Community Sequencing Program: Project Proposal

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Project Title: A Proposal Advocating Draft Sequencing the Genome of Rainbow Trout, Oncorhynchus mykiss
I. Abstract:

This proposal has been assembled by those in the scientific community interested in obtaining a whole genome sequence for rainbow trout (*Oncorhynchus mykiss*). The 85 authors represent a broad diversity of scientific disciplines and geographic distribution, hailing from multiple departments located at 46 institutions in 12 countries. In addition, 56 letters of support have been submitted by some co-authors to supplement and/or expound upon the arguments outlined in this document. These serve to individually demonstrate their need for a rainbow trout genome sequence and the impact it will have on the scientific community.

Rainbow trout are of the family Salmonidae, native to the Pacific coasts of North America and Russia, and have been widely introduced around the world. Considerable basic biological knowledge has been developed about this species as an outgrowth of their widespread cultivation as a food and sport fish. As outlined in this proposal, rainbow trout are excellent model organisms for studying the evolutionary process (comparative genomics, evolutionary fate of duplicate genes, and genetic architecture of complex phenotypes), carcinogenesis, toxicology, comparative immunology, disease ecology, physiology and nutrition. More is known about the physiology and biology of rainbow trout than any other fish species. Closely related species within the *Oncorhynchus*, *Salmo* and *Salvelinus* genera have also been studied extensively. In the past 20 years there have been over 40,000 reports on the ecology, behavior, physiology and genetics of these species, with rainbow trout specifically being used in half of these studies. Sequencing the trout genome will combine a set of diverse advantages that are not available in any other research organism. Such sequence information will enhance scientific progress by facilitating the association of genes with functions contributing to complex phenotypes. Scientists currently using rainbow trout to investigate biological questions falling within the disciplines listed above are anxious to enhance their research approaches with the benefit of genome sequence information.

The resources currently available for rainbow trout genomics include doubled haploid clonal lines, genetic linkage maps, an EST database (over 120,000 sequences), microarrays, well-characterized BAC libraries, physical maps, and established models for gene transfer. We believe that the availability of these resources, vast amounts of accumulated biological information, and the economic and scientific importance of this species make it an excellent candidate for genome sequencing at this time.

II. Scope of Work:

We propose that through the Community Sequencing Program the JGI:

- Shotgun sequence 8X genome coverage from a representative of the doubled haploid Swanson strain (clonal line) developed at Washington State University (Robison et al., 1999).
- Assemble shotgun sequences into contigs.
- Determine open reading frames.
- Work with community through the Proposers to develop repeat masking tools.

We propose to use DNA from the homozygous Swanson clonal line developed at Washington State University. Genomic DNA from this YY-male line was used by the USDA/ARS National Center for Cool and Cold Water Aquaculture (NCCCWA) to construct a 10X BAC library. Recent physical mapping of loci of interest has demonstrated the advantage of working with a single homozygous individual as duplicated loci were clearly distinguished by DNA
fingerprinting (Palti et al. 2004). Additionally, a large mapping panel of two hundred OSU x Swanson doubled haploids will be available by December 2004 at Washington State University to facilitate high-density genetic mapping.

III. Technical Information:

**Genome Size:** Estimated genome size is $2.4 \times 10^9$ base pairs (Ohno and Atkin, 1966). This estimate is supported by the findings of Palti et al. (2004) which confirmed a 10X genome coverage by the Swanson BAC library. The combined insert size for this library was estimated at $24 \times 10^9$ (184,704 clones x 130,000 bp average insert size).

**Genome Organization:** The number of chromosomes in rainbow trout varies between populations but the number of chromosome arms is constant at 104. The Swanson strain has 2N=58 chromosomes.

**G:C Content:** The rainbow trout genome has 42.9% GC content (Bernardi and Bernardi 1990).

**Polymorphism Level:** Wild populations of rainbow trout have the greatest levels of average heterozygosity (0.059) among salmonids as revealed by protein electrophoresis (Allendorf & Utter 1979). Although most of the total variation is maintained within populations (92% Hershberger 1992; 85% Ryman 1983) the remaining variation reflects substantial subdivision. Similar observations have been made in domesticated broodstock using microsatellite data (Silverstein et al., 2004). The use of a doubled haploid clonal line will alleviate difficulties that would result from such a high degree of polymorphism.

**Repeat Structure:** Repetitive elements are abundant and interspersed throughout the rainbow trout genome. They typically contain conserved sequence motifs as well as features unique to salmonid genomes. Tc1-like transposons have widespread occurrences (Radice et al., 1994; Leaver 2001), and SINE elements were shown by fluorescent in situ hybridization to be interspersed throughout all the chromosomes except for heterochromatin-positive areas (Perez et al., 1999). Current software for masking repeats in the assembly and annotation process is not sufficient and should be customized for typical teleost and salmonid repeats (Hansen and Palti, unpublished data).

IV. Available Resources:

**Germplasm:** Major advantages for using rainbow trout for genetic research include:

- **High fecundity** – A single 3 to 4 kg female produces 3000 eggs, and a very large amount of sperm (10 ml) is available from a single male.
- **Gamete manipulation** – The hardiness of rainbow trout gametes facilitates transportation (air shipment world-wide) before and after fertilization from a broodstock holding facility to grow out farms or research institutions. Chromosome set manipulations by means of heat shock or hydrostatic pressure shock are commonly utilized by farmers and scientists to produce gynogens (all-female populations which avoid deterioration of flesh quality due to early sexual maturation), androgens (Young et al. 1996), and to induce triploidy (employed for producing sterile fish to reduce genetic contamination of wild populations by farmed fish, Thorgaard, 1992; Palti et al., 1997).
- **Clonal lines** – Seven clonal lines of rainbow trout have recently been established to provide valuable experimental uniformity (well-established as an advantage by research with inbred lines of mice) and will provide opportunities for analysis and genetic
dissection of traits as differences among the lines are identified (Ristow et al., 1995; Robison et al., 1999) and genetically characterized (Robison et al., 2001).

**Gamete availability** – Year round supply of gametes due to a wide natural variability in spawning time (Siitonen and Gall, 1989; Sakamoto et al., 1999) and the use of photoperiod control.

**Semen cryopreservation** – Well-developed and widely-used methods (e.g., Wheeler and Thorgaard, 1991) allow for crosses between strains that spawn at different seasons and between different generations (e.g. for backcrossing mating design).

**Transgenics** – Gene transfer is well-developed for the trout model. The transfer of new genetic information into species has proven and will continue to be a powerful tool for studying the physiological, phenotypic, and fitness consequences of specific genes (Pereira, 2000; Tyagi and Mohanty, 2000). A great deal of research has been undertaken with model fish species, including zebrafish and medaka, as well as with salmonids, carps, catfish, tilapia, loach, and shellfish (Bachere et al., 1997; Sin, 1997; Ivetac et al., 2000; Maclean and Laight, 2000; Hew and Fletcher, 2001). To date, germ-line transgenic trout have been produced by microinjection of gene constructs into the fertilized egg shortly after fertilization in numerous studies (see Devlin et al., 2001a; Hew and Fletcher, 2001 for references). Other approaches utilizing sperm-mediated incubations, biolistic (gene gun), or electroporation methods have been explored in fish, but have produced equivocal or negative results (Chourrout and Perrot, 1992; Sin, 1997), and no confirmed cases of germ-line transformation of trout to date.

**Genetic Maps:** Genetic linkage maps are also developing for this species (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003). Two of the maps (Young et al., 1998; Nichols et al., 2003b) were based on a cross of two of the clonal lines. A larger panel of OSU x Swanson doubled haploids is currently being propagated in Dr. Thorgaard’s lab for high density linkage mapping. The two populations are diverse in many traits of scientific interest and genomic DNA from each line has been used for constructing BAC libraries. Over 500 polymorphic microsatellite markers have been developed for rainbow trout at the USDA/ARS National Center for Cool and Cold Water Aquaculture (NCCCWA) in the past 3 years (e.g. Rexroad et al., 2001). Approximately half of the microsatellites developed for Atlantic salmon work for rainbow trout (Roy Danzmann, unpublished data). Microsatellites developed for the Pacific salmon (Oncorhynchus) species also generally work for rainbow trout. This brings the total number of microsatellites currently available to well over 1,000. Currently all available microsatellite markers are being genotyped for the creation of a high-density microsatellite genetic map at NCCCWA.

**Mitochondrial Genome:** The mitochondrial genome of rainbow trout has been fully sequenced (Zardoya et al., 1995) and will be useful when assembling contigs from whole genome shotgun sequences. There are likely to be excellent opportunities to examine the effect of mitochondrial variation on development and physiology because using androgenesis it possible to create lines with the same nuclear but different mitochondrial genomes (Brown and Thorgaard, 2002).

**EST Data:** A major effort is now focused on increasing the number of sequences in the rainbow trout EST database (Rexroad et al., 2004). The most recent TIGR rainbow trout gene index release January 6, 2004 was composed of 109,857 expressed sequences in 21,303 Tentative Consensus sequences (TCs, EST contigs) and 26,431 in singletons for a total of 136,288 sequences (http://www.tigr.org). The total number of rainbow trout protein sequences in
GenBank as of January 31, 2004 was 1436, nucleotide sequences numbered 144,371 with 13,458 UniGene clusters. An additional 80,000 ESTs are currently being sequenced by the collaborative genome project of NCCCWA and West Virginia University and another 20,000 will soon be available from the French INRA project. The total number of ESTs available in the summer of 2004 is expected to be well over 200,000.

**Functional Genomics:** As the volume of publicly available expressed sequences expands, our research community is quickly moving into functional genomics. In addition to many targeted microarrays including select genes for studying pathways of interest, two publicly available high-throughput microarrays will be available in March 2004. A rainbow trout PCR based microarray has been constructed by Dr. Paul Coussens at Michigan State University to represent the first 8000 TCs from the first version of the TIGR gene index. A second PCR based microarray has been generated by the GRASP project and represents 16,000 expressed sequences. Over 15% of these sequences are derived from rainbow trout; experimental evidence with previous versions of this array (3700 gene array) has shown that cross-species hybridization works well between salmonids.

