INGA NEWS

Editor's note:

The successful genetic improvement of Nile tilapia through selective breeding has created an interest among a number of INGA member countries in developing a saline tolerant tilapia. Discussions held during the Third Steering Committee meeting of INGA in Cairo, Egypt on 8-11 July 1996 addressed the positive and negative aspects of developing saline tolerant tilapia and concluded that a saline tolerant strain once developed, is likely to escape into natural waters and may pose potential risks to the environment and biodiversity. The meeting further suggested that before embarking on such an endeavour, it is important that an environmental impact assessment be undertaken and that benefits of a saline tolerant tilapia should be great enough to warrant the risks to the environment and biodiversity. The recent Expert Consultation on Environmental Impact of Genetic Enhancement and Introduction of Improved Tilapia/Alien Species in Africa jointly organized by ICLARM - The World Fish Center, Technical Center for Agriculture and Rural Cooperation (CTA), Food and Agriculture Organization (FAO), World Conservation Union (IUCN), United Nations Environment Program (UNEP) and Convention on Biological Diversity (CBD) in Nairobi, Kenya on 23-26 February 2002 concluded that movement of fish across a natural ecological boundaries may endanger biodiversity and that there is a need for wider application of protocols, risk assessment methods and monitoring programs.

Studies to develop a saline tolerant tilapia for culture in vast areas of brackishwater swamplands and ponds in the Philippines are in progress and the paper in this issue provides progress made to date by some Philippine institutions.

Tilapia Broodstock Development for Saline Waters in the Philippines

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Abstract

Four *Oreochromis* species were used in the study. Progenies from the 27 cross combinations (5 pure breds and 22 crossbreds) were evaluated in 10 environments with different salinity levels and agro-climatic conditions using communal rearing concept. Among the different cross combinations reared across environments, *O. aureus x O. spilurus* gave the highest body weight and *O. mossambicus* x *O. spilurus*, the highest survival rate. Positive percent mean heterosis were observed in the crosses between *O. mossambicus* x *O. niloticus,* FAC selected line and *O. aureus* x *O. spilurus*.

Introduction

The Philippines has 232 065 ha of brackishwater swamplands and about 239 323 ha of brackishwater fishponds (BFAR 2000) indicating the high potential for coastal aquaculture. Traditionally, milkfish (*Chanos chanos*) and penaeid shrimp have been cultured in brackishwater fishponds. However, because of a shortage of milkfish fingerlings and penaeid fry, tilapia with all its positive attributes for culture has been identified as suitable species if a strain that could withstand saline conditions could be developed. There are already documented studies on salinity tolerance of various species of tilapia (Philippart and Ruwet 1982; Stickney 1986; Villegas 1990). PCAMRD (1997) recommended the culture of milkfish and Mozambique tilapia *(Oreochromis mossambicus)* which can tolerate water temperatures higher than 32°C and salinities higher than 32 ppt. At present, only *O. mossambicus* and the red tilapia hybrid (*O. mossambicus* x *O. niloticus*) can thrive in brackishwater fishponds in the Philippines, hence the need for a fast growing, saline tolerant tilapia.

The National Freshwater Fisheries Technology Center of the Philippine Bureau of Fisheries and Aquatic Resources (NFFTC/BFAR), and the Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU) with funding from the Philippine Bureau

of Agricultural Research (BAR) have undertaken a project to develop a saline tolerant breed of tilapia for aquaculture.

Materials and Methods

The fish used in the study were *O. spilurus*, *O. aureus*, *O. mossambicus* and three genetically improved strains of *O. niloticus* namely: sixth generation improved GIFT strain, FAC selected line and YY tilapia. Broodstock of *O. mossambicus* were obtained from BFAR-NIFTC, Bonuan, Pangasinan and from the wild in Bulacan. The *O. spilurus* fingerlings were provided by the Mariculture and Fishery Develop-ment, Kuwait Institute for Science and Research. FAC selected line and YY broodstock were provided by the FAC and GIFT fingerlings were obtained from NFFTC/BFAR. The genetic groups used in the study were composed of 27 cross combinations, 5 pure-bred and 22 crossbred.

