MEETING REPORT SYMPOSIA "GENE TRANSFER IN AQUATIC ORGANISMS: FROM THE LABORATORY TO THE MARKET". GENETICALLY MODIFIED FISH: FROM BASIC SCIENCE TO BIOTECHNOLOGY

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The history of human beings is closely linked to the study of nature. Among all animal kingdoms, aquatic organisms have concentrated most attention because of their abundance and importance as a source of animal protein.

The first ichthyological references are found in the "History of Animals" of Aristotle (384-322 BC) and in the "Natural History" of C. Plinio II (29-79 AC). Educated in these principles, scientists in many countries studied the biology of aquatic organisms.

Cuba, being an island, is tightly ligated to the aquatic life. Our aborigines depended on fish, shellfish and crustacean for subsistence. References about Cuban aquatic organisms are found in the chronicles of Spanish conquerors: Fernández de Oviedo G. "Historia General y Natural de las Indias", Madrid 1851. However, the first work published about Cuban ichthyology appeared in Havana in 1787 by Parra A "Descripción de diferentes piezas de Historia Natural, las más del ramo marítimo". Later, in the 19th century, the encyclopedical work by Ramón de la Sagra "Histoire Physique, Politique et Naturelle de L´Ile de Cuba", Paris 1838-1842 and Felipe Poey "Memorias sobre la Historia Natural de la Isla de Cuba", Habana 1851-1861; "Observations on different points of the Natural History of the Island of Cuba, with reference to the ichthyology of the United States", Annals of the Lyceum of Natural History of New York 1854;6(5):133-136; "Ictiología Cubana", La Habana 1955 & 1962, situated Cuban ichthyological research in the science's front line. The pioneering work by these scientists was continued by others as Guitart DJ "Sinopsis de los peces marinos de Cuba", La Habana 1978-1979 and crystallized in the last 40 years in important contributions to scientific knowledge (now more than 20 institutions in Cuba have groups involved in research with aquatic organisms).

All the knowledge accumulated in Cuba and in many other countries has produced an impact, not only on ichthyological sciences, but also on the development of aquaculture technologies with domesticated fish species.

In recent years, when gene manipulation techniques produce a revolution in biological sciences, the genome of aquatic organisms have been modified to gain more knowledge on the biology of these organisms and to produce improved strains for aquaculture.

The decline of marine living resources and the stagnation of the wild harvest world fish stocks at the maximum sustainable level of 100 million tonnes per annum, together with the increase in human population and in the need for seafood, have stressed the importance of ichthyological research and the role of aquaculture and mariculture in producing high quality food.

In this context, we held the symposia "Gene transfer in aquatic organisms: form the laboratory to the market", organized by the Center for Genetic Engineering and Biotechnology in Havana, Cuba (November 16-21, 1998) with the participation of leading scientists from Chile, Finland, Norway, Spain, Taiwan, USA and Cuba.

Contributions covering the fallowing subjects were presented at the meeting:

a) genetic engineering in fish, shellfish and crustacean,
b) growth manipulation in economically important and model fish species,
c) public involvement in the application of transgenesis in fish,
d) effect of growth hormone on fish metabolism and physiology,
e) manipulation of fish metabolic functions,
f) mechanisms of disease resistance in aquatic organisms,
g) regulatory sequences for gene transfer in fish and shrimp for DNA vaccination and for transgenesis,
h) generation of ES cells in fish,
i) microsatellite genetic markers in tilapia. If one important conclusion must be drawn from this meeting is that gene transfer in aquatic organisms has produced a great impact in ichthyological research and has evolved from the laboratory to the market with a promising impact on world aquaculture production.

Cuba is among countries with more advanced research in the area of gene transfer in aquatic organisms. As evidenced during the meeting, genetically modified tilapia with improved growth performance are being introduced into national aquaculture.

Major challenge for the near future focuses on:

a) the modification of disease resistance, metabolic pathways, reproductive performance and other traits in fish, mollusks and crustacean,
b) the characterization on case-by-case basis of behavioral, physiological and toxicological param-

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eters of new strains to assure environmental and food safety,

c) the optimization of aquaculture technologies to fully exploit the genetic potential of improved strains,

d) the design of expression vectors and gene transfer methodologies through the use of somatic cell cloning and ES cells to improve the efficiency of transgene transfer and expression

e) the characterization of the genome of these species for targeted genetic manipulations and population studies.