**BAC Libraries and Physical Mapping:** Four BAC libraries of the rainbow trout genome have been constructed to date. Two were constructed in Japan by Katagiri et al. (2001). These contain an average insert size of 58 kb and 110 kb, and provide haploid genome coverage of 6.7 fold and 5.3 fold, respectively. Two BAC libraries from the OSU female homozygous line and the Swanson male homozygous line were commercially constructed by Amplicon Express Inc.. The OSU BAC library has 96,768 clones arrayed in 384 well plates with an average insert size of 110 kb (4.5X coverage). The Swanson BAC library has 184,704 clones arrayed in 384 well plates with an average insert size of 130 kb (10X). DNA fingerprinting was used for local physical mapping of 20 genes in the Swanson library, which demonstrated its utility for identifying duplicated loci and confirmed its 10X coverage (most probes yielded two sets of contigs with an average of ~10 BAC clones each, Palti et al. 2004). Both libraries are being used for genomic sequencing and mapping of type I markers (ESTs), and can be used to produce a sequence-ready BAC contig map (Phillips et al. 2003; Gahr et al. 2004). BACs from the OSU and Swanson libraries have been used as probes in fluorescence in situ hybridization to anchor the genetic linkage map to specific chromosomes (Philips, 2001; Phillips et al., 2003). A publication using BAC probes and type I and II markers to anchor every chromosome to the linkage groups on the genetic map of Nichols et al., (2003) is in preparation.

**Importance of Scientific Information:**

Researchers in a number of countries, including the US, Australia, Canada, Chile, Denmark, Finland, France, Germany, Ireland, Japan, Netherlands, Norway, Spain, Sweden, and the United Kingdom are currently conducting genetic, evolution, carcinogenesis, toxicology, reproductive physiology and immunology research with this species. The 85 co-authors of this proposal represent this community as described in their 54 letters of support. We anticipate that the availability of full genome sequence data for rainbow trout will expand the community and lead to increased research with this attractive model species.

Trout and salmon, in addition to being important animals in aquaculture and sport and commercial fisheries, are some of the most biologically fascinating vertebrates on earth. Their migrations, ability to move between fresh and salt water and home to specific rivers of origin
constitute one of the great puzzles of nature. Genomic studies promise to help to address these and other interesting biological features of these fishes.

Two fish species for which complete genome sequences will soon be available are the zebrafish, a freshwater species native to south Asia which is smaller than a goldfish, and the pufferfish (*Fugu*), a marine fish with an unusually small genome. The zebrafish is a superb experimental species for development and genetic research. Although the pufferfish is not widely used as an experimental model, its small genome allows interesting comparisons with other species. Trout are a very distinct evolutionary lineage from the zebrafish and fugu. Their large size and native distribution in North America have facilitated a wide range of research (TABLE 1). Sequence information for trout will ultimately also be of considerable importance to issues related to conservation and agriculture.

**TABLE 1.** Comparison of numbers of publications in PubMed for rainbow trout, zebrafish and pufferfish. Numbers in parentheses are the percentage of total publications from the last 3 years. As shown, nearly half of the publications for zebrafish and pufferfish are from the last three years, during which time draft and whole genome sequencing projects have been completed, respectively. With the sequencing of the rainbow trout genome, we would expect the diversity of research areas to continue, yet we would see a similar increase in publication rate.

<table>
<thead>
<tr>
<th></th>
<th>Rainbow Trout</th>
<th>Zebrafish</th>
<th>Pufferfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total publications</td>
<td>5507 (19)</td>
<td>4010 (45)</td>
<td>344 (51)</td>
</tr>
<tr>
<td>Physiology</td>
<td>4633 (18)</td>
<td>3480 (42)</td>
<td>286 (47)</td>
</tr>
<tr>
<td>Hormone</td>
<td>1040 (18)</td>
<td>213 (49)</td>
<td>23 (61)</td>
</tr>
<tr>
<td>Nutrition</td>
<td>208 (20)</td>
<td>9 (44)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Genetics</td>
<td>1070 (29)</td>
<td>2541 (43)</td>
<td>263 (46)</td>
</tr>
<tr>
<td>Transgenic</td>
<td>32 (31)</td>
<td>208 (53)</td>
<td>24 (58)</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>277 (15)</td>
<td>238 (40)</td>
<td>5 (60)</td>
</tr>
<tr>
<td>Evolution</td>
<td>255 (31)</td>
<td>571 (55)</td>
<td>165 (62)</td>
</tr>
<tr>
<td>Behavior</td>
<td>177 (29)</td>
<td>177 (47)</td>
<td>5 (80)</td>
</tr>
<tr>
<td>Cancer</td>
<td>138 (7)</td>
<td>85 (55)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>65 (6)</td>
<td>11 (45)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Toxicology</td>
<td>29 (24)</td>
<td>23 (52)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Toxicity</td>
<td>660 (23)</td>
<td>177 (40)</td>
<td>9 (56)</td>
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<tr>
<td>Immunology</td>
<td>581 (24)</td>
<td>102 (37)</td>
<td>12 (33)</td>
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<td>451 (22)</td>
<td>132 (36)</td>
<td>4 (25)</td>
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<td>Environment</td>
<td>867 (21)</td>
<td>185 (39)</td>
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<tr>
<td>Development</td>
<td>709 (26)</td>
<td>1945 (47)</td>
<td>26 (100)</td>
</tr>
</tbody>
</table>


**VI. Post-sequencing Plans:**

The rainbow trout genome research community, through coordination of the 4 Co-PIs, will work to obtain funding from US, foreign governments and international funding organizations to finish and functionally annotate the genome sequence.
Some of the Proposers are conducting ongoing MHC sequencing projects and will immediately integrate draft sequence data as a test run for finishing the genome sequence. This will represent roughly 1.4 Mbp of finished/annotated sequence.

- Conduct high-throughput end sequencing of the Swanson YY BAC library BACs to close gaps.
- Conduct in partnership with JGI gene annotation jamboree.
- Work with JGI to develop repeat masking tools.
- Dr. Dan Lee at The Institute for Genome Research (TIGR) has developed the rainbow trout gene index (http://www.tigr.org/tdb/tgi/rtgi). In his letter of support he stated that:
  - 1) TIGR has developed an efficient pipeline to assemble and annotate prokaryotic and eukaryotic genomes.
  - 2) TIGR would like to provide all the necessary bioinformatics support for annotating the genome and further functional genomic research including the EST assembling, gene modeling by mapping the known trout genes back to genome, SNP prediction, oligo array design and comparative genomic analyses involving the pufferfish, zebrafish and trout genomes etc. All of the data generated will be available for general public use as soon as possible.
- Data availability – The Proposers have the ability and will create a database similar to ZFIN (http://zfin.org/cgi-bin/webdriver?MIval=aa-ZDB_home.apg) to make genome sequence and functional annotation publicly accessible for data mining through the internet.

Several DNA chips have been developed for rainbow trout as discussed in section IV. However, the complete sequence of the genome will improve bioinformatic interpretation of the results of functional genomics experiments and will lead to the development of additional DNA chips. Functional genomics coupled with high throughput bioinformatics has been recently demonstrated to be a powerful tool for better understandings of gene interactions and the functions of living systems. The availability of the entire genome sequence will also open numerous opportunities for proteomics research, which is very limited without a complete genome sequence.

VII. Technical Challenges:

The most significant technical challenge in assembling the rainbow trout shotgun sequences into contigs will be dealing with the recent genome duplication event resulting in a semi-tetraploid state (Allendorf and Thorgaard 1984). Therefore, the use of a homozygous line for sequencing should be useful for detecting gene duplication in the process of constructing shotgun sequencing contigs. Our recent physical mapping experience with BAC from the Swanson library has demonstrated that duplicated loci can be easily detected by basic DNA fingerprinting (Palti et al. 2004). The duplicated Id1 loci were recently sequenced by Gahr et al. (2004). Approximately 4 Kb of genomic sequence was obtained from each gene (Id1-1 and -2). Each gene was composed of two exons of about 500 bp separated by a 200 bp intron. The sequence identity between the two genes was 83% for the transcribed region (exons and intron) and 65% for the untranscribed upstream and downstream sequences. Similar information has been observed in the genomic sequencing of the duplicated MHC class I region (John Hansen, unpublished data). Additionally, BACs that represent one of two duplicated loci were shown by FISH to distinctly hybridize to a specific chromosome pair. Therefore, it appears that the vast
majority of the duplicated loci contain enough sequence variation that will allow for correct assembly from shotgun sequencing.

VII. Scheduling Requirements:

An additional 80,000 ESTs from the collaborative genome effort of NCCCWA and West Virginia University will be available on GenBank by September 2004 and an additional 20,000 from INRA. The MHC sequencing project will be completed by February 2005. An extended and improved 15x Swanson BAC library will be available by spring of 2005 and the new OSU x Swanson mapping panel will be ready in December 2004.

VIII. Project Description:

Genome Evolution: The rainbow trout, and all other species in the family Salmonidae, are descended from a single tetraploid event that occurred approximately 25 million years ago (Allendorf and Thorgaard 1984). The presence of duplicate genes can complicate the interpretation of genetic information because very similar sequences and gene products may, in fact, represent different genes. The positive aspect of the complexity of salmonid genomes is that it provides a natural laboratory for following the process of evolution by gene duplication. This process, which is generally acknowledged to be a central one in evolution (Ohno, 1970; Force et al., 1999), is still taking place in these fishes. Only disomic inheritance has been detected in females. However, some loci are inherited tetrasomically in males from some populations but disomically in males from other populations. The salmonid duplication is more extensive and recent than the similar event which has been studied in the zebrafish (Amores et al., 1998).

Many of the duplicate pairs of protein coding loci produced by the salmonid-specific polyploid event show divergent patterns of tissue-specific expression (Allendorf and Thorgaard 1984). In addition, polymorphisms in tissue-specific patterns of gene expression (Allendorf et al., 1983; Danzmann et al. 1985) and gene silencing (Ferguson et al., 1988; Leary et al. 1993) occur in rainbow trout at some of these duplicate genes. Salmonid fishes are an ideal group to study the evolution of regulatory elements in a phylogenetic context because there is a large body of information on their physiology and natural history, and there are a large number of closely related species that exist in wild and laboratory populations. Wider-scale sequence information for rainbow trout will provide an excellent and distinctive system for studying the aftermath of a genome-wide duplication event and the associated structural and regulatory gene changes. The extension of current bioinformatics approaches (e.g., the Lek algorithm of Venter et al. 2001) will likely be needed to identify and characterize the internal genomic homologies resulting from this tetraploid ancestry and obtain full benefit from this dataset.

