Book Review

Aquaculture and Fisheries Biotechnology: Genetic Approaches

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With the increased global demand for aquaculture products comes the need for developing more efficient production systems. The emergence of aquatic biotechnology over the past 25 years poses the possibility of manipulating a wide variety of traits valued by aquaculturists and fisheries managers. In addition to benefits for aquaculture and fisheries, however, aquaculture biotechnology also presents a number of controversies. Against this background, Rex Dunham presents *Aquaculture and Fisheries Biotechnology: Genetic Approaches*. His aim, stated in the preface, is to explain to students, farmers, fisheries biologists, and scientists how theory relates to reality and to provide a strong review of the current status of key biotechnology topics, illustrating concepts with key research results. He aims to be objective regarding controversial topics, presenting various viewpoints and then discussing differing perspectives in the context of the available data. The book has 19 chapters, with a supporting glossary, extensive references to the technical literature, and an index.

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Chapter 1 presents a short, concise history of genetics and biotechnology, placing aquaculture biotechnology within its larger context. Genetics is traced from Robert Hooke (cell theory) through Charles Darwin (natural selection), R.A. Fisher and Sewall Wright (quantitative and population genetics), and the rise of molecular genetics and biotechnology. Developments in the breeding of aquatic organisms are traced from non-scientific carp breeding by the Romans and Chinese to the emergence of science-based selective breeding, to the rise of aquaculture biotechnology, including ploidy manipulation, sex reversal, gene transfer, and genomics. The chapter effectively provides the historic context for aquatic biotechnology, and the few glitches (e.g., Frank Ruddle's group produced the first transgenic mouse – see Gordon *et al.* 1980, Dolly the sheep was cloned in 1996) will not distract most readers.

To effectively measure and exploit the genetic components of variation for purposes of selective breeding, environmental sources of phenotypic variation must be recognized and then controlled for experimentally or corrected. Chapter 2 proceeds from a brief explanation of partitioning of phenotypic variance to a discussion of the numerous types of environmental effects, including stocking density, age, and maternal effects. Making the point that many published papers in molecular genetics and biotechnology ignore details of how the fish were cultured, Dunham provides important background for evaluating the validity and contribution of aquaculture biotechnology studies.

Manipulation of entire chromosome sets has proven a key area of aquatic biotechnology, producing triploid stocks that are sterile or rapidly growing for particular species. In Chapter 3, Dunham reviews the literature on polyploidy induction in fish and shellfish, considering methods for ploidy manipulation, ploidy assessment, and performance of triploid stocks. He then considers the implications of using existing triploids stocks for a range of potential applications in aquaculture and fishery management. This is generally a strong chapter; Dunham explains why triploidy is or is not a viable option for various species in aquaculture or fisheries management contexts, and concludes that polyploidy has good potential for genetic conservation.

Chapter 4 begins by considering gynogenesis and androgenesis, respectively, chromosome set manipulation-based methods for propagating individuals with all-maternal or all-paternal inheritance. Methods for induction of gynogens and androgens are explained, and their use for

producing monosex stocks and clonal lines is considered from the viewpoints of phenotypic variability, regeneration of genetic variation, growth, disease resistance, and production of clonal hybrids. I would have valued a presentation of the author's evaluation of the utility or limited utility of gynogens and androgens in aquaculture and fisheries. Material on chromosome set manipulations is followed by material on nuclear transplantation, because both sets of methods relate to cloning. Dunham begins by explaining the cloning of individuals by transplantation of nuclei from donor cells to enucleated eggs, citing key studies outside of aquatics and then focusing on fishes. While the piscine literature is covered rather fully, some key issues are overlooked. The different implications of transplanting nuclei from embryonic cells or from fully differentiated cells on subsequent gene expression and performance of the cloned individual (see, for example, NRC 2002) are not discussed. A section on possible applications of nuclear transplantation, e.g., for rapid generation of true-breeding transgenic lines would have added to the practical utility of the chapter.