Diallele crossing of the different species/strains was carried out following the GIFT Project procedure (De Vera 1998). Ten 1 m^3 breeding hapas for each of the 30 combinations were stocked with two females and one male, adding up to a total of 300 breeding hapas in a 1 ha earthen pond. To avoid female mortality due to male aggression, the premaxilla of the male breeders was clipped before stocking in hapas. A week after stocking, breeders were inspected and fry were collected over a period of two days. Once a female had spawned, males were transferred to another hapa. Fry collected from each breeding hapa were counted, weighed and stocked at 200-250 fry per m^3 in separate fine mesh hapas for each family. Fry collection was carried out every week to minimize the age difference between groups of fry collected. Out of 30 combinations tried, fish in 27 combinations only bred. The total number of families collected was 143 from the 27 successful crosses. Only 127 families were used in the study because of the age difference of some of the fry. Fry collected during the same period were referred to as a batch.

Growth performance trials were undertaken using 10 environments with different conditions. Fish were tagged with an external tag four days before stocking and were acclimatized to the salinity level where the fish would be stocked. A total of 250–391 fingerlings from each of the 27 cross combinations were individually tagged/sampled. This was determined by the number of females contributing to the progeny in each cross combination. Stocking density used was three fingerlings/m^2 using a communal rearing system. Every month 30% of the total fish stocked in each environment were sampled for length and weight. Sex was noted as soon as it could be determined. After 120 days of culture, final weights and lengths of all fish were measured. Dissolved oxygen, temperature and salinity were measured during the growth trial.

Results and Discussion

Growth and Survival

Top ranking cross combinations based on the least square means after 120 days of culture are shown in Table 1. Among the different cross combinations reared across environments, S2xS1 (*O. aureus* x *O.spilurus*) gave the highest body weight of 108.90 g followed by the cross of S5xS5 (*O. niloticus* FAC x *O.niloticus* FAC) with 103.10 g. The third ranked cross by weight was S1xS1 (*pure breed O. spilurus*) with 100.43 g followed by S1xS5 (*O. spilurus* x *O.niloticus* FAC) with a mean body weight of 99.10 g and S4xS5 (*O. niloticus GIFT* x *O.niloticus* FAC) and S4xS4 (*pure breed O. niloticus GIFT*) with mean body weights of 98.92 g and 98.63 g respectively.

Fig. 1. A battery of fine mesh hapas in ponds for breeding of 6 *Oreochromis* species/strains

Fig. 2. A battery of b-net hapas in ponds used for rearing of tilapia families until tagging size

It is interesting to note that the pure cross of S3xS3 (*O. mossambicus*) gave the lowest mean body weight of 33.95 g. Hybrid crosses were faster growers than the pure cross. These results are consistent with the observations of Auperin and Prunet (1996) and Guerrero (1994) who reported poor growth performance of this salt tolerant species. High tolerance to salinity of the FAC selected line and GIFT strain tilapia could be explained by their origins. Israel, Singapore and Taiwan strains used to create FAC selected line and GIFT populations were descendants from a founder stock of Ghanaian origin (Eknath et al. 1993). It is assumed that the distribution of *O. niloticus* in Ghana was in the Volta River System, which flows through Lake Volta to the Bight of Benin in the Gulf of Guinea. Tolerance of this species may have evolved from its

Table 1. Mean body weight (g) and ranking of the different crosses[1] of tilapia across environments[2], based on the gain in weight (W) and survival (S) after 120 days of culture[3].