Comparative Genomics: Sequencing the trout genome will also inform the sequence of two other fish species whose genomes were sequenced, the pufferfish (Fugu rubripes) and the zebrafish (Danio rerio), and the medaka (Oryzias latipes) genome which is currently being sequenced in Japan. Comparative DNA sequence studies will provide insights into genome evolution among these species. These three bony fish (teleost) species are believed to have diverged from each other over 100 million years ago and represent three of the most important lineages of bony fishes: the superorder Acanthopterygii and the percomorph fishes (pufferfish), the superorder Ostariophysi and the cypriniform fishes (zebrafish), and the superorder Protacanthopterygii and the salmonid fishes (rainbow trout) (Helfman et al., 1997)
Sex Determination: The evolution of sex chromosomes and sex determination is another fruitful research area with the trout model. The X/Y sex determination system of rainbow trout is distinctive because of the existence of among-population variation in sex chromosome morphology, suggesting that they are in the early stages of differentiation (Thorgaard, 1983). Analyzing this variation at the sequence level will provide important knowledge about the earliest stages of sex chromosome evolution in vertebrates, something that is not possible in the highly derived mammalian models. Similarly, locating and isolating the factors responsible for sex determination in an easily manipulated model species such as rainbow trout will lead to beneficial comparative research into this important but inadequately studied aspect of vertebrate development.

Biomedical Research: The strongest biomedical applications of the rainbow trout model lie in its use for carcinogenesis and toxicology research, and as a comparative immunology model (Thorgaard et al., 2002). Excellent and complementary knowledge is also available in the related fields of physiology, nutrition (Hardy, 2002) and disease pathogenesis (Ozaki et al., 2001). Fundamental processes such as vision (Julian et al., 1998), olfaction (Laberge and Hara, 2001), exercise physiology (Kieffer, 2000), excretion (Wood, 2001), osmoregulation (Perry et al., 2000) and stress response (Iwama et al., 1998) have been intensively studied. Those fields will also be greatly enhanced by the availability of genome sequence data.

Carcinogenesis: An epizootic of liver cancer in Pacific Northwest trout hatcheries in the early 1960s contributed to the discovery of aflatoxin B1 (AFB1) as a potential human hepatocarcinogen, and led to the development of the rainbow trout as a sensitive alternative model for cancer research (reviewed in Bailey et al., 1996). The attributes of this model for cancer research include its non-mammalian comparative status, well-established husbandry and nutritional requirements, availability of multiple exposure routes, a well-defined tumor pathology, externalized gametes and embryos for experimental manipulation, tissue accessibility from animals in the milligram to kilogram size range, low spontaneous tumor background, and high sensitivity. For instance, a single microinjection of as little as 0.5 ng of AFB1 per embryo yields a 40% incidence of hepatocellular/cholangiocellular carcinoma nine months later (Dashwood et al., 1994) – this is 1 billion-fold less aflatoxin than was required to elicit the same incidence in the monkey. Perhaps the attribute most extensively exploited in the past decade has been the trout’s very low husbandry/per diem cost. This feature allows fundamental dose-response issues in carcinogenesis and chemoprevention to be addressed with the trout, using statistically rigorous study designs unaffordable or impossible with traditional rodent models. It was practical, for example, to employ pioneering study designs of up to 10,000 animals each to investigate the quantitative interrelationships between increasing carcinogen dose, increasing anti-carcinogen dose, level of target organ DNA adduction, and eventual tumor outcome (Dashwood et al, 1989; Breinholt et al., 1995). Sequencing of the genome will allow for the identification of novel genes involved in tumor suppression and the onset of cancer.

Toxicogenomics: The rainbow trout is also very useful model for biomonitoring of numerous chemicals in the aquatic environment. Rainbow trout are the standard cold water test species used for regulatory 96-hour acute lethality testing for chemicals entering freshwater aquatic ecosystems. An ASTM standard protocol was established and has been used in the US and elsewhere for over 20 years.

In recent years, a great deal of interest has been focused on the potential for environmental chemicals to act as “endocrine disrupters”. Exposures to such chemicals are
thought to perhaps play a role in breast cancers in women and declines in sperm quality in men, although the evidence for these effects is equivocal (Safe, 1995). It is well established, however, that feral populations of some aquatic organisms exposed to endocrine disrupters exhibit a number of adverse reproductive responses including lack of development of secondary sex characteristics or even sex reversal (Sumpter, 1995). The class of compounds known as xenoestrogens include $o,p'$-DDT, nonylphenol, hydroxylated PCBs and a number of other significant aquatic pollutants. Trout make an excellent sentinel and model for studying xenoestrogens in the environment as they express a protein in liver and blood that is induced in juveniles of either sex or in adult males. This protein is vitellogenin, an egg yolk precursor protein (Donohoe and Curtis, 1996).

The trout model has made, and will continue to make, important contributions in the study of cancer and its prevention, and in environmental toxicology research. At present there are sufficient gene sequences available to contemplate gene expression profiling using targeted genes in limited microarray analysis. However, data-rich exploration of unsuspected interactions within and among various developmental and regulatory pathways must await sequencing of the genome.

**Comparative immunology:** Immunogenetic studies of rainbow trout have served two very important functions. The comparable design and function of human and salmonid immune systems make trout an excellent biomedical model while, as an important agricultural commodity and object of enhancement programs, the same information becomes of direct and immediate importance to the aquaculture industry and to conservation efforts. Trout present the best fish model for disease studies because of the well-defined nature of their bacterial and viral pathogens and well-established disease challenge protocols. Immunologically, the availability of clonal/ syngeneic lines of trout as well as an ample repertoire of immunoreagents and probes, provide investigators with highly advantageous tools to begin extensive genomic analysis of immune system structure and function. The organization of the immunoglobulin, T cell receptor, major histocompatibility, and nonspecific cytotoxic cell (NCC) gene complexes / loci will enable us to precisely envision the basic repertoires by which an individual recognizes and responds to the gamut of pathogens in its environment. This knowledge of immune capabilities will be required to exploit the unique aspects of this simpler immune system.

**Innate Immunity** The human equivalent of natural killer cell equivalent in fishes are known as nonspecific cytotoxic cells (NCC). Current work has shown that the NCC activity difference between two clonal lines maps to a single chromosome region (Zimmerman et al., 2004). We thus are now well poised with the trout system to dissect one of the most critical elements of innate immunity.

**MHC** Class I and II sequences (mainly cDNAs) have been cloned from a variety of teleost species including zebrafish (Takeuchi et al., 1995), salmonids (Grimholt et al., 1993; Hansen et al., 1996; Shum et al., 1999), cod (Persson et al., 1999), catfish (Godwin et al., 2000; Antao et al., 2001) and pufferfish (Timon et al., 1998). The most unexpected discovery for MHC architectural arrangements came from studies in bony fish where the MHC class I and II loci do not co-segregate and, in fact, reside on different chromosomes altogether (Takami et al., 1997; Hansen et al., 1999) which differs from all other vertebrate classes including cartilaginous fish (sharks, rays and chimeras) (Flajinik and Kasahara, 2001; Ohta et al., 2002).
Specific Immune Response To date there have been no reported studies that attempted to determine the $V_H$ genes utilized in the production of specific antibodies in fish. All cloning and sequencing efforts, thus far, have employed primers derived from various consensus sequences, without regard to their probable specificity, and which are often based on sequences from phylogenetically distant species (Roman et al., 1996; Roman and Charlemagne, 1994; Andersson et al., 1995). Lewis (2000) determined that specific gene families are employed in the production of specific antibodies to TNP (Family V) and the G protein of infectious hematopoietic necrosis virus (Family IV).

Knowledge of the complete structure of these gene complexes can do much for our understanding of the evolutionary processes that mold the capacity of an individual's immune response. Our understanding of the components of the immune system of trout will advance dramatically with detailed sequence information in those regions.

Aquaculture: Rainbow trout are the most cultivated cold freshwater water fish in the US. Recent efforts for the genetic improvement of this species for aquaculture production efficiency through selective breeding have begun to employ molecular genetic technologies. Many qualitative/quantitative trait loci have been identified for production traits (Jackson et al., 1998; Danzmann et al., 1999; Sakamoto et al., 1999; Nakamura et al., 2001; Ozaki et al., 2001; Perry et al., 2001; Robison et al., 2001; Nichols et al., 2003a; Nichols et al., 2004). Fine mapping such regions will identify genotypes for use in marker assisted selection, and will ultimately pinpoint the exact DNA sequence variation responsible. The availability of a whole genome sequence would dramatically facilitate fine mapping efforts and the selection of positional candidate genes.

In contrast to other fish models, much is known about the natural populations of rainbow trout that can serve as a resource for addressing evolutionary questions (Behnke, 1992; Hershberger, 1992). Trout are more amenable to surgical manipulations than smaller species, and their size allows specific tissues and cell types to be isolated for biochemical, immunological and/or molecular biological analyses. They are also economically important for aquaculture and sport fishing and they represent the entire salmonidae family which encompass the majority of the economically important cold water fish species.

In this proposal we have provided an evidence base for extensive development of rainbow trout as a state-of-the-art model organism, the lines of study for which it is used as a model, and the considerable body of available resources that will contribute to swift progress in completing the draft sequence and in carrying out post-sequencing phase work. We believe that available resources, vast amounts of accumulated biological information, and the economic and scientific importance of this species make it an excellent candidate for genome sequencing at this time. A DOE-funded effort will benefit from complementary genomics efforts being conducted with the support of NIH, NSF and USDA extramural funds and intramural USDA funds in the US, and funding in Australia, Canada, Chile, Denmark, Finland, France, Germany, Ireland, Japan, Netherlands, Norway, Spain, Sweden, and the United Kingdom. This represents a significant “value-added” research opportunity.
REFERENCES


# APPENDIX B

Names and Affiliations of Co-authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2. Follow the sample format for each person. DO NOT EXCEED FOUR PAGES.

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Caird E. Rexroad

POSITION TITLE

Molecular Biologist

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

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<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
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</table>

A. Positions and Honors

Texas A&M University, College Station, TX, Genetics graduate student in the Department of Veterinary Pathobiology, Dissertation: Radiation Hybrid Mapping of Bovine Chromosome One

USDA/ARS Roman L. Hruska Meat Animal Research Center, Clay Center, NE, Post Doctoral Research Associate in the Productions Systems Research Unit fine mapping loci affecting carcass quality traits in cattle, pigs, and sheep.