The relevance of the results in this rapidly evolving area will have a great impact in basic research and in biotechnological applications. It will be possible to address challenging fundamental questions like the limits for growth efficiency in fish while having an impact on world fish production to help fulfill the increasing food demands of the population in the next millenium.

On behalf of the Biotecnología Habana'98 organizing committee I am indebted to all participants in the meeting and especially to those who contributed with their work and from which abstracts have been included in this report.

Increasing public involvement in enriching our fish stocks through genetic enhancement

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Seventy percent of the world's conventional commercial fish species are now fully exploited, overexploited, depleted or recovering from depletion. This dramatic crash in the capture world fisheries production has led to problems in foods distribution, balance of payments, employment, and ecological depletion. Public support for breeding programs with terrestrial farm animals and plants in agriculture have revolutionized this industry over the past few hundred years. However, new genetic rearing technologies to improve marine animal production through aquaculture that utilize modern biology to obtain sustainable aquaculture and preserve biodiversity provide a promise to address these problems. However, aquaculture has not been subject of public discussion and approval. Public involvement, not necessarily acquiescence, provides value added in the decision making process. Public understanding and involvement consist of three stages. Public concern over the pool of genetic information. If aquaculture is to respond to the fisheries crises with innovation, the knowledge gap between public understanding and scientific information must be bridged. Strategies must be developed for achieving this. Release of recombinant DNA to the environment, and handling exotic species, are useful case studies. Illustrations will be given of communication bridges to the public and ways to involve the public in making policy decisions.

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The control of the process of growth in tilapia: basic research and applications

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Growth manipulation in fish is one of the targets of gene transfer experiments to produce strains with improved growth performance. Although the transfer of growth hormone transgenes has been successful in many fish species, the knowledge of the molecular events that control growth in fish is necessary to efficiently manipulate this process. We have selected tilapia for our studies because these species are suitable for basic research as well as for the development of improved strains for aquaculture. Here we review the results of basic and applied research in the field of growth control and manipulation in tilapia. The experiments bring new results on the growth control in tilapia and resulted in an aquacultured line with improved growth performance. Many of these results are probably applicable to other teleosts.

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Serum growth hormone levels in transgenic and wild type tilapia

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Growth hormone (GH) plays a key role in growth regulation in vertebrates. Recent developments in gene transfer techniques have permitted the generation of fast-growing fish strains through the transfer of GH-expressing transgenes. However, only a few reports have supported the correlation between the transgenic genotype and the resulting fast growing phenotype. The availability of data in this field has been hampered mainly because of technical difficulties in showing and quantitating the expression of the transgenic GH, especially when using the endogenous fish GH in chimeric transgene constructs. Here, an ELISA for tilapia GH (tiGH) was established and validated. The plasma circulating tiGH levels were determined in transgenic fast growing (IG-91/03F70) and wild type tilapia. The results showed high variability in tiGH levels among different individuals. However, in hypophysectomized tilapia it was possible to establish that the transgenic line expresses low ectopic levels of tiGH, necessary and sufficient to accelerate growth in these species.

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Transfer of growth hormone (GH) transgenes into Arctic charr (*Salvelinus alpinus* L.). I. Growth response to various GH constructs

Tiina I Pitkänen, Aleksei Krasnov, Heli Teerijoki, Hannu Mölsä

Four constructs containing salmonid growth hormone (GH) genes were transferred to Arctic charr *(Salvelinus alpinus* L.). Cytomegalovirus (CMV) and piscine metallothionein B (OnMT) and histone 3 (OnH3) promoters connected to sockeye salmon growth hormone 1 gene (OnGH1) were used for ectopic expression, and Atlantic salmon growth hormone 2 gene with 5′ flanking region (SsGH2) was tested for pituitary-specific expression. Charr carrying the OnGH1 constructs showed a dramatic increase in growth rate. Ten-month old transformed fish were 14-fold heavier than control siblings. The ability of the CMVGH1 construct to promote growth was greater than that obtained in fish with piscine promoters. Analysis of individual growth curves of charr carrying the OnH3GH1 transgene indicated a stable ratio of specific growth rates in transformed and control fish regardless of fish size. No alteration in growth performance was found in fish carrying the SsGH2 transgene. There was an evidence that the rainbow trout *(Oncorhynchus mykiss)* were unable to produce SsGH2 mRNA in their pituitary glands. The presence of the transgene in various tissues was examined in trout to evaluate the reliabilty of one-tissue sampling.

