Published in final edited form as:

Comp Biochem Physiol C Toxicol Pharmacol. 2007 February ; 145(1): 1–4. doi:10.1016/j.cbpc.2006.11.013.

Aquatic Animal Models of Human Disease

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The study of disease is, at its core, the study of abnormal structure and function spanning levels of organization from molecules to cells to tissues to organisms (and including populations and ecosystems in the disciplines of epidemiology and epizootiology). Fish and invertebrates from marine and freshwater environments have long provided valuable models for the study of basic biological processes. Some of the earliest studies of non-self recognition and phagocytosis were conducted using sea urchins (leading to the 1908 Nobel prize for Mechnikov) while the principles of signal propagation in axons were elucidated from studies of the giant axon of squid (leading a Nobel prize in 1963 for Hodgkin & Huxley). Aquatic invertebrates and fishes have proved to be valuable model organisms based on specific physical features, such as giant axons, as well as by providing an evolutionary perspective on structure and function in higher vertebrate systems. Less recognized, however, are the contributions that these organisms have made to the study of disease. Historically, such studies have tended to fall into two very broad categories, natural versus laboratory experiments.

Natural experiments are those in which spontaneously occurring disease states are discovered in wild or laboratory animal populations as a result of injurious environmental factors, infectious agents and/or mutations. Outbreaks, or epizootics, of these diseases often serve as a first warning of serious environmental problems, such as when flatfish with liver tumors were found in high numbers in Eagle Harbor in the Puget Sound area of Washington State, USA (Myers et al, 2003). This observation led to the detection of high levels of polycyclic aromatic hydrocarbons in sediments at this site, resulting in the designation of the harbor as a "superfund site" and the eventual remediation of the toxic sediments. In laboratory experiments disease states are induced under controlled circumstances, often in an attempt to determine the causes and mechanisms of spontaneously occurring diseases or to evaluate the potential injurious effects of toxins or drugs. Disease research on aquatic animals involves a wide variety of disciplines such as physiology, pathology, toxicology, immunology, microbiology, embryology, molecular biology and genetics working and a wide phylogenetic range of organisms. To bring together investigators working in such varied fields of disease research,

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a series of meetings focusing on use of aquatic organisms as models of human disease was begun in 2000. This special issue of *Comparative Biochemistry and Physiology* contains 16 manuscripts from a conference entitled “Aquatic Animal Models of Human Disease” held from September 29 to October 2, 2005, at the University of Georgia, Athens, Georgia. This volume joins similar special issues developed from the two previous meetings in this series held in 2000 and 2003 (Walter 2001; Schmale, 2004). The studies presented in this volume demonstrate that fish models can be used to investigate a wide range of disease related questions and can provide unique models for human disease processes.

Aquatic models of human disease

The usefulness of fish as models for disease research is based on both general properties shared by many species of fish as well as features unique to a single species or related group of species. Some general characteristics which make many fish valuable for pathological and toxicological studies include high fecundity with externally fertilized, often transparent eggs and relatively brief generation times. Based largely on these features, over the past two decades the zebrafish, *Danio rerio*, has been developed into a versatile and robust system for the study of such diverse fields as genetic regulation of development, aging, cancer and high-throughput screening of drugs and toxins (Amsterdam and Hopkins, 2006). For toxicological studies, the ease of exposure of aquatic animals to waterborne compounds via the relatively vast epithelial surface area of the gills and skin and the potential to study the effects of body temperature on disease processes as a result of ectothermic physiology are great assets common to most fish species. Many aspects of the genomes of fishes have also provided valuable models for gaining insights into the evolution of genome structure. Most fish genomes reflect a whole genome duplication event which apparently occurred after the divergence of modern fishes from the ancient lineages which gave rise to tetrapods (Amores et al, 1998). Large scale loss of duplicate genes as well as non-coding sequences has resulted in the unusually small, compact genomes found in pufferfish (Brenner et al, 1993; Jallian et al, 2004). In contrast, large genomes are found in some families of fish such as salmonids which appear to have undergone a second, much more recent, whole genome duplication event resulting in tetraploidy these species (Allendorf and Thorgaard, 1984).

In addition to these shared features, many species exhibit unique features or disease syndromes which make them particularly suitable as animal models of specific disease processes. Some well known examples include the extreme sensitivity of rainbow trout to liver tumors induced by aflatoxins (Williams et al, 2003), the predisposition of genetic hybrids generated from backcrossing species of swordtails and platyfishes (genus *Xiphophorus*) to melanomas and other tumors (Nairn et al., 2001a; Walter and Kazianis, 2001) and transmissible neurofibromas in damselfish (Rahn et al., 2004) and papillomas in walleye (Rovnak et al., 2005). The advent of high throughput mutagenesis screens using zebrafish has yielded a wide variety of potential disease models in this species (Amsterdam and Hopkins, 2006). In addition, the ease of producing transgenic fish of several species, especially zebrafish and medaka, has led to development of fish engineered to express fluorescent reporter genes either constitutively or under the control of environmentally relevant promoters such as heat shock and retinoic acid response elements (Perz-Edwards et al, 2001; Carter et al, 2004; Grabher and Wittbrodt, 2004.). Transgenic fish of these species containing engineered mutation “targets” are also serving as new models for study of in vivo mutagenesis and for assessing environmental mutagens (Amanuma et al. 2000; Winn et al., 2000; McElroy, et al., 2006).