USDA/ARS National Center for Cool and Cold Water Aquaculture, Leetown, West Virginia, 2000 – present. Research focus is on the identification of genes affecting aquaculture production traits in rainbow trout and inclusion of molecular genetic information into a selective breeding program. Activities include the development of BAC libraries, high-throughput sequencing of ESTs, and the development of microsatellite genetic maps for QTL discovery.

B. Selected peer-reviewed publications (in chronological order)


C. Research Support (Selected recent or ongoing projects).

As part of the USDA I operate mostly of off base funds appropriated for genome research in cool and cold water aquaculture species.

Kent Sea Tech Corporation, 2003 - 2005. Molecular Markers for Genome Mapping and Selective Breeding of Striped Bass. This project is funded through the State of North Carolina (I am subcontracted to KST) and includes myself, Dr. Mark Westerman (KST), and Dr. Craig Sullivan of North Carolina State University. We are developing microsatellite markers for striped bass to use in selective breeding programs at the University and Industry locations.
Gary H. Thorgaard

**NAME**

**POSITION TITLE**

Professor

**EDUCATION/TRAINING** *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

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</table>

**A. Positions and Honors**

Oregon State University, Corvallis, OR, 1969-73, B.S., Zoology (Honors) received June, 1973.

University of Washington, Seattle, WA, 1973-77, graduate student in the Department of Genetics, Ph.D. received December 1977.
Dissertation topic: Chromosome Rearrangements and Sex Chromosomes in the Rainbow Trout and Sockeye Salmon.

University of California, Davis, CA, Department of Animal Science, April 1978-August 1979, Postdoctoral Fellow.

Washington State University, Pullman, WA, Program in Genetics and Cell Biology, September 1979-September 1981, Visiting Assistant Professor of Genetics, September 1981-September 1983, Assistant Professor of Genetics.

Washington State University, Pullman, WA, Department of Zoology and Program in Genetics and Cell Biology, September 1983- August 1986, Assistant Professor of Genetics and Zoology.

Oregon State University, Corvallis, OR, Marine/Freshwater Biomedical Center, January - May 1987, Visiting Scientist (professional leave from Washington State University).


Washington State University, Pullman, WA, August 1999- present, Professor, School of Biological Sciences. Honors:
American Association for the Advancement of Science (Elected Fellow, 1997)
Distinguished faculty award, College of Sciences, Washington State University, 2001
B. Selected peer-reviewed publications (in chronological order)


C. Research Support (Selected recent or ongoing projects).

NIH/NCI R21CA95909-01 (G. Thorgaard, PI, J. Brunelli, co-Investigator) 7/1/02-6/30/04
Analysis of expression and LOH in trout nephroblastomas
This project seeks to identify genetic changes occurring during carcinogenesis of nephroblastoma in the rainbow trout. Loss of heterozygosity analysis and differences in AFLP (amplified fragment length polymorphisms) associated with expression and methylation differences are sought.

NIEHS 1RO1ES012446-01 (J. Nagler, Univ. of Idaho, PI, G. Thorgaard, J. Brunelli, J. Cloud and I Schultz, co-Investigators) 7/1/03-5/30/08
Xenoestrogen effects on reproduction in male trout.
This project will investigate methylation changes in the genomic DNA of the brain, pituitary gland, and testis in isogenic male rainbow trout in response to in vivo xenoestrogen exposure during three different periods of development (i.e., embryonic, juvenile, sexually maturing). We will also determine whether methylated changes in sperm DNA (in exposed fathers) due to xenoestrogen exposure are heritable and transmitted to F1 and F2 male progeny. Our laboratory would also supply isogenic trout to help address other aims of the project.

NSF IBN0082773 (G. Thorgaard, PI) 8/16/00-8/15/03
Control and evolution of seawater adaptation in Oncorhynchus mykiss
This project seeks to map quantitative trait loci associated with the physiological process of smoltification in clonal line crosses of rainbow/steelhead trout.

USDA 2001-03251 (G. Thorgaard, PI, Raymond Lee, co-PI) 1/1/02-12/31/2004
Physiological effects of mitochondrial substitution in rainbow trout
This project examines the cellular and organismal physiology of rainbow trout lines with identical nuclear genotypes but varying mitochondrial haplotypes.

USDA 2001-03123 (G. Thorgaard, PI, S. Ristow, co-PI) 7/1/01-6/30/04
Aquaculture Washington Subproject: Genetic control of IHNV resistance in rainbow trout
This project seeks to identify major loci associated with viral resistance in crosses of clonal rainbow trout lines.

U.S. Fish and Wildlife Service 101401G0019A (G. Thorgaard, PI) 7/1/01-6/30/04
Detection of genetic damage in trout sperm using all-paternal inheritance
This project uses the chromosome set manipulation method of induced haploid androgenesis to screen for levels of genetic damage in rainbow trout of different genetic backgrounds.

Bonneville Power Administration 2000-071-00 (Thorgaard) 3/1/02-2/28/04
Behavioral and physiological changes during salmonid domestication
This project seeks to develop reproducible methods for characterizing behavioral differences related to domestication among chinook salmon and steelhead trout.

USDA 2001-03123 (S. Ristow, PI, G. Thorgaard, co-PI) 7/1/01-6/30/04
Aquaculture Washington Subproject: Innate immunity in rainbow trout
This project seeks to identify major loci associated with nonspecific cytotoxic cell activity in crosses of clonal rainbow trout lines.
BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2. Follow the sample format for each person. DO NOT EXCEED FOUR PAGES.

<table>
<thead>
<tr>
<th>NAME</th>
<th>POSITION TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yniv Palti</td>
<td>Research Geneticist</td>
</tr>
</tbody>
</table>

EDUCATION/TRAINING  
(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebrew University, Jerusalem, Israel</td>
<td>B.Sc.</td>
<td>1991</td>
<td>Agricultural Economics</td>
</tr>
<tr>
<td>Washington State University, Pullman, WA</td>
<td>Ph.D.</td>
<td>1997</td>
<td>Genetics and Cell Biology</td>
</tr>
<tr>
<td>Agricultural Research Organization, Beit Dagan, Israel</td>
<td>Postdoc</td>
<td>1998-1999</td>
<td>Genetics</td>
</tr>
<tr>
<td>The Technion, Haifa, Israel</td>
<td>Postdoc</td>
<td>1999-2000</td>
<td>Genetics</td>
</tr>
<tr>
<td>Thomas Jefferson University, Philadelphia, PA</td>
<td>Postdoc</td>
<td>2000-2001</td>
<td>Genetics</td>
</tr>
</tbody>
</table>

A. Positions and Honors


Dept. of Food Engineering and Biotechnology, The Technion, Israel, Visiting Lecturer, September 1999 – September 2000.


USDA/ARS National Center for Cool and Cold Water Aquaculture, Leetown, West Virginia, September 2001 – present. Research focus is on the identification of genes affecting aquaculture production traits in rainbow trout and inclusion of molecular genetic information into a selective breeding program. Activities include the development of BAC libraries, physical mapping, sequencing and characterization of immune response genes and the development of microsatellite genetic maps for QTL discovery.

B. Selected peer-reviewed publications (in chronological order)


C. Research Support (Selected recent or ongoing projects).

As part of the USDA I operate mostly of off base funds appropriated for genome research in cool and cold water aquaculture species.

USDA-SBIR grant number 2003-33610-13074 (James Parsons, PI; Yniv Palti, co-PI) 2003 - 2005. Genetic and diet effects on growth rate and body size in rainbow trout.

USDA-NRICPG grant number 2002-034722002 (John Hansen, PI; Yniv Palti, co-PI) 2002 – 2004. DNA sequencing and fine mapping of the rainbow trout MHC class I and class II regions.
Biographical Sketch:
John David Hansen, Ph.D.  Assistant Professor
Univ. of Wisconsin at Eau Claire  B.S.  12/88  Molecular Biology
Oregon State University  Ph.D.  1/95  Genetics
Basel Institute for Immunology, Basel, CH Member 95-01  Immunology/Genomics

Professional Experience
88-91  Senior Lab Tech, Immunobiology Unit, VA Hospital, Minneapolis, MN
92-94  Teaching assistant, Genetics and Cell Biology, Department of Zoology and the
         Genetics Program, Oregon State University
95-01  Scientific Member of the Basel Institute for Immunology, Basel, Switzerland
May. 01-present  Assistant Professor, Center of Marine Biotechnology, Univ. of MD Biotechnology
                   Institute, Baltimore, MD. Adjunct Professor, University of Maryland at Baltimore,
                   Molecular and Cellular Biology Program.

Editorial Board:  Immunogenetics 2001-present
Faculty of 1000:  2002-present (www.facultyof1000.com)
2001-present:  Ad hoc reviewer: USDA Animal Health and Well-being /Animal Genomes
2001-present:  Ad hoc reviewer: NIH/NIAID-Molecular Immunology (R29 Panel member 5/02)
2001-present:  Ad hoc reviewer: NSF MCB Signal Transduction unit
2003-present:  Co-course Master-MMCB716 Applied Bioinformatics-UMB School of Medicine.

Research Projects Ongoing or Completed in the last 3 years
Active/Pending

Hansen-2002-03472 11/15/02-11/14/05
USDA-NRICGP Animal Genomes $622,000 (3 yrs)

“Genomic Resources for Examining Disease Resistance in Rainbow Trout”
The major goal of this project is to develop genomic tools for assessing genes and pathways involved in disease
resistance for rainbow trout. A major goal of our research is to elucidate the genomic content and organization
of the rainbow trout major histocompatibility genes as an initial assessment for their role in disease resistance.
We will also investigate the regulation of these critical loci during an immune response. In our second aim we
will develop and utilize focused microarray technology for the identification of molecular pathways and
candidate genes that are relevant to fish health and the production of effective DNA vaccine technologies.

Hansen-2003-0321617
NSF-MCB Signal Transduction Unit 10/03-9/05  $200,000.

“Interactions of the TCR co-receptors and p56LCK in an ectothermic model”.
This project is designed to elucidate the factors governing T lymphocyte development in a fish model to provide
functional insight on the evolution of adaptive immunity as it pertains to T-cell lineage commitment.
Selected Publications (12 of 29)

1. Ohta, Y., Boulay, T., Flajnik, F., and Hansen, J.D. Isolation and characterization of a putative Dendritic cell marker from elasmobranch and teleost fish. Accepted pending revisions-Journal of Immunology.