Cross (MxF)	ENV 1	ENV 2	ENV 3	ENV 4	ENV 5	ENV 6	ENV 7	ENV 8	ENV 9	ACROSS ENV.	SURV. (%)	RANKING W	RANKING S
S1XS1	68.94	48.07	84.35	146.4	51.50	84.98	100.19	112.8	107.4	100.43	41.98	3	8
S2XS1	109.27	71.97	91.33	107.2	74.81	42.90	84.66	125.8	169.56	108.9	45.19	1	4
S3XS1	44.46	38.04	71.62	96.88	31.16	39.59	31.57	69.29	61.54	60.34	51.92	16	1
S4XS1	65.26	44.26	81.92	83.74	52.06	30.10	48.22	93.83	91.09	75.14	44.70	13	5
S5XS1	52.48	30.69	93.76	67.17	43.68	29.38	36.43	93.08	75.56	68.28	50.95	14	2
S6XS1	76.25	55.11	92.16	95.45	48.64	35.37	64.01	137.8	94.34	88.73	41.31	10	8
S1XS2	92.35	55.95	100.0	114.4	70.47	94.09	51.49	102.4	93.33	88.68	44.08	10	5
S2XS2	77.97	68.43	39.06	67.44	60.54	-	54.89	97.68	75.05	65.78	30.14	15	13
S4XS2	96.48	68.82	57.14	119.9	72.87	-	85.27	145.52	105.6	97.71	35.71	6	11
S5XS2	84.25	64.01	79.95	92.08	76.05	28.32	78.19	115.34	99.97	90.26	41.75	8	8
S6XS2	59.82	53.45	-	59.18	75.63	-	53.73	52.67	55.26	59.29	34.29	17	12
S1XS3	51.81	33.75	56.36	74.23	21.13	28.60	34.95	60.9	39.87	47.91	40.95	20	9
S2XS3	67.68	43.94	81.29	46.65	43.48	34.68	29.92	63.7	69.61	55.76	35.71	19	11
S3XS3	34.26	43.69	51.96	35.07	15.79	22.95	24.35	48.2	28.35	33.95	23.11	21	16
S4XS3	67.72	44.23	102.3	83.75	51.11	31.22	37.89	63.77	56.58	65.9	43.33	15	6
S5XS3	67.34	56.54	93.09	94.8	43.15	30.98	50.56	113.7	97.38	82.11	46.19	12	3
S6XS3	80.34	44.93	72.46	86.69	49.46	32.98	46.69	82.25	53.72	65.21	41.59	15	8
S1XS4	84.69	64.28	98.62	105.3	59.93	38.02	67.73	123.2	125.8	96.76	42.86	7	7
S4XS4	102.6	81.92	115.3	144.5	76.11	-	88.3	107.2	60.35	98.63	30.66	5	13
S5XS4	93.46	74.59	49.35	123.1	77.38	-	74.69	174.5	86.41	97.58	29.05	6	14
S6XS4	94.04	82.15	51.16	131.4	83.07	-	65.47	113.9	92.72	89.61	23.81	9	16
S1XS5	84.54	59.66	107.4	113.6	59.91	31.87	66.87	117.8	129.0	99.10	43.81	4	6
S2XS5	49.93	44.9	74.43	50.25	42.39	28.02	36.34	63.05	87.15	58.94	44.76	18	5
S3XS5	87.71	61.94	105.7	141.7	65.8	38.09	35.57	131.1	100.2	96.67	42.86	7	7
S4XS5	104.2	68.47	45.83	136.6	68.25	-	82.57	134.7	125.6	98.92	24.17	5	15
S5XS5	104.7	92.94	80.7	151.54	67.85	23.21	96.35	95.5	126.6	103.1	30.8	2	13
S6XS5	88.56	44.05	54.26	109.5	73.41	50.42	86.96	104.4	72.19	83.45	36.45	11	10
MEAN	77.45	57.07	78.14	99.2	57.62	38.79	59.77	101.6	88.16	80.63			

[1]Strains:
S1 = *O. spilurus*
S2 = *O. aureus*
S3 = *O. mossambicus*
S4 = *O. niloticus* (GIFT)
S5 = *O. niloticus* (FAC selected line)
S6 = *O. niloticus* (YY Males)

[2]TEST STATION:
ENV 1 = BFARP1 (0 ppt)
ENV 2 = BFARP2 (0 ppt)
ENV 3 = La Union (29-32 ppt)
ENV 4 = Bulacan (0-12 ppt)
ENV 5 = Butuan (10-20 ppt)
ENV 6 = Davao (23-35 ppt)
ENV 7 = Claveria (0-10 ppt)
ENV 8 = Pagbilao Pond (15-29 ppt)
ENV 9 = Pagbilao Cage (15-30 ppt)
ENV10 = Ilo Ilo (18-42 ppt)

[3]Ranking:

1	2	3	4	5	6	7	8	9	10

Table 2. Mean heterosis and % heterosis for body weight of tilapia crosses within and across test environments.