Utilization of monosex or sterile populations of fish is a solution or partial solution to problems associated with sex-related differences in performance, early sexual maturation, and unwanted reproduction. Techniques available for producing monosex or sterile populations – including chemical and mechanical sterilization and hormonal sex reversal – are discussed in Chapter 5. There is a strong section on genetics of sex determination in Nile tilapia, and a strong concluding section shedding light on practical issues constraining commercialization of monosex aquaculture stocks.

Development and screening of genetic markers has had a huge impact on aquaculture and fisheries management, an impact that will grow in the future. Chapter 6 discusses the development of biochemical and genetic markers and selected applications, mostly on management of aquaculture species. Methods are discussed in their historical order of development, and include isozymes, DNA restriction fragment length polymorphisms, mitochondrial DNA, randomly amplified polymorphic DNA, amplified fragment length polymorphism, microsatellite DNA, expressed sequence tags, and single nucleotide polymorhisms. The relative costs and effectiveness of the respective marker methods are compared. While the chapter covers methodological and aquaculture-related topics well, the huge impact of genetic marker methods on fishery management

is not well addressed. Landmark publications are not mentioned in the context of discussions of the respective marker methods, e.g., Ryman and Utter (1987) in the context of isozymes, and the work of John Avise and many other researchers regarding mitochondrial DNA. Microsatellite DNA markers are now the most widely used markers for population genetics and have had a huge impact on fisheries management, which is not discussed or referenced. The last section of the chapter begins to address issues posed by management of natural populations, but does not draw on numerous case studies that could illustrate applications of molecular markers in fisheries management, for example, in management of anadromous salmonids.

Interactions of hatchery and wild fish – a focus of continuing controversy among aquaculturists, fishery managers, ecologists, and population geneticists – are discussed in Chapter 7. The first part of the chapter is intended to provide a foundation for understanding population genetics. The reader would be served by mention and definition of all population genetic processes, but the section is very uneven, covering some processes in detail, while covering others only in cursory fashion. For example, discussion of coadaptation and outbreeding depression does not address the multilocus nature of coadaptation, as modeled in Sewall Wright's (1932) adaptive landscape. There is a growing literature on coadaptation in fishes (Hallerman 2003) to which interested readers should refer. Mention of many landmark studies or reviews of population genetics of fish is omitted, e.g., regarding inbreeding, Allendorf and Leary (1986) on fluctuating asymmetry, or Kincaid (1976a, 1976b, 1983) on inbreeding depression. Reviews of the huge literature on how population genetics affects fisheries management (e.g., Ryman and Utter 1987, Utter and Ryman, 1993) might have been cited to give depth to what is necessarily limited coverage in a single chapter. The issue of how population genetics affects conservation was not given sufficient coverage; critical concepts such as evolutionarily significant units (ESUs - Waples 1991, Moritz 1994, Nielsen 1995) should have been defined in depth, if only to refer the reader to the technical literature. Instead, the issue of what unit to conserve (page 109) was approached from the viewpoint of "performance" as opposed to adaptive characters. Mention that negative impacts of cultured fish upon wild gene pools might be ameliorated by natural selection was not appropriately qualified by discussion of the long time required, of the impact of intermittent introductions, and of loss of

between-population genetic diversity. These are perhaps the best examples of how, while thought-provoking and citing many interesting studies, the chapter is uneven and written from an aquaculture geneticist's view. Discussion of geographic distribution of genetic variation did not mention such key driving factors as the recolonization of North America by fishes after deglaciation. Discussion of the effects of stocking hatchery brook trout did not mention the landmark work of McCracken's group (e.g., McCracken *et al.* 1993), and of interspecific hybridization did not mention introgression of rainbow trout into cutthroat trout gene pools (Leary *et al.* 1995). The concluding discussion of an integrated management strategy, describing designation of areas where different degrees of preservation or manipulation of aquatic gene pools would be practiced, omitted discussion of such key operational issues as how areas would be designated and subtleties of management.