APPENDIX D

LETTERS OF SUPPORT
February 6, 2004

Dr. Caird Rexroad  
USDA / ARS National Center for Cool  
and Cold Water Aquaculture  
11876 Leetown Road,  
Kearneysville, WV 25430

Dear Caird:

I am writing this letter in enthusiastic support for the sequencing of the Rainbow trout genome and quantum advances that would result for the field of salmonid genetics.

Our work has focused on Atlantic Salmon and Rainbow trout and since the two species are so close (approximately 95% in noncoding regions) genomics resources for one will benefit the other. Over the past 2 years we have completed about 100,000 ESTs, constructed of an Atlantic Salmon BAC library of 300,000 clones of which 2/3rds have been fingerprinted and contigs made. I believe this is a tremendous resource to all salmonid researchers and our commitment to making all of the data public as soon as possible (well before publication) is an effort to encourage the international salmonid community to better share resources for the benefit of all. An additional 120,000 ESTs from the closely related Rainbow trout is in Genbank largely through yours, ours and others efforts. These resources provide a powerful baseline for further genomics work.

It is also clear that the rainbow trout work and work on Atlantic salmon greatly benefit from collaborative efforts. Our group has focused on Atlantic salmon microarray development. We have made a 4000 gene array available and a new 16,000 gene array has just been completed and has been made publicly available. These arrays, EST clones and BAC clones are available to everyone. Of the 16000 genes on the array, over 15% are from rainbow trout. Results clearly show that all salmonids including salmon and trout work equally well with the microarray and genomic resources can be easily shared. In addition we are developing a large clone BAC gene map complete with linked microsatellite markers, SNP markers and gene markers. To make the leap to the next level in salmonid research it is clear that we must build collaborations and take advantage of the extremely powerful genomics technologies. Our emphasis on microarrays, genomic and EST sequencing, proteomics and genetic markers and the facilities and resources around these pursuits benefit the entire salmonid community.

Genomic research and other larger projects can only be successful if researchers develop good working collaborations. This is one of the strengths of the salmonid genome
I enthusiastically support your efforts to sequence the Rainbow trout genome and will work diligently to ensure its success.

Best Regards,

Ben F. Koop, PhD.
Professor & Canada Research Chair Department of Biology
Director Centre for Biomedical Research
February 17, 2003

Caird Rexroad
USDA/ARS National Center for Cool and Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430

Dear Caird:

I am writing this letter to support the white paper requesting sequencing of the genome of rainbow trout. Trout have unique advantages compared to other vertebrate models. Although they are native to western North America, they have been widely cultured and introduced around the world for human food and sport fishing. They have been the most widely used model fish for physiology, immunology, toxicology, carcinogenesis, reproductive biology, and evolutionary biology for decades.

An important factor in the decision to sequence a genome is the size of the scientific community and the number of related species that will benefit. The availability of sequence information from rainbow trout would have a large impact on ongoing salmonid genetic research around the world. Rainbow trout is a member of the family Salmonidae with 60 species world-wide and the subfamily Salmoninae that includes about 30 species, half of which are native to North America. Each species is divided into a group of stocks or strains that are marvelously adapted to their native streams or lakes. Many of these populations are currently threatened and it has been difficult to reintroduce fish into streams where they have been extirpated. Genome studies have the potential in the future to identify the genetic basis of these adaptations.

Susumu Ohno’s theory that two rounds of genome duplication occurred early in the evolution of vertebrates remains controversial, although it is clear that the human genome contains a large number of duplicate genes. Part of the problem is the lack of consensus on what the expected structure of a genome of a vertebrate paleopolyploid would be after 300 million years. The genome duplication that occurred 50-100 Mya prior to the radiation of the salmonid fishes is well documented and salmonids are an important model group in which to examine the processes involved in diploidization in vertebrates. The rainbow trout genome sequence would facilitate the use of salmonids as a model system for comparative work on sub-functionalization of duplicate genes in different species, and for investigations of the relationship between chromosome location, genetic recombination and rates of molecular evolution of duplicate gene pairs.
Most genome projects have greatly benefited by having data from a closely related species. The most detailed genome maps currently available for salmonids are for rainbow trout and Atlantic salmon (*Salmo salar*), although framework maps are available for a half dozen other species. The Canadians and Norwegians have a joint genome research project on Atlantic salmon (GRASP) sponsored by Genome Canada and are currently making contigs from a 10X BAC library in preparation for future sequencing. This information could be very useful for the trout project.

The focus in the US has been on rainbow trout, which has been a model organism for many years and for which more genetic resources are available. These include five homozygous clonal lines have been produced that differ in phenotypes of interest. Fortunately we in the trout community had the foresight to make our BAC libraries from these clonal lines. Although the genome duplication could cause problems for a preparation of contigs prior to sequencing, preliminary data suggests that this will not be the case with these BAC libraries from clonal lines. Duplicate contigs have been readily identified using BAC fingerprinting and cytogenetic mapping of 75 BAC clones showed that 90% of them hybridized to one chromosome pair.

In conclusion, salmonid fishes have been one of the most intensively studied fish groups because of their importance for aquaculture, recreation and conservation as well as a model organism for all areas of biology including evolutionary biology. The availability of the trout genome sequence would have a tremendous impact on many areas of biological research in the world-wide scientific community.

Sincerely yours,

Ruth B. Phillips
Adjunct Research Professor
February 16, 2004

Gary Thorgaard, Ph.D.
Professor
Center for Reproductive Biology
School of Biological Sciences
Washington State University
Heald Hall Room 205D
Pullman, WA 99164-4236

Dear Gary:

I am happy to provide this letter of support for your DOE proposal to sequence the trout genome. As you know, the Center for Reproductive Biology involving both Washington State University and the University of Idaho has a fish reproduction program and over 15 investigators use the salmonid model in their research. The availability of a sequenced genome would provide a technical advance to significantly impact and enhance the research activities of all these investigators. This would put the salmonid research in the genomic era to address advanced biological questions. This impacts basic research and understanding for endangered salmonid populations and disease states. The proposal to sequence the trout genome is essential for all these investigators.

The Center has numerous advanced technology service laboratories to assist members of the Center. This includes Genomics, Bioinformatics, and Molecular Biology Cores that would allow the availability of the trout genome sequence to be immediately utilized by those doing functional genomics and molecular research. As you know these Core Laboratories are available to all the members of the Center including the fish reproduction group.

I am very supportive of the proposed DOE program to sequence the trout genome on both a scientific level and an administrative level. The impact of the information obtained could revolutionize research in fish biology. I am happy to provide support in any way I can. Please let me know if you need any additional information and good luck with your application.

Sincerely,
Department of Energy
Joint Genome Institute
Community Sequencing Program

Dear Sir:

I am submitting this letter to strongly support the proposal submitted by Dr. Caird E. Rexroad, III, to obtain a draft sequence of the rainbow trout (*Oncorhynchus mykiss*) genome. The availability of a well-annotated genome sequence for rainbow trout is very important and will be very beneficial for the advancement of knowledge about one of the most broadly distributed and most extensively produced cold water fish species.

In the laboratory where I am currently Director (USDA ARS National Center for Cool and Cold Water Aquaculture in Leetown, WV) we have a staff of nine Ph.D. scientists in a variety of disciplines conducting research to improve aquaculture production traits and develop a more efficient strain of rainbow trout for aquaculture. A major part of this research effort is the identification, characterization and use of molecular tools to enhance our understanding and application of these tools to problems in the aquaculture industry. The ability to coordinate and refer these molecular findings to a well-annotated genome sequence would allow more thorough and rapid progress toward achieving our goals.

In addition to the more directly applied utilization of sequence information, the genome sequence for rainbow trout offers a potential for providing an exciting and wide range of biological information on a species about which there is an extensive amount of basic biological data. Based on the 30+ years I have worked with various species of the family *Salmonidae* (salmon, trout, char, whitefish, and grayling), I would say rainbow trout is one of the most adaptable and resilient. From its original distribution along the coastal and inland areas of the eastern Pacific, this species has been distributed in temperate regions circum-globally and exhibits both freshwater and anadromous life styles. With such a diversity of biological characteristics and its adaptability to artificial culture, it has been one of the most extensively studied cold water species. Consequently, there is a vast biological database on which to draw to complement and enhance the molecular information made available from a genome sequence.

Further, rainbow trout evolution has fairly conclusively occurred from a historic genome duplication (i.e., tetraploidization) and subsequent rediploidization. This offers some challenges for interpretation of a genome sequence; however, it also offers a unique opportunity to provide some insights into the evolutionary process and yield a better understanding of the reasons for the persistence of some of the historic gene duplications. A genome sequence for this species

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National Center for Cool and Cold Water Aquaculture
11861 Leetown Road • Kearneysville, WV 25430
Voice: 304 724-6340 • FAX: 304 725-0351
An Equal Opportunity Employer
could provide some valuable information on the importance of gene duplication in evolution and a better understanding of the resultant genome structure.

In summary, obtaining a well-annotated genome sequence of the rainbow trout will provide an crucial dataset that will be of scientific assistance to both the applied research and the basic understanding of a very important fish species. A very robust database already exists with which to enhance analysis and interpretation of the genome sequence and will go along way toward making the sequence data a very well used and thoroughly interpreted body of molecular information.

Sincerely,

William R. Hershberger

WILLIAM K. HERSHBERGER, PH.D.
Director
Dear Dr. Thorgaard,

On behalf of the Departments of Animal and Poultry Science, Pathobiology, and Biomedical Sciences at the University of Guelph, Ontario, Canada, we would like to include a letter of support for the proposal to draft sequence the rainbow trout genome. The University of Guelph has numerous programs including, Toxicology, Endocrinology, Immunology, Nutrition, Microbiology, Quantitative Genetics, Biomedical Science, and Pathology that all rely on the use of rainbow trout for their respective areas of research. I have compiled a list of a few of the scientists whose research will directly benefit from this sequencing project.

Dr. N.A. Karrow’s research program is focused around comparative neuroendocrine-immunology, and immunoregulation. His laboratory is specifically involved in studying the genetic regulation of inflammation, and the effects toxins on the genetic re-programming of neuroendocrine-immune axis during early-life development. The RT Genome Sequencing Project, increased availability of microarrays containing developmental genes, and the development of RT proteomics will enhance the utility of RT as a comparative, and developmental neuroendocrine-immune model in our laboratory.