The emergence of the new generation of genomic tools and databases has revolutionized the way in which normal developmental and regulatory processes as well as perturbations induced by disease or environmental toxins are studied. The application of these genomic tools to organisms other than humans and traditional laboratory models such as mice and rats has lagged

considerably due to the large investments required to produce these tools for each model species. However, this situation has recently begun to change with more widespread access to high-throughput sequencing technology and bioinformatics database tools. This effort has been aided by completion of genome sequencing projects on several aquatic organisms, including the zebrafish, medaka, pufferfish and stickleback. Other genomes will certainly be added to this list in the near future. The application of such advanced approaches to understanding alterations in the transcriptome and proteome in selected aquatic model species was a major topic of this conference and is reflected in the number of the manuscripts presented in this volume on this subject. Other topics included the development and use of models for toxicology, infectious disease, vertebral morphology and cell culture.

Models of altered gene expression

The review by Ju et al. (2006a) provides an overview of the major issues involved in applying microarray technology to aquatic model species. At least ten species of fish have at least 10,000 ESTs sequenced, including the zebrafish, medaka, rainbow trout, Atlantic salmon, stickleback, killifish, Japanese pufferfish, channel catfish, blue catfish and common carp (Ju et al., 2006a). Microarray studies have been published for most of these species. Many of these studies focus on toxicogenomics, the study of how gene expression patterns change in response to toxins or other stressors in the environment.

An original study by Ju et al. (2006b) in this volume reports on changes in genome-wide expression levels in multiple tissues from Japanese medaka exposed to hypoxia, illustrating the usefulness of microarray technology for the study of an important stressor in many disease processes. A complementary approach to finding hypoxia induced genes in medaka, discussed by Oehlers et al. (2006), is the analysis of differential protein expression using difference gel electrophoresis combined with tandem mass spectrometry. Hook et al. (2006) report the effects of an environmentally relevant xenoestrogen, ethynyl estradiol, on gene expression profiles in rainbow trout using a 16,000 spot cDNA microarray. Page et al. (2006) used another aquatic animal, the salamander *Amblystoma mexicanum*, as a model of hormonal changes induced by environmental endocrine disruptors. Here they report that exogenous application of thyroid hormone results in dramatic changes in keratin gene expression as measured by microarrays.

Two papers in this volume investigate quantitative changes in expression of selected genes in *Xiphophorus* (platyfish and swordtail) interspecies hybrids which may be associated with tumor formation in these fish. Using real-time PCR methodology, Butler et al. (2006) measured mRNA and protein levels of the *Xiphophorus* homolog *CDKN2AB* and retinoblastoma (RB) genes in melanized skin and melanomas. A significant, positive correlation between overexpression of the *XMRK* oncogene and overexpression of the *CDKN2AB* putative tumor suppressor was observed, but *RB* expression did not exhibit the inverse correlation with *CDKN2AB* often seen in mammalian tumors. Such comparative studies have the potential to reveal common and distinctive pathogenic pathways in aquatic vs. mammalian disease models. Focusing on other genes of potential importance in tumorigenesis in these models, Heater et al. (2006) report significant increases in expression of several DNA repair genes in F1 hybrids of several *Xiphophorus* species which may be associated with processes leading to melanoma development in backcrosses involving these species. These studies are representative of the breadth of scientific issues being made accessible in aquatic animal models through the application of gene expression technologies.

Toxicology models

Several studies in this volume focus on toxicological model systems using medaka or zebrafish. Kashiwada et al. (2006) report on the use of a new mutant of the medaka which is essentially transparent, allowing in situ observation of CYP1A activity in the livers of fish exposed to 3-