Development of large numbers of genetic markers gave rise to genetic mapping of entire genomes, detection of loci affecting expression of quantitative traits (QTLs), and application of such knowledge for genetic marker-assisted selection (MAS) (Chapter 8). This is a very thorough chapter in terms of reviewing research to date with aquatic organisms. However, the chapter has its quirks and minimizes treatment of certain key issues. For example, in a very thorough section on isozyme-based genetic maps is a long passage on DNA marker-based mapping of puffer fish. The section on QTL mapping does not mention that segregation is followed *within families*. The section on MAS might have discussed how marker and phenotypic performance information are combined in a selection index. There is a huge literature on these topics, and these should have been major sections with appropriate citations to the technical literature. Certain omissions will make the chapter more difficult for most readers to understand. For example, there is no figure showing a genetic map, nor a figure comparing genetic maps to show conservation of linkage groups.

Elucidation of gene function is a rapidly-developing area of genomics, and Chapter 9 discusses gene expression, isolation, and cloning. These procedures are discussed in the context of what we have learned of such key processes as development and growth, reproduction, disease resistance, brain function, cold tolerance, and osmoregulation. Genome-level issues such as genetic imprinting, transposable elements, and ribosome function are described, and the chapter ends with a very brief presentation on

proteomics. This is generally a well-done chapter, but has its quirks and omissions. Microarrays are not described or shown, leaving the reader with only a description of their applications. A DNA dot-blot is shown, but neither the genes spotted on it, nor the probe used to screen it are specified. Much more progress has been achieved on understanding of MHC (major histocompatibility complex) function and on proteomics than is alluded to. The notion that differential performance of reciprocal crosses of many species may be attributable to genetic imprinting was not considered.

Applied with highly visible success in fishes, gene transfer technology is discussed in Chapter 10. Given the author's notable involvement in gene transfer in common carp and channel catfish, it is not surprising that this is one of the stronger, more thorough chapters in the book. Dunham covers gene transfer techniques, expression vectors, integration and transmission of gene constructs, and pleiotropic effects of transgenes. He considers the performance of transgenic fishes for traits relating to growth, cold tolerance, and disease resistance. In a more prospective analysis, he discusses transgenic production of pharmaceuticals, gene knock-out technology, and potential role of mitochondrial DNA in gene transfer. The chapter is well illustrated. However, there are a few questionable assertions and glitches. The suggestion that use of active transposons for gene transfer is a good idea should be questioned (NRC 2002). Neither the Anderson *et al.* nor Liang *et al.* citations seem related to cold tolerance. Hybrid breakdown in F_2 and F_x generations is widely regarded as caused by disruption of coadapted gene complexes (Hallerman 2003), not by nuclear-mitochondrial DNA interactions.

Maximum genetic progress in development of genetic lines of aquatic organisms will come from combining different sorts of genetic enhancement programs. A brief Chapter 11 considers combinations of, for example, sex reversal and triploidy and gene transfer and crossbreeding. The author is correct in his assertion that combinations of approaches may be especially powerful when combined, and cites many interesting examples. However, some biotechnological approaches were not combined for the purposes suggested by their inclusion in this chapter. Notably, Devlin et al. (2001) did not crossbreed a transgenic line with a wild population in order to increase the effect of transgene expression, but rather to assess the risks posed by introgression of a transgene into a wild stock. In the context of a passage on gynogenesis and selection to improve immune response, it is never asked whether a high antibody response

is necessarily a desirable trait. These aspects of the chapter can leave readers unfamiliar with the material without a critical perspective.