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Transfer of growth hormone (GH) transgenes into Arctic charr (*Salvelinus alpinus* L.). II. Nutrient partitioning in rapidly growing fish

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To examine whether the utilization of protein and lipids is altered in the genetically modified, rapidly growing charr, we compared CMVOnGH1 transgenic and sibling fish. Muscle composition and rates of gas exchange were analysed. Plasma metabolites were determined in the recently fed and post-absorptive state. No difference was found in muscle composition. At equal rates of protein accretion, the rate of NH_4^+ excretion was 43% greater in sibling charr. The lower molar ratio of NH_4^+ to O_2 exchange implied the reduced expenditure of metabolized protein in transgenic charr. Plasma NH_4^+ concentration in transgenic fish did not differ from that in sibling charr whereas the greater level of total CO_2 indicated enhanced oxidation of non-proteic nutrients. Decreased plasma triglycerides concentration and lower triglyceride to cholesterol ratio showed a faster utilization of ingested lipids in transgenic charr, especially of the energy-containing fraction. However, this was not accompanied with a reduced lipid content or altered fatty acid composition of muscle triglycerides or phospholipids. Comparative studies suggested that the transgenic charr had acquired features of domesticated salmonid fish. Their increased metabolic rate and enhanced utilization of dietary lipids, especially triglycerides, resembled the characteristics of domestic rainbow trout rather than of the wild counterparts.

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Growth hormone effects on essential amino acid absorption, profile, N-retention and nutritional requirements

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Improvements in modern commercial aquaculture are linked to the utilization of biotechnological methods and processes. The most visible approach has been the use of growth hormone (GH) and/or insulin-like growth factor I & II (IGF-I & II), which it mediates, to accelerate the growth of fish. The growth of many species of aquacultured fish has been successfully accelerated using various preparations of exogenous homologous or heterologous GH delivered via injection, orally, or by gill permeation.

A more sophisticated approach has been to construct and produce suitable GH plasmid DNA fragments and introduce these into fish eggs or at early embryonic stages by different transgenesis methods. Then, one hopes for survival and the ultimate expression of the desired downstream GH and IGF peptides. Growth hormone and IGF profoundly influence the intestinal absorption of amino acids, total N-retention and the amino acid profile of the fish muscles. Feed amino acid (protein) quantity and quality (profile) must be optimized under biotechnological growth acceleration. Previously we have reported that the injection of bGH in striped bass hybrids increased the specific growth rate and food conversion efficiency without significant alteration of food consumption rate. In this paper we present the results of experiments in which growth, food consumption, conversion efficiency, ammonia excretion, and amino acid absorption were monitored for individual fish after bGH injection. The specific growth rate was stimulated by 50% without significant change in the relative food consumption rate. Food conversion efficiency increased by 51%. Intestinal L-leucine absorption was increased by 25-40% at various concentrations tested. The relative N-retention was stimulated by 20% when computed raw. When a correction factor derived from the elevated amino acid absorption was introduced into the computations, the calculated relative N-retention was increased by 56%. Muscle amino acid profile was significantly altered.

We conclude that the post-translational effects of GH used in a biotechnological approach in aquaculture profoundly alter the physiological and nutritional conditions of fish and should be investigated in order to optimize growth.