Dr. J.S. Lumsend’s lab is currently investigating two aspects of RT biology that would greatly benefit from a draft of the RT genome. We are identifying plasma proteins that are induced by tissue damage/inflammation and that are part of the acute phase response of RT. We have identified numerous proteins that are induced following intraperitoneal injection of lipop polysaccharide, however the majority of these have N-terminal sequences that do not match any known proteins found in databases. A separate project has identified several novel RT plasma proteins that bind to bacterial surfaces that also have N-terminal amino acid sequences that cannot be matched at present to known proteins. The availability of a draft RT genome would allow us to develop degenerate DNA sequences to facilitate identification of these proteins.

Prof. Ian McMillan and research associate Laura McKay are examining the genetic relationships within schools of rainbow trout to select a strong and progressive parent stock without the fear of inbreeding. The researchers are defining how much impact genetic selection can have on improving the physical traits of rainbow trout. Using DNA analysis, they’ll develop a new strain of rainbow trout – one that grows more rapidly, reaches sexual maturity at a later age, and spawns in the spring rather than the fall. Early spawning is of particular interest because it would provide producers with a second annual harvest and consumers with a more consistent source of fresh Ontario fish.

Dr. J Leatherland’s laboratory is currently involved in sequencing genes for thyroid hormone, growth hormone and IGF-I receptors and for GH and IGF-I protein. We are currently collaborating with Matt Vijayan (Biology, U of Waterloo) using microarray analysis of the gene expression during early development of RT. The microarray currently screens for expression of 158 genes, and this number is to be increased to over 200 by the summer of this year. We are also engaged in studies that examine the effect
of stress on steroidogenic pathways and growth of juvenile trout. The genome project would add considerably to our knowledge of the genetic sequences that might be of interest to us, and allow us to expand the scope of our work. This approach has great significance in enhancing our understanding of growth processes, and thus offers opportunities for enhancing the efficiency of growth, and adapting growth rate regulating strategies to varying environmental conditions, and food quality and content.

Dr. Bureau and his colleagues of the Fish Nutrition Research Laboratory are carrying out a research program whose goal is to develop a more comprehensive understanding of nutrient (amino acids, glucose, phosphorus) utilization by fish. This involves understanding of the role (and effects) of specific absorbed nutrients explicitly rather than just that of aggregates, such energy and protein. This greater understanding of nutrient utilization is then used to devise solutions and tools (feed formulae, genetic selection markers, production strategies, models, software) to improve the economic and environmental sustainability of fish culture operations.

Of particular interest to this application is the current research program of D. Bureau’s research team on the “Efficiency of Amino Acid Utilization for Protein Deposition in Different Salmonid Species”. This program focuses on the dramatic differences in efficiency of utilization of amino acid between rainbow trout and Atlantic salmon, two fish from the salmonidae family. Significant differences in the efficiency of amino acid utilization have also been observed for fish of different sizes/ages in recent studies with these two species. These differences translate into dramatic differences in feed efficiency, and, consequently, production costs among fish species and fish of different sizes/ages. It is, therefore, of great interest to investigate the regulation of the main metabolic pathways of amino acid utilization in these animals. This could then be translated into the development of approach for genetic selection or metabolic modulation.
February 17, 2004

Dr. Caird Rexroad
National Center for Cool and Cold Water Aquaculture/ARS/USDA
11861 Leetown Rd.
Kearneysville, WV 25430

Dear Dr. Rexroad:

I read your white paper, entitled "A White Paper Advocating the Complete Sequencing of the Genome of the Rainbow Trout, Oncorhynchus mykiss", with great interest and enthusiasm. I am writing to provide the strongest possible support for your collective efforts to secure sequencing services from the Department of Energy's Joint Genome Institute's Community Sequencing Program, to sequence the rainbow trout (Oncorhynchus mykiss) genome.

As you are no doubt aware, more is known about the environmental physiology of rainbow trout (salmonids in general) than in most other vertebrates. In this regard, research in salmonids has far-reaching consequences to environmental remediation, fisheries stocking programs, human nutrition and food safety, and unequivocal contributions to human medicine. For example, in human medicine, salmon calcitonin continues to be used for treatment of hypercalcemia, Paget's disease, osteoporosis and is used as a potent analgesic for the relief of bone pain. More recently, another hormone, Urotensin II, who's activity was first described in rainbow trout, has recently been identified in the human central nervous system, and been shown to be a potent vasopressor as well as a vasodilator, depending upon the tissue examined. More importantly, however, the finding of Urotensin II in humans has permitted the characterization and identification of human G-protein-coupled orphan receptor(s) (GPCR) (rat homolog: GPR14) that have a wide distribution (first identified in a human genome research project) in the human cardiovascular system. Given the potent vasoactive characteristics of the human Urotensin II/GPCR system, it is the center of intense focus for development of pharmacological agents (new ligands) that activate/inactivate GPCRs in the cardiovascular system. Ideally, engineered ligands can be used to treat hypertension and cardiovascular disease.

As part of the Agricultural Research Service, our center (NCCGWA) is interested in the improvement of growth, nutrition and immune function in cold-water species, namely the rainbow trout. The focus of our laboratory's work is on understanding the mechanisms (molecular and protein) that underlie growth. Genome sequencing will, 1) immediately increase the number of research tools that can be used for research into growth and immune function, 2) identify molecular markers that can be used for genetic selection, and tracking, of trout strains that exhibit a desirable phenotype, and 3) aid in conservation efforts by understanding the genetic mechanisms that lead to population differences in the timing of development and migration in endangered salmonid populations (e.g., Atlantic salmon and Steele head).

Sincerely,

Brian S. Shepherd, Ph.D.
Research Physiologist and Adjunct Assistant Professor, Dept. of Biology, Univ. of Kentucky
Dear Dr. Caird Rexroad,

I am writing you in support of the proposal to sequence the rainbow trout genome. I have been using the clonal trout model system to study genetic mechanisms of sex determination, endocrine disruption, cancer formation, and epigenetic effects of CpG methylation. I have found 5-methylcytosine patterns to be both tissue and gender specific, by using the Methylation-Sensitive Amplified Fragment Length Polymorphism (MS-AFLP) analysis upon clonal populations of trout. Furthermore, these tissue and gender-specific patterns are established early in embryonic development. This technique allows the comparison of tissue-specific genomic methylation patterns between individuals, using isoschizomers to methylated restriction sites, and has demonstrated the establishment of genomic methylation to be highly consistent between clonal individuals in the comparison of a given tissue. These results indicate genomic methylation is playing a significant role in differentiation and development. In order to characterize these methylation differences, I have been excising the 5-methylcytosine polymorphic fragment from acrylamide gels, and characterizing the sequence. The figure below shows a striking difference in the methylation patterns of brain DNA between males and females. Females show the failure to amplify due to methylation of the specific locus. I have included two BlastX alignments derived from sequences which demonstrated clear methylation differences between male (Xy) and female (XX) brain DNA.

Note the MS-AFLP fragment overlaps the 5' end of the FGF4 gene, and relevance to sexual differentiation is revealed in citation. (HST-1/FGF-4 gene activation induces spermatogenesis and prevents adriamycin-induced testicular toxicity. Yamamoto et al., Oncogene. 2002; 21(6): 899-908.)

gi|10179934|gb|AAG13950.1| fibroblast growth factor 4 precursor [Danio rerio]
Expect = 4e-14 Identities = 39/48 (81%), Positives = 41/48 (85%)

Query: 85 MAFQSALLPILVLGMLTSLVRCAPFGGLNGTVERWETLYSRLARI 228
M+ QSALLPILVLGLMTS VRCAP PG +G VER WETLYSRLARI

>gi|4758520|ref|NP_004658.1| hect domain and RLD 2; HERC2 [Homo sapiens]  
Expect = 2e-06 Identities = 24/27 (88%),
Query: 140 IRLELLPDVLVHRLKMIVDPADSSYMP 60
IRLE+PDVLVHRLKMVDPADSSYMP
Sbjct: 2820 IRLEIFPDVLVHRLKMIVDPADSSYMP 2846

This homology is shocking, because the phenomena of imprinting is presumed to be peculiarly mammalian, and indeed the ability to “easily” generate clonal fish argues against any significant imprinting. However, to have recovered this fragment in comparison of methylation patterns distinguishing males and females indicates the mammalian phenomena of imprinting is soundly grounded in an ancestral process which employs methylation in differentiation, and is being revealed by comparison of the trout 5-methylcytosine patterns.

I can not emphasize enough how difficult determining the relevance of these comparisons are, when the NCBI Blast searches of trout sequences depends predominately upon homology to widely diverged species. As I am sure you know, much analysis of the role of CpG methylation in regulation of gene expression has shown the methylation events to be involved in heterochromatin condensation, and may be distant to the targeted locus. Therefore the recovery of the methylated fragment may not correspond to any obvious relevant gene sequence. However, if the trout genome were sequenced, the direct linkage of a given affected locus to near-by candidate genes would allow insight into developmental processes, disease formation, environmental impacts upon genomic methylation and ultimately late-onset disease, and much more.

I am absolutely convinced this model organism is going to compliment the Zebrafish project in a way which will only facilitate our understanding of species evolution and developmental processes. The advantages of the trout model include the clonal populations for simplifying the process of characterizing genetic mechanisms, the large size enhances tissue recovery and analysis (expression, development, differentiation, pathology), the sensitivity to environmental agents, as well as being an important agricultural species.

I hope my petition is received sympathetically if not enthusiastically!

Sincerely Yours;

Joseph Peter Brunelli
Assistant Research Professor
School of Biological Sciences
Washington State University
Pullman, WA 99164-4236
Dear Dr. Rexroad,

This letter to support the proposal you are leading for draft sequencing the genome of rainbow trout (Oncorhynchus mykiss). This support comes from a collaborative network (Stressgenes project) supported by EU and aiming to characterise genes involved in molecular pathways associated with stress responses in trout using a functional genomic approach (see bellow list of participating European laboratories). As a mid-term goal, this project will develop genetic approaches that will allow characterisation of genetic markers for markers-assisted selection breeding for stress or disease resistance.

In this context, access to a draft sequence of the rainbow trout genome will be very important and useful for the success of our project. Access to the complete sequence of the genome will be of great help for interpretation of our functional genomic data and identification of duplicated genes having very similar sequences and expression products. Moreover, this information will also be necessary for positional cloning and identification of candidate genes for QTL related to stress and disease resistance.