CROSS	ENV 1		ENV 2		ENV 3		ENV 4		ENV 5		ENV 6		ENV 7		ENV 8		ENV 9		ACROSS ENV.	
	H	H%	H	H%	H	H%	H	H%	H	H%	H	H%	H	H%	H	H%	H	H%	H	H%
S1XS2	27.35	37.23	5.71	9.80	-9.46	-12.20	33.98	55.07	-	-	3.90	3.65	16.62	29.68	40.22	44.09	8.87	8.43	15.90	21.97
S1XS3	-3.47	-6.72	-9.99	-21.77	-29.01	-46.59	-6.36	-9.33	-19.87	-36.82	-5.15	-5.68	-7.50	-22.29	-17.17	-25.09	-15.39	-19.12	-12.66	-21.49
S1XS4	-10.82	-12.62	-10.72	-16.49	-36.27	-38.48	-9.55	-9.56	-	-	-50.94	-35.02	-7.81	-12.24	24.58	29.30	-1.46	-1.33	-12.87	-12.05
S1XS5	-18.33	-21.11	-25.33	-35.93	-46.62	-47.44	18.05	21.87	-23.47	-43.39	-58.55	-39.31	-7.88	-13.20	-14.72	-12.58	1.32	1.27	-19.50	-21.09
S2XS5	-24.27	-26.56	-26.23	-32.51	-18.35	-24.27	17.31	28.91	-	-	-38.32	-34.99	-4.98	-7.76	-7.26	-7.20	-7.39	-7.65	-13.69	-14.00
S3XS5	8.03	11.55	-9.07	-13.28	-17.28	-28.63	33.05	49.83	11.45	49.61	24.92	26.71	12.66	30.27	21.32	27.52	50.55	70.35	15.07	24.88
S4XS5	-4.85	-4.68	-15.9	-18.18	-13.7	-14.84	-50.41	-51.44	-	-	-18.19	-12.29	0.84	1.17	12.53	13.41	53.25	52.54	-4.55	-4.29

Table 3. Number (n) of male and female tilapia breeders of the 14 best performing strain combinations used to build the base population.

RANK	CROSS	MALE (n)	FEMALE (n)	% Contribution toBase Population
1	S2 x S1	7	17	12
2	S5 x S5	6	16	11
3	S1 x S1	6	15	10.5
4	S5 x S1	5	15	10
5	S4 x S4	5	14	9.5
6	S4 x S2	4	14	9
7	S1 x S5	4	12	8
8	S4 x S5	3	10	6.5
9	S5 x S4	3	9	6
10	S1 x S4	2	8	5
11	S3 x S5	2	8	5
12	S1 x S2	1	6	3.5
13	S5 x S3	1	4	2.5
14	S5 x S2	1	2	1.5
Total		50	150	100

marine ancestors (Villegas 1990).

Percent survival was highest in test environment ENV 4 (0-12 ppt) and relatively low in ENV 7 (0-10 ppt), ENV 8 (15-29 ppt) and ENV 3 (29-32 ppt). There was an incident of almost total mortality in ENV 10 (18-42 ppt) caused by entangling of identification tags, intolerance to high salinity and tagging infection, hence ENV 10 was excluded in the ranking, as was ENV 1 and ENV 2 (freshwater environments). Mortality (including tag loss) of the different strain/species combina-tions, varied from 10.47% to 66.95%. Tag loss across test environments (excluding ENV 10) was 13.14%. The high rate of tag loss in pure breds was due to tagging infection (due to high salinity) and fish size exceeding a certain limit (during fish tagging, an allowance of 3.75 cm of thread was given for growth).

Heterosis

Least square means were computed for percent heterosis for body weight at harvest within test environments (Table 2). Since percent heterosis measures the non-additive genetic effects relative to the additive genetic and reciprocal effects, the species showing the highest non-additive genetic effects did not show the highest heterosis in percent. The highest mean percent heterosis across test environments was observed in the crosses between the S2xS1 (*O. aureus* x *O.spilurus*) and the S3xS5 (*O. mossambicus* x *O. niloticus* FAC selected line) (21.97 and 24.88% respectively). Hybrids with genes of *O. mossambicus* have demonstrated excellent tolerance to high salinity and grow well over a wider salinity range compared to *O. niloticus* (Villegas 1990). All other crosses showed negative mean percent heterosis across test environments.