methylcholanthrene by visualization of fluorescence of resorufin (a metabolite in a key CYP1A pathway). A bioencapsulation methodology for quantitative oral dosing of both water soluble and lipophilic compounds in Japanese medaka is presented by Schultz et al. (2006). This study demonstrated that absorption of model compounds was similar to that seen in rodents when fish were fed compounds encapsulated into newly hatched *Artemia* nauplii. Berry et al. (2006) describe the use of zebrafish embryos as an assay for developmental toxins from marine and freshwater microalgae. These algae, often associated with harmful algal blooms, produce complex arrays of toxic molecules with a variety of acute and chronic effects. Many features of zebrafish embryos and embryogenesis make this species an excellent model for study of developmental effects of toxins. Several distinct developmental phenotypes are documented following exposure to algal extracts from a number of species. Another study using zebrafish as a model of developmental effects is the report by Chun and Chen (2006) of the effects of exposure of embryos to exogenous E-peptides (related to insulin-like growth factors) from human and rainbow trout. Zebrafish embryos microinjected with these peptides exhibited dose-dependent developmental defects in heart, vasculature and red blood cells. Tsyusko et al. (2006) examined radiation induced germline mutations in medaka, demonstrating that this system is useful for measurement of the effects of chronic radiation exposures.

Infectious disease models

A number of infectious disease models have been developed using fish. Two studies included here, by Watral and Kent (2006) and Broussard and Ennis (2006), focus on mycobacterial infections. Watral and Kent (2006) have investigated the pathogenicity of various species of mycobacteria in zebrafish. While all species studied were capable of causing granulomas in at least some fish, only *Mycobacterium marinum* was found to be highly virulent and pathogenic, causing 100% morbidity and 30–100% mortality in exposed zebrafish. Broussard and Ennis (2006) have proposed using medaka infected with *M. marinum* as a model for long-term, chronic *M. tuberculosis* infections in humans. In this study *M. marinum* expressing green fluorescent protein were used to monitor colonization patterns of bacteria in isolated fish tissues and in intact fish of the same transparent strain of medaka used by Kashiwada et al. (2006).

Other disease models

Fish have been used as model organisms in studies of a wide variety of other types of disease processes, including growth and aging and alterations in immune system function, to name a few. Gorman and Breden (2006) provide a review of the usefulness of teleosts fishes as models for the study of genetic and environmental factors which affect vertebral stability and deformity. This represents a relatively new, and as yet largely untapped, area of research using fishes. A major type of resource which is often essential for successful development of animal models is cell culture systems. Fish and particularly the elasmobranch fishes (sharks and rays) have generally lagged far behind mammalian model systems in the development of species-specific cell cultures and cell lines. In this volume Parton et al. (2006) report establishment of the first cell line from an elasmobranch, in this case the spiny dogfish shark. This line, derived from embryos and expressing features suggestive of mesenchymal cells, represents a major breakthrough for studies of all aspects of elasmobranch biology.

Future directions

As exemplified in the papers collected in this special issue of *Comparative Biochemistry and Physiology*, fish and other aquatic animals provide compelling models for research into the mechanisms of human disease, combining ease of generating large numbers of animals and ease of manipulation and observation of developing embryos. In many cases inbred lines and strains are already available. An increasing variety of sophisticated tools and resources for genomics and proteomics are being applied to the generation of robust and tractable

experimental systems using these animals. Fish and other aquatic models are uniquely positioned to take full advantage of advances across the fields of biomedical and basic research, by focusing on developing comparative approaches which enhance and complement investigations using more traditional animal models.

The meetings in this series on aquatic animal models of human disease have provided a unique forum for investigators working in a diverse range of disciplines to exchange information derived from these aquatic model systems and to discuss priorities for development and dissemination of resources essential to advancement of research using these models. Such resources include animal stocks, inbred, transgenic and mutant lines, cDNA and BAC libraries, microarrays, antibodies and cell lines. Development and dissemination of such resources are facilitated by the existence of a number of resource centers, funded by the National Center for Research Resources (NCRR) of NIH as well as by a trans-NIH initiative to support development of tools for zebrafish research. Resource centers currently in place involving aquatic organisms include the National Resource for Zebrafish, the *Xiphophorus* Genetic Stock Center, the National Resource for Aplysia (sea hares used for neurophysiological research), the National Resource Center for Cephalopods (providing squid and octopus), the Sea Urchin Genome Resource and the Ambystoma Resource for Model Amphibians (providing animal and genomic resources for research using salamanders). We believe that conferences such as the ones in this series can improve efficiency of resource sharing and increase awareness of the usefulness of these models and thereby help generate new opportunities for fish and other aquatic models to be used in biomedical research.

Lastly, we wish to acknowledge those who supported this conference which was hosted at the University of Georgia. This meeting would not have been possible without the generous support of the National Center for Research Resources and the National Institute of Environmental Health Sciences of the NIH, the Roy F. and Joann Cole Mitte Foundation, the Marine and Freshwater Biomedical Sciences Center at Oregon State University, the Office of the Vice President for Research and the Warnell School of Forestry and Natural Resources of the University of Georgia. We would especially like to thank the members of the organizing committee, Ron Walter (Texas State University), James Lauderdale (University of Georgia), Marjorie Oleksiak (University of Miami) and Dave Williams (Oregon State University).

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