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Biochemical and metabolic correlates of growth rate in fast-growing transgenic tilapia expressing low ectopic levels of homologous growth hormone

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Growth performance has been manipulated in economically important fish species to improve aquaculture production. Growth acceleration has been achieved by the exogenous administration of homologous and heterologous growth hormone (GH) preparations and by the transfer of GH-transgenes to generate transgenic fish. In these experiments, GH has shown to have a profound impact on fish physiology and metabolism. However, detailed studies in transgenic fish have not been conducted. We have characterized the food convertion efficiency, lipid and protein profiles and biochemical correlates of growth rate in fast-growing transgenic tilapia expressing the tilapia GH cDNA under the control of human cytomegalovirus regulatory sequences. Transgenic tilapia exhibited about 3.6-fold less food consumption than non-transgenic controls ($P < 0.001$; Student t-Test). The food conversion factor was significantly ($P < 0.05$; Student t-Test) higher (3.4x) in transgenic tilapia (2.8 ± 0.6) when compared to the control group (9.7 ± 3.0). Distinctive metabolic differences were found in transgenic juvenile tilapia. The energy required for the accelerated growth in transgenic tilapia is produced from hepatic glucose and the gluconeogenic amino acids alanine and aspartic acid in muscle. The decrease in plasma cholesterol levels may be associated with increased membrane synthesis in fast growing tilapia. These differences were supported by increased enzymatic activity in target organs. Quantitative analysis of non-esterified fatty acids in muscle reported higher unsaturated fatty acid content in transgenic tilapia. We conclude that GH-transgenic tilapia with accelerated growth show altered physiological and metabolic conditions and are also biologically more efficient.

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Gene transfer for targeted modification of salmonid fish metabolism

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The reviewed studies addressed the possibility of using gene transfer for correction of L-ascorbic acid biosynthesis and carbohydrate utilization in rainbow trout. Analyses of enzymatic activities in the L-AAB pathway indicated that reasons for the lack of L-AA production could be common in fish and scurvy-prone animals. Rat guconolactone oxidase cDNA was transferred into trout. Regardless of the fact that rGLO transcription occurred in embryos, neither GLO protein, nor enzyme activity were detected. There was no production of L-AA in transgenic fish raised on vitamin C-free diets or injected with L-guconolactone. These results indicated that the conditions required for translation or stability of rGLO were not present in trout tissues. To augment carbohydrates utilization, human glucose transporter 1 (hGLUT1) and rat hexokinase II (HKII) cDNAs were tested. In the transfected embryos, HK activity, rates of hexose uptake and glucose oxidation were increased. The effect of hGLUT1 on glucose metabolism was greater than that of rHKII. Trout carrying hGLUT1 and rHKII with viral or piscine promoters were created. Though interpretation of the metabolic effects of the transgenes was complicated with mosaicism, a tendency to improved carbohydrate utilization was revealed in some of the transgenic individuals.

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Transgene transmission and expression in zebrafish studied by immunofluorescence and fluorescence *in situ* hybridization

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NLS peptides derived from SV40 T antigen enhance nuclear import of plasmid DNA in zebrafish embryos when non-covalently complexed at a molar ratio of 1:100 DNA to peptide. Nuclear uptake of a 5.5 kb CMV-LUC vector was measured by fluorescence *in situ* hybridization (FISH) in interphase nuclei isolated from 1 to 8 h post-injection (p.i). Using 104 copies of plasmid complexed to NLS over 70% of nuclei were FISH positive at 5 h p.i, compared to 20% after microinjection of 106 copies of naked plasmid DNA. The average number of FISH signals were 2-6 for 104 + NLS and 1-3 for 106 copies of naked DNA. Transmission frequencies were measured by PCR detection of LUC sequences in offspring embryos from crosses between microinjected founder fish (F0) and wild type. Comparing 104 + NLS with 106 without NLS revealed 43% vs 14% germline transgenic F0's and 47% vs 7% transgenic germline cells (Collas and Aleström, Trans Res 1998;7:303-309). To study position effects on CMV-LUC transgene expression a group of transgenic F1 individuals containing both expressers and non-expressers were analyzed by combined immunofluorescence and FISH. The results revealed a strong correlation between histone H4 acetylated chromatin and transgenes from LUC expressing individuals coinciding with immunofluorescence signals from antibody staining of RNA pol II and spliceosome complex. Transgenes from non-

expressers only rarely were co-localized with immunofluorescence signals of acetylated histone H4, RNA pol II and spliceosome complex. Inheritance patterns of F2 offspring from LUC expressing F1 transgenics segregated into both expressors and non-expressors demonstrating the importance of overcoming position effects for the generation of stable lines of transgenic fish.