Base Population

Since only two crosses showed positive heterosis or hybrid vigor in terms of gain in weight, the effect of crossbreeding was too low to be of significance in an applied breeding program. A simple pure breeding strategy will be adopted by selecting the best growing individuals from the 14 best performing purebred and crossbred groups (out of the 27 crosses evaluated) to build a genetically mixed base population (synthetic breed). Table 3 shows the number of male and female breeders of the 14 best performing species combinations used to build a new base population.

Future Direction

The base population established from this experiment will be used as the basis for a further generation of selection (2nd Phase Experiment).

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China's National Certification Committee Regulates New Release of Aquatic Germplasm and Seed Farms

Making available good quality seed has been recognized as one of the important national strategies to develop China's inland and marine aquaculture. In view of this, the National Certification Committee of Aquatic Wild and Bred Varieties (NCCA) was established by the Government of China in 1991 under the Ministry of Agriculture. The geneticists, aquaculturists and administration of research institutions, universities and officials of Bureau of Fisheries under Ministry of Agriculture, are represented in the Committee.

The NCAA has been mandated to: (i) certify "new species for aquaculture" (i.e. genetically improved varieties, hybrids and alien species). Any new aquaculture species for introduction has to be evaluated and certified first by the NCAA before this gets approved by the Ministry of Agriculture, which is the fisheries authority of the National Council; (ii) evaluate and certify the National Aquatic Wild and Bred Seed Farms in terms of their capacity to carry out the major functions of national seed farms and the technical approaches used for maintaining and providing quality breeders to hatcheries and *ex situ* conservation of genetic resources; and (iii) establish the certification regulations and policies related to the above two major activities.

Since establishment of the Committee in 1991, 42 new species/strains/varieties have been certified for use in aquaculture. Of these, 21 are carps, 5 tilapias and the rest include other species/strains of commercial importance: catfish, large mouth bass, rainbow trout, *Colossoma* sp, giant prawn, oyster, scallop, kelp and frog. Nineteen fish farms have been certified as national seed farms. Of these, 16 are concentrating on carps, 4 on tilapias and the rest are for various aquaculture species. The national seed farms are the accredited source of good quality breeders for supply to hatcheries for production. For a new species/strain/variety to be certified by NCAA, the institution involved has to submit to NCAA a description of the species/strain: origin; species characterization in morphology, chromosome, biochemical and molecular genetics; breeding procedures used on-station; and on-farm performance; disease resistance; socio-economics; risk assessment; etc.

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Training for African Scientists

The World Fish Center is organizing a training program on *Estimation of Genetic Parameters* with funding support from the United Nations Development Programme/ Technical Cooperation among Developing Countries (UNDP/ TCDC). About 15 participants from African countries are expected to participate in this training. This will be held at the World Fish Center's Regional Research Center for Africa and West Asia, Abbassa, Egypt in May 2002. The training will focus on analysis of datasets from selective breeding experiments.

Training for INGA member country scientists

In the past, a number of training programs have been organized by World Fish Center/INGA for scientists from member countries in quantitative genetics and its application to aquaculture. A needs assessment has been undertaken through questionnaire to assess the training needs of these scientists. The feedback from this survey will be used in formulating training programs to be organized by the network in early 2002.

Germplasm transfer

The third batch of GIFT strain Nile tilapia (*Oreochromis niloticus*) fingerlings from sixth generation of selection families being kept in the Philippines by the Center and maintained by the GIFT Foundation International Inc. were transferred to Malaysia on 7 November 2001. Quarantine protocols developed by the INGA members were strictly followed during the transfer. The germplasm will be used in genetic improvement program being carried out in Malaysia.

Consultation meeting in India on shrimp genetics

An Indo-Norwegian consultation on *Genetic Evaluation and Improvement of Prawns* was organized at Central Institute of Fisheries Education, Mumbai, India on 28 - 29 September 2001. Scientists from national research institutions in India and Institute of Aquaculture Research Ltd. (AKVAFORSK), Norway and representative of Norwegian Agency for Development Cooperation, New Delhi participated.

During the meeting, research carried out in India on genetics and breeding and aquaculture of freshwater prawn (*Macrobrachium rosenbergii*) and marine tiger shrimp (*Penaeus monodon*) was reviewed. The consultation meeting identified the need for collaboration between Indian national research institutions (Central Institute of Fisheries Education, Central Institute of Freshwater Aquaculture, Central Institute of Brackishwater Aquaculture, and University of Agricultural Sciences) and AKVAFORSK on: (i) "Genetic improvement of the fresh water prawn *M. rosenbergii"* and; (ii) Domestication and disease resistance in *P. monodon"*. The principle of selection in crustaceans as applied to Columbian prawn *P. vannamei* will be extended by the Norwegian partner.