Aquaculture stocks genetically improved in a research environment are not necessarily high-performance stocks in a commercial production environment, and such genotype-environment (GE) interactions are considered in a brief Chapter 12. The material is straightforward, leading to the appropriate conclusion that GE interactions on performance should be evaluated before manipulated stocks are commercialized. More detail on how to set up, analyze, or evaluate studies of GE interaction would have been helpful to many readers

Commercialization of transgenic aquatic organisms on a large scale poses environmental hazards (Chapter 13). Consideration of these hazards from the theoretical and empirical viewpoints comprises a large literature. Dunham draws from the literature but sometimes adds inferences that ought to be questioned, resulting in a mix of well-reasoned and less well-reasoned arguments. The reader must consider these critically for him/herself. For example, empirical estimations of fitness components generally show that fitness-related characters are decreased in transgenics. Recent work on the net fitness of transgenics indicates, however, that it is not enough to identify *a priori* a key fitness-determining factor (e.g., vulnerability to predators), show lessened fitness for this trait, and then conclude that selection will remove the transgene from a receiving population. The *net* effect of the transgene on *overall* fitness will determine the genetic impacts of transgenics on a receiving population, impacts that may take many generations to reach equilibrium. To argue that selection would remove maladaptive genotypes from a population, posing only temporary harm (pp. 203, 205) does not consider either the (generally slow) rate at which selection operates in natural populations or the effect of recurring introductions of such genotypes into a receiving population. The assertions that transgenic fish may not have greater genetic impact on natural populations than domestic conspecifics (p. 204) or that "all available data indicate that transgenic fish are less fit than non-transgenics fish and would probably have little, if any, environmental impact" (p. 206) are bold speculations. Dunham goes on to suggest that escaped transgenic fish, by adding genetic diversity to populations, could increase fitness and render such populations more viable, an assertion made by no one else in the sector. This part of the chapter does not come to the key bottom-line conclusion, that risk assessment

and risk management must be done on a case-by-case basis, considering species, transgene construct, integration event, and receiving ecosystem. Conspicuously missing from the chapter was discussion of studies of non-transgenic fish injected with the growth hormone (GH) protein, a ready model for GH transgenic fish which can be stocked in the wild for realistic ecological risk assessment. For example, brown trout injected with GH were more willing to risk exposure to a predator than non-injected fish (Johnsson *et al.* 1996). Similarly, among non-transgenic rainbow trout, aggression was lowest in the control pairs, intermediate in the control/GH-injected pairs, and highest in the GH-injected pairs (Jonsson *et al.* (1998a), supporting the hypothesis that GH increases aggression levels. Reproductive confinement would go a long way toward addressing genetic and ecological hazard posed by transgenic fish and Dunham presents a detailed consideration of progress towards achieving transgenesis-mediated sterility. This is generally a strong passage, although hazard posed by progressive reversion of triploid oysters towards diploidy is not mentioned.

Foremost in the minds of many consumers is the issue of safety of food products from transgenic organisms. The safety of GM foods has been examined thoroughly and is reviewed in Chapters 14 and 15. Chapter 14 provides a brief general review and correctly identifies allergenicity as posing the greatest potential for harm to consumers. U.S. oversight by the Food and Drug Administration and international guidelines promulgated by the Codex Alimentarius Commission are briefly described. Interested readers might note that subsequent to the book going to press, FAO and WHO (2004) published the recommendations to Codex Alimentarius of a workshop on food safety of products of animal biotechnology including fish. Issues posed by labeling of GM foods are briefly discussed. Against this background, Chapter 15 presents a very detailed case study on safety of consumption of transgenic salmon expressing elevated levels of growth hormone and insulin-like growth factor. Dunham leads the reader through the technical literature to show lack of bioavailability to the human consumer and lack of bioactivity of these piscine hormones in humans. Appropriately, he briefly reviews results of Cuban studies of food safety of growth hormone transgenic tilapia. In the best review of the subject that I have read, he concludes that GH and IGF levels in transgenic fish products pose no hazards to human health.

