., and *Phyllospongia foliascens* also inhibited the fungi. *Mycale* sp., the eleventh species, did not show any antimicrobial activity.

IDENTIFICATION OF CHROMOSOMAL REGIONS UNDERLYING SOME QUANTITATIVE TRAITS IN RICE USING RFLP, RAPD, AND AFLP MARKERS

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The development of molecular genetic maps has facilitated the identification of genes controlling quantitative characters referred to as quantitative trait loci (QTLs). A molecular map based on a tropical japonica x indica cross (Labelle/Black Gora) was constructed using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) markers. The map spanned 1476 centi Morgans (cM) with markers on all 12 rice chromosomes and an interval spacing of 11 cm. QTL analysis was performed using Mapmaker/QTL with a log-likelihood threshold of 2.5. An average of 3.3 putative QTLs were identified for two reproductive and five seed traits studied.

Four QTLs controlled the number of spikelets per panicle, filled seeds per panicle, seed length, and seed breadth. Three controlled percentage fertility and seed shape and were mapped for seed weight. Several genomic regions influenced

more than one trait. The trait variation accounted for by each QTL ranged from 6 to 27%. QTLs with large effects may be useful for marker assisted selection strategies. The multiband and high-resolution AFLPs were randomly distributed across the rice genome indicating their suitability for gene tagging and DNA fingerprinting in rice.

**FINE MAPPING OF THE GREEN LEAFHOPPER
(GLH-*Nephotettix virescens* DISTANT)
AND RICE TUNGRO SPHERICAL VIRUS (RTSV)
RESISTANCE GENES IN RICE CV. ARC 11554**

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The GLH and RTSV resistance genes were mapped on chromosome 4 of ARC 11554. In this first report, several molecular markers were linked to the resistance genes. Using the non-radioactive enhanced chemiluminescent (ECL) method of molecular marker labeling and detection, a fine map was constructed around the GLH resistant gene, and several markers were found putatively flanking the RTSV (ARC 11554/7TN1) plants. This mapping population was phenotyped for both GLH and RTSV resistance using GLH antibiosis and enzyme linked immunosorbent assay (ELISA). Another F₂ population was also used to find flanking markers within 5 cM from the GLH resistance gene and these have been identified. Molecular markers flanking the RTSV resistance gene have also been found. We are now using the tightly linked molecular markers to design and test sequence tagged site (STS) markers.

**THE HEMOLYMPH OF *Pomacea canaliculata*
AFTER EXPOSURE TO
SUBLETHAL CONCENTRATIONS OF CADMIUM**

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Laboratory-reared *Pomacea canaliculata* snails were exposed for two weeks to sublethal concentrations of cadmium chloride after which the hemocyte composition of hemolymphs was studied. Both granulocytes and agranulocytes

were observed to occur. The total hemocyte count was found to increase with increasing concentration of the cadmium salt. Differential cell count showed that intermediate (0.1875 ppm) and high (0.375 ppm) sublethal concentrations of CdCl_2 caused significant increases in granulocyte number. SDS-PAGE profiles of soluble hemolymph proteins from control and experimental snails showed differences in banding patterns.