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Publications of interest

Book on Fish Biodiversity in India

The North East Region of India is endowed with very rich and varied freshwater fish biodiversity. Information on its germplasm resources is important for the conservation and sustainable utilization of the rich natural wealth. Generation of primary data on freshwater fish biodiversity and linking it to the development programs could be the right step in this direction. In line with this initiative, India's National Bureau of Fish Genetic Resources (a member institute of INGA) held a workshop on *North East Fish Germplasm Inventory and Conservation* in India on 10-11 February 2000. The proceedings of the workshop entitled *'Fish Biodiversity of North East India'*, edited by Dr. A.G. Ponniah and Dr. U.K. Sarkar was published in 2001. The 228-page document contains background papers, extended abstracts and working group recommendations on germplasm resources, potential food and ornamental fishes and conservation aspects. The book will be useful to students, scientists and policy makers involved in research and management of fish genetic resources of Northeast India.

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Genetic Resources, Biotechnology and Intellectual Property Rights - CGIAR Booklet

The International Agricultural Research Centers of the Consultative Group on International Agricultural Research (CGIAR) have developed and agreed on various policy instruments, guidelines and position statements to guide and validate their decisions regarding genetic resources, biotechnology and intellectual property rights. The System-wide Genetic Resources Programme (SGRP) and the CGIAR Genetic Resources Policy Committee have compiled all these into a booklet for adoption by the Centers. The 40–page document entitled *Booklet of CGIAR Centre Policy Instruments, Guidelines and Statements on Genetic Resources, Biotechnology and Intellectual Property Rights* contains the current version of the System-wide policies and guidelines for acquiring, managing and transferring plant, animal, aquatic and microbial genetic resources. It also presents CGIAR and Center Committee statements on a number of genetic resources and related issues. Published in Rome in September 2001, the booklet is intended primarily as a reference for the Centers but is available to outside parties upon request.

For more information, contact: the Secretariat of the CGIAR System-wide Genetic Resources Programme, c/o International Plant Genetic Resources Institute (IPGRI) Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Rome, Italy; Tel: 39-0661181; Fax: 39-0661979661; E-mail: SGRP-Secretariat@cgiar.org

Access to genetic resources and benefit sharing

The Biodiversity Action Network (BIONET) announces the availability of the book *Access to Genetic Resources and Benefit Sharing*. The book edited by Lyle Glowka, Balakrishna Pisupati and Sanjiv de Silva is based on a regional workshop held in Southeast Asia to facilitate the exchange of ideas and information through discussion of case studies. It includes sections on: mechanisms and approaches to control access to genetic resources and promote benefit sharing; tools for safe-guarding traditional knowledge; planning mechanisms; the state of access legislation in South and Southeast Asia; and case studies from the region. Published in 2001, the 203-page book is a useful reference for policy makers, scientific research communities, NGO's and other stakeholders.

For further information and orders contact: IUCN-Regional Biodiversity Program, Asia; 48 Vajira Rd, Colombo 05, Sri Lanka; Tel: 074-510-517; Fax: 941-580202; E-mail: iucn-rbp@sltnet.lk; internet: http://www.rbp-iucn.lk; Source: Bionet; http://www.bionet-us.org

Handbook of the Convention on Biological Diversity

A handbook of the Convention on Biological Diversity (CBD) has been published by the CBD Secretariat. This handbook contains the CBD's rules of procedures; Subsidiary Body on Scientific, Technical and Technological Advice and Clearing House Mechanism modus operandi; the text of the Convention; and decisions from Conference of the Parties, as well from the Conference addressing the Cartagena Protocol on Biosafety.

For further information and orders contact Earthscan Publications, 120 Pentonville Rd., London N1 9JN, UK; Tel: 44—20-7278-0433; Fax: 44-20-7278-1142; E-mail; earthinfo@earthscan.co.uk; internet:http://www.earthscan.co.uk. Source: Bionet; http://www.bionet-us.org