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Studying the retinal-specific regulatory element of photoreceptor gene by using transgenic fish

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Rod opsin, rhodopsin, is one of the photoreceptors but locates at rod cells, which mediates dim-light vision. We have cloned rhodopsin gene from the common carp *(Cyprinus carpio)*. The deduced amino acid sequence of carp rhodopsin showed 95.7, 85.5 and 74.4% identity with that of goldfish, sand goby and lamprey, respectively. However, few significant homologous regions could be found in the 5' regulatory region compared with corresponding sequences of other terrestrial species. To define the *cis*-acting DNA elements required for rhodopsin expression, we generated transgenic fish carrying sequences upstream the carp rhodopsin gene fused to the green fluorescent protein cDNA as a reporter gene. Upstream sequences extending from positions -6000 to +66 and from -146 to +94 were able to drive retinal-specific expression, whereas from -53 to +94 segment not. This suggests that the element regulating retinal-specific expression may locate within -146 to -53. Interestingly, little homology of polynucleotide sequences was shown between the region from -146 to -53 and any other known retina-specific protein binding sites of terrestrial animals.

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Evaluation of eukaryotic promoters for the construction of DNA vaccines for aquaculture

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Although several research groups have shown that DNA vaccines can protect finfish against infectious diseases, no DNA vaccines have been approved for use in aquaculture. Our goal is the development of safe and efficient expression vectors for the construction of DNA vaccines for aquaculture following guidelines from the Center for Biologics Evaluation and Research of the Food and Drug Administration. In search for efficient non-viral regulatory sequences, we compared the transcriptional activity in fish muscle tissue of two fish promoters, the carp β-actin (CBACT) and the *Fundulus heteroclitus* lactate dehydrogenase-B (LDHN500) promoters, to the activity of the widely used cytomegalovirus enhancer/thymidine kinase promoter (CMVtk). The highest levels of reporter gene expression were observed when using the CMVtk promoter, although the CBACT and the LDHN500 promoters also drove similarly high levels of expression in Atlantic salmon muscle. In order to avoid the possibility of integration of plasmid DNA into fish and human genomes we identified regions of homology between the promoters and fish and human sequences. No regions of extensive homology with human sequences were found in the CBACT and LDHN500 promoters. These promoters may be useful for the design of DNA vaccines for aquaculture.

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Chimeric genes for *in vivo* transient expression studies in shrimp *(Penaeus schmitti)*

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The genetic manipulation of shrimps is of interest for biological studies and for biotechnological applications. For gene transfer experiments, chimeric genes must be designed and tested and gene transfer protocols established. We have studied the function of carp ß-actin and SV40 promoters in conjunction with the *E. coli lacZ* reporter gene in shrimp (*Penaeus schmitti*) tissues. Transient ß-galactosidase activity was detected in shrimp embryos and nauplii after microinjection or electroporation of early embryos and in adult muscle after injection of naked DNA. Endogenous ß-galactosidase activity was localized and characterized in the hepatopancrea of adult shrimps. Our results demonstrated that it is possible to transfer naked DNA to shrimp tissues. The SV40 and carp ß-actin promoters are functional in shrimp and the *E. coli lacZ* gene is a suitable reporter for transient expression studies.