Policies adopted by nations and international bodies will have major bearing on the pace and extent of adoption of aquatic biotechnology. Chapter 16 considers regulation of transgenic fish, reviewing development of biotechnology policy in the United States, the European Union, Canada, and the United Kingdom. Developments in Cuba and China, countries with transgenic lines in advanced stages of development, are not discussed. Development of biotechnology policy under the U.N. Convention on Environment and Development is discussed in terms of its impact on adoption of GM organisms in developing countries. The effects of World Trade Organization decisions and intellectual property rights (IPR) will have a large impact on international trade in GM products. While generally a sound treatment, there is no consideration of how IPR came to be applied to living materials and to animals in particular, a surprising omission given that the landmark case, *Ex parte Allen*, involved triploid oysters and that a key review (Hallerman and Kapuscinski 1990) appears in the list of references. For interested readers, a detailed review of 10 states' regulatory policies on GM aquatic organisms is presented by Stenquist (1998).

Certain products of biotechnology are reaching commercial application (Chapter 17). Triploid salmon, rainbow trout, grass carp, and Pacific oysters are now produced, variously for reasons of reproductive confinement and maintenance of product quality after age of maturation. Sex reversal is used to produce all-female salmonid and silver barb and all-male Nile tilapia stocks. Dunham also mentions progress towards possible commercialization of transgenic fishes, notably the AquaBounty Atlantic salmon (which was not approved by FDA in 2004 as the author had expected). Subsequent to the book going to press, GloFish fluorescent zebrafish were commercialized as ornamentals (Hallerman 2004).

Chapter 18 – discussing strategies for genetic conservation, gene banking, and maintaining genetic quality – in many respects picks up where Chapter 7 leaves off. The first section, discussing population size, inbreeding, and maintenance of genetic quality – while a review of interesting studies – does not convey a straightforward picture of principles and procedures that a breeder should apply when establishing and maintaining a captive population. For straightforward guidelines, interested readers might consult Tave (1993) for aquaculture broodstocks or Frankham *et al.* (2002) for stocks of imperiled species. Discussion of how to avoid inbreeding notably lacked mention of rotational line crossing

(Kincaid 1977). A particular level of inbreeding where inbreeding depression becomes likely was put forward without qualification; the reader would have been better served by mentioning that the critical level varies among species, stocks, and environments and supporting the statement with reference to case studies. The possibility of purging deleterious alleles from small captive populations omits mention of the classical work with Speke's gazelle (Templeton and Read 1984), and of studies questioning the approach (e.g., Hedrick 1994). Discussion of genetic drift does not acknowledge the relevant case where a transgenic line is established by one founder with one integration event. Pathbreaking isozyme-based studies of sperm competition in mixed milt of salmonids by Gharrett and Shirley (1985) and Withler (1988) are not cited. The chapter ends with a section on genetic conservation emphasizing biotechnological approaches such as cryopreservation and cloning. Considerations regarding living gene banks are not discussed. The flagship genetic conservation program – the Norwegian living and cryopreserved gene banks for Atlantic salmon (Gausen 1993) – is not mentioned. For a complete assessment of the range of issues, interested readers might consult Cloud and Thorgaard (1993).

Various environmental, research infrastructure, economic, and political issues will have to be resolved for aquaculture genetics to make its maximum contribution. Chapter 19 presents recommendations for resolution of these issues made by a working group chaired by Dunham in anticipation of the 2001 International Conference on Aquaculture in the Third Millennium. These recommendations provide a succinct and appropriate way to put the development of aquatic biotechnology into perspective at the end of the book.

While providing a useful resource, the glossary and references sections have some omissions. Key undefined terms pertaining, for example, to genome mapping, include ortholog, paralog, Kosambi cM, paint probe, and centirays. The references section lacks the supporting citation for certain key studies cited.

While I have my criticisms, *Aquaculture and Fisheries Biotechnology: Genetic Approaches* is the best review to date and will prove useful to a variety of readers. The general strength of this book is the thorough review of applications of aquatic biotechnology to aquaculture, and it will serve as a useful reference to a range of professionals. Its general weakness regards applications to population genetics and fishery

management. For teachers, combining a book focusing on selective breeding with this volume focusing on biotechnology would support a state-of-knowledge course on genetics in aquaculture.

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