In conclusion, we think that a support from the Community Sequencing Program for a draft sequencing of the genome of the rainbow trout is very important and will be a major scientific progress for the fish scientist community.

Best regards,

Dr. Patrick Prunet
Coordinator of the Stressgenes project

European laboratories involved in the Stressgenes project:

- **Dr. Patrick PRUNET**, INRA/SCRIBE, campus de Beaulieu, 35042 RENNES Cédex
- **Professor Chris SECOMBES**, Department of Zoology, University of Aberdeen, 2 Tillydrone Avenue, AB24 2TZ Aberdeen, UK.
- **Dr. Michael CAIRNS**, National Diagnostic Centre, University road, Galway, Ireland.
- **Professor Andrew COSSINS**, University of Liverpool, School of Biological Sciences, Derby Building, Brownlow Street, L69 3BX Liverpool, UK.
• **Dr. Tom POTTINGER**, Centre for Ecology and Hydrobiology, Windermere Laboratory, Far Sawrey, LA22 0LP Ambleside, Cumbria, UK.

• **Professor Svante WINBERG**, Evolutionary Biology Centre (EBC), Department of Comparative Physiology, Uppsala University, Norbyvägen 18 A, SE-752 36 Uppsala, Sweden.
To: Caird Rexroad

RE: Proposal for sequencing the rainbow trout genome

This letter is in support of the Rexroad et al. proposal for the DOE Joint Genome Institute to produce a draft sequence of the rainbow trout genome. For the last two years I have worked with GRASP (Genomic Research on Atlantic Salmon Project), developing genomic resources (e.g. sequence database, cDNA microarrays) for salmonid research. I have used these tools to study variability of gene expression within and between salmonid species, transcriptome dynamics over rainbow trout embryogenesis, and the responses of salmonid transcriptomes to rickettsial infection and toxin exposure. I have now started a toxicogenomics lab at the University of Wisconsin-Milwaukee. Because rainbow trout are a favorite toxicology model species, their gametes are available year-round, and they can be easily and inexpensively cultured, I am primarily using this species to assess impacts of stressors on gene expression and morphology in salmonid early life stages. Microarray-based experiments allow the identification of embryonic genes responding to stressors such as temperature change, anoxia, or toxin exposure. Microarrays will also aid in identifying trout strains having eggs and early embryos with high levels of protective transcripts, such as those encoding proteins involved in redox balance or xenobiotic metabolism. The identification of trout strains producing embryos with higher incidence of successful embryogenesis under stressful conditions will aid aquaculturists and conservationists in broodstock selection. Having a draft sequence of the rainbow trout genome will aid my research immensely. Levels of protective maternal transcripts are regulated during oogenesis; a well-annotated genome sequence will facilitate investigation of these regulatory regions. It is likely that several thousand rainbow trout genes have yet to be identified; the genome sequence will promote gene discovery. I would like to study the impact of environmental toxicants on the embryonic rainbow trout proteome, but effective proteomics research requires a fully sequenced genome. With a rich literature documenting the use of rainbow trout in a myriad of toxicity tests, this species is poised to become a prominent toxicogenomic model organism. A sequenced rainbow trout genome will improve the utility of this model for studying the effects of toxicants on fish and human health.

Sincerely,

Matthew L. Rise
Great Lakes WATER Institute, UWM, 600 E. Greenfield Ave., Milwaukee, WI 53204
Phone: (414)382-1700 Fax: 414-382-1705 email: mrise@uwm.edu
Madrid, February 14, 2004

Dr Gary Thorgaard,
School of Biological Sciences and Center for Reproductive Biology
Washington State University
Pullman WA 99164-4236, USA

REF: Draft Sequencing of the Genome of the Rainbow Trout, *Oncorhynchus mykiss*

Dear Dr Thorgaard,

I have read with great interest the manuscript “A White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout, *Oncorhynchus mykiss*” which I have found of maximal scientific importance to the future of fish research.

Most housekeeping functions in fish have been studied and modelled in *O. mykiss* and there is not other fish species whose genome is critical to sequencing to substantially advance in the functional and physiological analysis of teleosts that are the major group within the vertebrate species (about 25,000) and which shows a high diversity potential and adaptability.

As senior author of the complete sequencing of the mitochondrial genome of *O. mykiss*, published earlier in 1995, and present coordinator of the major European effort for create a Molecular Genetic Catalogue of more than 200 European Marine Fish Species (FihTrace: funded by the European Commission), I for one am sanguine about the major impact that the sequencing of *O. mykiss* genome will have to pave the way for integrative genomics, proteomics and transcriptomics of the key representative vertebrates.

Through the present letter I am fully supporting the scientific criteria to choose *Oncorhynchus mykiss* as a necessary genomic project to be afforded and therefore I adhere to the principles defined in the White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout.

Sincerely,

José M. Bautista, PhD
Professor of Molecular Biology

José M. Bautista, PhD
Departamento de Bioquímica y Biología Molecular IV, Universidad Complutense de Madrid, Facultad Veterinaria
Ciudad Universitaria, 28040 Madrid, Spain. Tel.: + 34 91 394 3823. Fax: + 34 91 394 3824. E-mail: jmbau@vet.ucm.es
Dear Dr. Thorgaard:

I wish to provide a strong letter of support for your proposal on sequencing the genome of rainbow trout (*Oncorhynchus mykiss*).

The past decade has seen a very dramatic transformation in the way Canada harvests salmon and other fish species. With declining wild populations and emerging global commercial opportunities, Canada’s aquaculture industry has emerged as an important economic sector which provides direct jobs in coastal areas as well as associated support employment (processing, feed manufacture, production of therapeutics) in major centres. Although the aquaculture industry is moderately diversified, many salmonids have become the major species utilized in aquaculture on both Canadian coasts. This important industry will require continuous innovation as well as scientific and technological support to remain internationally competitive in the coming decades.

In parallel, the past decade has also witnessed a new appreciation of the complexity of natural aquatic ecosystems and man’s need to preserve them through enhanced understanding and management. Such conservation goals, particularly for a species such as rainbow trout, will require new tools to fully understand impacts occurring to individuals and populations which arise from intensifying anthropogenic activities and changing natural forces.

Improved understanding of the genetic control of production characteristics (for aquaculture) and fitness parameters (for survival in nature) of rainbow trout is critical for Canada, one area within the native range of this species. The development of draft sequence data for rainbow trout genome will provide a landscape on which genetic maps of phenotypic traits and markers can be positioned and ultimately utilized as predictive tools in selective breeding programs. Coupling BAC contig mapping and EST analyses, followed by proteomic approaches, will provide a comprehensive view of the salmonid genome and salmonid physiology. Of equal importance, this information will also provide very useful tools for individual investigators to study specific genetic and physiological pathways controlling phenotypic traits. Such tools, if sought by conventional approaches, would in many cases be unattainable or prohibitively expensive, whereas a genomics approach will yield such information both abundantly and economically.

The utility of your proposal is not limited to rainbow trout. Sequence information derived from rainbow trout will provide information that can be used in many other fish species, and indeed many other vertebrates. For my own genetic research programs with Pacific salmon, I can envisage extensive use (conventionally, and in smaller scope genomics applications) of sequence information and gene organization data from rainbow trout. In particular, we have been studying the evolution of the Pacific salmon Y chromosome now for a decade and I can see exciting
possibilities derived from the trout sequence information to further our understanding of the evolution of sex chromosomes and sex-determination systems. Further, we are involved in applying transgenic technologies as a basic research tool, and the gene sequences uncovered from sequencing the trout genome will surely allow extensive examination of questions in the area of functional genomics.

I strongly support the proposal to undertake sequence analysis of the rainbow trout genome.

Robert H. Devlin

Robert H. Devlin, Ph.D.
Head, Molecular Biology Program
West Vancouver Laboratory
Dear Caird,

I am writing this letter in support of the upcoming JGI application for draft sequencing of the rainbow trout genome. This project, if funded, will greatly improve our capabilities to explore the genome of this autotetraploid derivative species, and will undoubtedly accelerate the characterization and understanding of gene function in this important teleost model species. As outlined in the ‘white paper’, rainbow trout is very much one of the primary experimental teleost model species. The species has been used extensively in physiological, toxicological, and immunological studies over the past decades. It is also one of the most important freshwater aquaculture species worldwide, and represents a source of income from high-scale commercial operations to moderate and low-scale single family farming operations. Within North America it is the most important freshwater aquaculture species. The propagation of rainbow trout also represents a source of cultural pride and heritage and connection to the ‘wilderness’ for all cultural groupings that call North America their home.

The research will provide us with knowledge of the duplicated gene regions within the genome of rainbow trout. This is the most intriguing and perhaps challenging aspects of the proposed research, with respect to how this information will be compiled and understood. Our understanding of the diploidization process and gene silencing following polyploidization events is still greatly lacking. This is in large part due to the fact that we have very few animal polyploid models in which we may study this process. Salmonid fishes (including the rainbow trout) provide researchers with an ideal model system in which we may ‘dissect’ and characterize the processes of multi-gene family regulation, duplicate gene expression, duplicate gene organization among chromosomes (i.e. specific homeology [i.e. duplicated homologues] arrangements within the genome), integrated gene cassette organization within chromosomes, and functional genomics studying the relative contribution of single, duplicated, or differentially regulated [or divergent] duplicated gene functions within the genome.

Draft sequencing of the rainbow trout genome will allow researchers to more accurately pinpoint duplicated gene regions by designing species-specific primers that may be used to more accurately characterize homeologous gene regions in concert with
other genetic techniques (i.e. genetic mapping, BAC contig mapping, and physical mapping). By applying a compendium of complementary techniques to unraveling the mysteries of duplicate genome evolution, researchers will be able to gain a wider understanding of not only the rainbow trout genome, but of genome evolution following polyploidization. It has been suggested by many researchers that the evolution of genomes by genome duplications (eg. Ohno, 1970) have been fundamental in the process of evolution among all living organisms. The rainbow trout provides us with a ‘window’ in which we may view the aftermath of this process.

It is hoped that the JGI committee will view this application with favour and recommend funding for this important project.

Yours truly,

Roy G. Danzmann
Associate Professor
Dr. Gary Thorgaard  
School of Biological Sciences  
Washington State University  
Science Hall Room 312  
Pullman, WA 99164-4236

Dear Gary,

I enthusiastically support the proposal to the Department of Energy's Joint Genome Institute's Community Sequencing Program to sequence the genome of the rainbow trout (*Oncorhynchus mykiss*). The resulting information from this project would have enormous benefits for basic and applied research with this valuable species.