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Transfección de vectores reporteros en el interior de una línea celular de peces mediada por una lipopoliamina catiónica

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La importancia evolutiva, nutritiva y comercial que poseen los peces, especialmente los del grupo de los salmonídeos, los ha convertido en una interesante fuente de experimentación en mejora genética clásica, y últimamente, en un sistema clave para realizar experimentos de transgénesis. Sin embargo, en la práctica, la eficiencia de expresión y/o de inmortalización de genes foráneos en un organismo receptor ha resul-

tado ser de muy baja frecuencia. En este trabajo se presentan experimentos de transfección *in vitro*, con dos vectores alternativos, con el fin de optimizar, en lo posible, la eficiencia de transgénesis en células en cultivo, evaluando la mantención sostenida de los vectores en las células blanco o bien midiendo la calidad y cantidad de la expresión de los genes de interés en el sistema modelo. Los vectores utilizados fueron los plásmidos pCMVL y p103, ambos conteniendo el gen reportero LUC como indicador selección. El vector p103 se diferencia de su progenitor pCMVL, por tener el gen LUC flanqueado por dos módulos idénticos, pero de polaridades opuestas, de la secuencia tipo de la familia *Sma* I específica del genoma de peces salmonídeos. Se postula que estas secuencias, por estar polidispersas y altamente repetidas en el genoma blanco, favorecerían la integración del gen indicador a través de una recombinación homóloga no recíproca. La introducción de los vectores al interior de las células se logró a través de un proceso de transfección facilitada por el uso de una lipopoliamina catiónica comercial y se definieron las relaciones de concentración óptimas lipopoliamina/DNA plasmidial para ser aplicados en experimentos de transgénesis. Se realizaron experimentos de mantención de los vectores hasta después de 5 pasajes de cultivo para evaluar la poten cial integración del gen reportero LUC al genoma celular, así como también se midió la expresión del gen en fracciones nucleares, citoplasmáticas y mitocondriales. Los experimentos de optimización indicaron que una razón de 6,25 mg/2,5 mg de lipopoliamina/DNA plasmidial, 4 veces menos que la reportada, es suficiente para introducir DNA foráneo a las células, lo cual disminuye el riesgo de toxicidad y por ende permite una mayor asimilación de DNA por parte del sistema rececptor. La presencia de secuencias *Sma* I en el vector p103 parecen favorecer su integración, pero no así su expresión, la que presenta niveles basales al compararse co las logradas con el vector pCMVL. La detección de DNA plasmidial de pCMVL asociado al genoma mitocondrial, así como los niveles de expresión del gen indicador en estos organelos, es el primer antecedente reportado de transfección extranuclear y se establece como una potencial alternativa de transgénesis en organismos eucariontes por cuanto se aseguraría la transmisión vertical de los genes de interés, disminuyéndose el riesgo de escape génico al medio circundante.

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Assays for measuring immune response in marine decapods *Callinectes sapidus* and *Penaeus schmitti*

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Decapod crustaceans possess both humoral and cellular components involved in the immune system. The hemolymph of these animals presents humoral and cellular effectors capable to inhibit the bacterial growth. The most common defense cellular reaction is phagocytosis, which involve the hemolymph cell, called hemocytes. These, during phagocytosis, are able to produce microbicide metabolites highly reagents by means of the reduction of molecular oxygen, this reaction is kwon like oxidative or respiratory burst. This immune response was determined in two species of crustaceans when the hemolymph of this decapods were incubated with the bacterium Vibrio parahemolítico. The techniques standardized let determined the production of O^{2-} y H_2O_2 in the hemolymph and the results demonstrate that under *in vitro* conditions the hemocytes from *Callinectes sapidus* and *Penaeus schmitti* are able to release measurable quantities of O^{2-} and H_2O_2.

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Towards obtaining ES cells in the marine fish species *Sparus aurata*; multipassage maintenance, characterization and transfection

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Animal embryonic-stem (ES) cells represent an excellent tool for *in vitro* obtaining targeted genetic changes, whose phenotypic effects can be detected *in vivo*, after transferring the cells to a recipient embryo. ES-cells can be applied either to study early development, or to genetically improve productive traits by manipulating the genome. Only ES cell lines from mice proved to be successful so far, though putative ES cells from other species have been reported.

The interest in obtaining ES cells in fish is due to their advantageous embriology and to the recent development of suitable constructions for transgenesis. Deriving ES cells from the seabream *(Sparus aurata)*, seems attractive because of its commercial value and the application of the ES-cell strategy to increase its productivity. With this aim, suitable short-term culture conditions, based on a feeder free protocol of medakafish have been set up for *S. aurata*. In the present work the multipassage maintenance of a cell culture initiated from mid-blastula cells (SaBE1) is reported. It has been kept for about 6 months (30 passages), showing typical morphology of undifferentiated cells and no signs of instability or senescence. Both monolayer cultures and colonies showed very strong alkaline phosphatase activity and the chromosome counts at several passages, indicated an apparent normal karyotype. Additionally, the SaBE1 cells have been successfuly transfected with a plasmid containing the GFP protein as reporter. The results obtained so far are indicative of ES-like properties. However further *in vitro* tests have to be performed and the final recognition as true ES cells will come after demonstrating the ability of producing chimeras.

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Variation in microsatellite sequences between tilapia belonging to the *Oreochromis* genus

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Polymorphic animal microsatellites have proved valuable genetic markers. In this report, conditions were established to examine the variability of 6 tilapia *(Oreochromis niloticus)* microsatellite loci between tilapia belonging to two species *(O. niloticus* and *O. aureus)* and one transgenic *O. hornorum* hybrid (F70) of the genera *Oreochromis*. The heterozigosity of the microsatellites was determined and the paternity index and power of exclusion calculated, showing that tilapia microsatellites are powerful tools both in regard to gene mapping, population genetic analysis and for individual identification.

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Population structure of the shrimp *Penaeus notialis* assessed using allozymes and mitochondrial DNA

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In the present study we investigate the genetic variation among populations of the shrimp *Penaeus notialis*, the most abundant penaeid specie around Cuba. In this regard, samples of shrimps from five localities in the south central platform of the island (Ana María Gulf) were analyzed using nine polymorphic allozyme loci and PCR-RFLP of a segment of 2072 pb of the mtDNA comprised between the COI and COIII genes.

Of the 25 allozyme loci studied 9 resulted polymorphic and presented no deviation from Hardy-Weingber proportions. Among them three loci contributed significantly to the genetic variation observed Gdh, Est-3 and Fos-2. In contrast to mtDNA at the Ana María Gulf, the level of genetic differentiation from allozyme was significant among some localities. Manatí and Florida were different compared to the rest and among them and a limited genetic flow was evidenced. However, homogeneity of the mtDNA suggested that differentiation al allozyme loci should correspond rather than to recent events to a historical isolation of this subpopulations. The current pattern in the region and some other biological parameters support the results. The genetic distinction of mtDNA among subpopulations from Batabanó Gulf and Ana María Gulf indicates that this marker will be useful to study the genetic structure of *P. notialis* along the Cuban platform.

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MAMMARY GLAND TRANSGENESIS: TODAY AND TOMORROW

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The field of transgenesis in mammals is moving quite fast. In 1983, there were only a few reports about transgenic animals in the main stream of scientific literature. By the end of 1998, in less than 15 years, we have to face the fact that somatic nuclear transfer transgenic livestock has been produced. Needless to mention the establishment of gene replacement in murine embryogenic stem cells, the production of high-value pharmaceutical proteins in the milk of transgenic mammals or pigs for the xenotrasplantation of human organs, just to mention some of the more quickly developing areas of research and investment.

The annual meeting Biotecnología Habana '98 is a good witness of these developments. In our previous edition devoted to transgenesis in mammals during Biotecnología Habana '95 there were important contributions from relevant scientists on the regulation of milk protein gene expression, from the basic science to biotechnology. A lot of effort was then centered on the use of transgenic bioreactors for the production of human therapeutics in the milk.

In this edition the field moved further, as it can be noticed in the following examples:

- Knock-out mice lacking important regulatory elements for milk gene transcription have been produced and characterized.
- Gene ablation of the crucial prolactin receptor was achieved *in vivo* in mice.
- Evidences are on hands about the existence of a casein gene locus controlling region at least in the bovine.
- The role of internal ribosome entry sites (IRES) in the expression vectors has been deeply questioned and instead new data appeared about the role of these sequences in the rescue of translation stimulators (RTS).
- The post-translational machinery of the mammary gland has been challenged with new hard tasks such as the expression of vitamin K-dependent gamma-carboxilated proteins in pigs and recombinant antibodies in rabbits.
- New emerging technologies have gained a leading role in the generation transgenic animals express-