As you know, my primary interest is evolutionary and population genetics. The ancestral polyploidy of salmonid fishes and the ongoing process of diploidization in this family of fishes make sequencing the rainbow trout genome a challenging but potentially enormously rewarding endeavor.

We have just begun two exciting research projects with rainbow trout that would benefit tremendously from this project. We have initiated a genomic approach to understand the genetic basis of the anadromous versus freshwater residence life-history polymorphism of many populations. We suspect that genes associated with this polymorphism are located on the sex chromosomes because of sexually antagonistic selection. The undifferentiated sex chromosomes of rainbow trout make this a potentially important project to understand sex chromosome evolution.

We have also initiated a project to test for the effects of the mitochondrial genome in rainbow trout on male fertility. The best evidence for the effects of mitochondrial genes on male infertility is in humans. We believe that the rainbow trout is an ideal model system to study this relationship.

Please let me know if there is anything else that I can do to help you in this endeavor.

Sincerely,

Fred W. Allendorf  
Professor of Biology
February 18, 2004

Dr. Caird Rexroad
USDA/ARS National Center for Cool and Cold Water Aquaculture
11861 Leetown Road
Kearneysville, West Virginia 25430

Dear Dr. Rexroad,

I wish to provide my strong support for your proposal to the U.S. Department of Energy’s Joint Genome Institute’s Community Sequencing Program to sequence the rainbow trout genome.

The Agricultural Research Service strives to sustain a competitive agricultural economy and enhance the natural resource base and the environment. Basic information from the rainbow trout genome will enable our laboratories to advance our research toward both of these key parts of the mission. Our work to improve the farming attributes of the rainbow trout will be greatly strengthened by having the genome sequenced. Traits of interest include growth, disease resistance, reproductive capacity and product quality attributes. The genome sequence will enable us to go from defining phenotypic differences to identifying the genes and proteins responsible for the phenotypes much more quickly and cheaply than traditional positional cloning procedures. The benefit to US producers and consumers of aquacultural products could be tremendous. Additionally, the benefit to each individual researcher working on trout or salmon or fish or even comparative physiology is potentially enormous. The draft genomic sequence data would be useful for physiologists trying to understand a particular metabolic pathway and for evolutionary geneticists working to understand evolution in a taxon that has undergone genome wide duplication event. The outcomes of this sequencing effort would have far reaching consequences.

Furthermore, I sincerely believe that better use of domesticated lines of trout, such as the Swanson strain, will improve the chances for natural populations of salmonids to flourish with less fishing pressure due to harvests of wild animals.

This project would have a large and lasting impact on genetic improvement from a selective breeding perspective and I am glad for the chance to support this proposal,

Sincerely,

Jeffrey Silverstein
Lead Scientist, Broodstock Development
Re: Rainbow trout genome sequencing project:

Dear committee members:

This letter is written to express my unqualified support for the proposed project of sequencing the rainbow trout genome. My research primarily involves the molecular physiology of ion and acid-base regulation in rainbow trout. Primarily, research projects in my lab involve A) examining the transporters involved in regulation of ion balance during salt water adaptation B) examining the transporters that regulate both intracellular and extracellular pH during such stresses as exercise and C) understanding how toxic metals can interfere with these transport systems. An example of one of my recent research programs that would directly benefit from this proposal focuses on cloning and characterizing the SLC26 family members in rainbow trout, important bicarbonate regulating proteins. Until we recently (December 2003) submitted our 2 cloned transporters in the family, there we no members of this family represented in the NCBI database for rainbow trout, and only one complete sequence in this family for any fish species (eel). Also, there were no matching EST’s for rainbow trout in the database as well. Currently, we know there are at least 13 members of this important family in the human genome database so our work is far from complete. An annotated genome sequencing project would greatly facilitate a research program by allowing us to go directly to the functional physiology of these transporters, the area of real interest. There are also many other transporter families represented that I would be greatly interested in the molecular identity in rainbow trout but the cost and time required to clone these members is simply too prohibitive. The advancements that would come with a genome sequencing project would be great and allow for a rapid advancement of our understanding of the molecular mechanism involved in salinity transfer, exercise recovery and toxicological impairment. This has important implications for our growing aquaculture industries that are constantly looking for means to lower husbandry costs and mortality during parr-smolt transformations. A rainbow trout sequencing project will certainly be a great a boon for my research program and I urge you wholeheartedly to support this project.

Sincerely,

Greg G. Goss Ph.D.
Associate Professor
University of Alberta
Edmonton, Alberta
Dear Panel,

I am writing this letter in very strong support of the proposal ("A White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout, Oncorhynchus mykiss") being submitted by Caird E. Rexroad III on behalf of the salmonid research community. I am a researcher who has continually worked for the past 25 years on research covering reproduction, growth and immunology in brook trout and rainbow trout. Over the past 10 years a large focus of my research in these areas has been on gene discovery and molecular genetics. I have used trout for several reasons: 1) they are one of the most important aquacultured species in the world; 2) they are good model organisms for physiology because of their size, and for genetics because of the number and size of their eggs; and 3) they are members of a phylogenetic group of organisms that have worldwide distributions that impact all people, and they have the most diverse and interesting life histories exhibited by vertebrates.

I have also worked extensively with zebrafish and fugu; the 2 fish species for which genome sequencing has already been conducted. While each of these species has something to contribute to the advancement of modern science, it is also clear that they have significant limitations. In the case of fugu, genomic resources in the form of animals, RNA, tissues, ESTs, etc. are nonexistent; making this useful only as a virtual tool. In addition, there is basically no scientific background in the literature for this species. While zebrafish have great attributes as a genetic model, they are nearly impossible to use for meaningful physiological studies at the organismal level. Next to zebrafish, the rainbow trout is one the most well studied fish species and there is a significant literature base for this species covering reproduction, growth, immunology, endocrinology and metabolism. While a large number of ESTs are begin generated for trout, what is lacking for the rapid development of studies is genomic sequence. Anyone who has worked in gene discovery and has utilized the zebrafish and fugu genomes realizes what an advancement that can be. Having a sequenced genome for the rainbow trout would provide for an exponential jump in what can be accomplished with this species and I hope JGI will act favorably on this proposal for a draft sequence of the rainbow trout genome.

Sincerely,

Frederick William Goetz
Senior Scientist and Leader,
Program in Scientific Aquaculture
Dear Caird,

This letter is to confirm my strong support for the sequencing of the rainbow trout genome.

As you know my institute [the French Institute for Agricultural Research (INRA)] initiated a vast project on animal genomics. This program is called AGENAE (Analysis of Animal Genomes) and its main objective is the understanding of physiological function regulations in different species of economic importance including one fish species, the rainbow trout, *Oncorhynchus mykiss*. Our goal is to be able to perform systematic studies on a large number of genes either implicated in a function or characteristic of an interesting phenotypic trait. This will be carried out through micro-array expression studies together with mapping information to select traits of agronomic interest.

We already constructed high quality cDNA libraries and initiated a high throughput ESTs sequencing project. Right now, more than 100 000 sequences have been performed by this AGENAE program. All this information has been already released in international databanks. Together with the important USDA EST collection it now brings rainbow trout as one of the major fish model in term of EST sequences just after the zebrafish, *Danio rerio*. Using these sequenced transcript collections we already initiated micro-array expression studies on different fish physiological aspects i.e., reproduction including gametogenesis, gamete quality, sex differentiation, growth and nutrition, stress and welfare.

We also have programs on gene mapping and a trout radiation hybrid panel is currently under construction. When available, this panel will be used to link genetic and physical maps, to assemble BAC contigs, and will help developing comparative maps between trout and other genomes.

This trout INRA scientific community gathers more than 30 PI scientists working on trout genetics, physiology, pathology, ecotoxicology, nutrition ... and as a resource person for this INRA-AGENAE trout community I again express my very strong support for this initiative.

Sincerely,

Dr Yann GUGUEN
INRA-SCRIBE
Campus de Beaulieu
35042 Rennes Cedex

Institut National de la Recherche Agronomique
Établissement public à caractère scientifique et technologique placé sous la tutelle conjointe des ministères chargés de la recherche et de l'agriculture
Campus de Beaulieu - 35042 Rennes Cedex - Tél. : 02 23 48 50 02 - Fax : 02 23 48 50 20
17 February 2004

Dr. Caird Rexroad
USDA/ARS National Center for Cool and Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430

Dear Dr. Rexroad,

I am writing to provide my strongest possible support for your proposal to the U.S. Department of Energy’s Joint Genome Institute’s Community Sequencing Program to sequence the genome of the rainbow trout. As part of the U.S. Department of Interior, our laboratory is extremely interested in this work because it offers to provide critical information on basic elements of the salmonid genome that will advance our work on infectious diseases and the immune system as well as to make available important research tools that can lead to improvements in the health of fish reared by both the private and public sectors. In my opinion, the ability to select a clonal line of trout (Swanson) for this project is an especially strong feature of the proposal that will provide researchers with a much superior laboratory model for future research.

Of particular interest to the U.S. Department of Interior will be the development of scientific information and research tools that can enhance the survival of populations of threatened and endangered salmonid species (e.g. genetically unique stocks of steelhead trout and sockeye, chinook and Atlantic salmon) in the western and eastern U.S. against losses from a variety of enzootic diseases. Likewise, research on infectious diseases of salmonids conducted in our laboratory will be greatly aided by results from this proposal. Genomic sequence data are needed to improve our understanding of basic features of the fish immune system and to further our research on the response of salmonid fish to a variety of infectious agents, antigens and vaccine formulation. Additionally, the availability of a clonal line of rainbow trout on which the sequence is based will provide a source of animals to the research community that will be critical to extend our work on development of new generation vaccines that can be used to protect salmonid fish against important diseases.

If there is anything I can do to further your chances of success, please do not hesitate to ask.

Sincerely,

James R. Winton, Ph.D.
Chief, Fish Health Section
Dear Caird,

I would like to register my strong support for the proposed trout genome programme, that is being put forward by scientists in the USA. The programme would have major ramifications for work throughout the world, including on-going fish immunology research in Scotland. We use trout as our model species, since it is THE fish species for physiological work, and has a large number of cell lines available for use, as well as a growing EST database. It is also an excellent model for the major farmed fish species in Europe and much of North America. Our work on fish cytokines has illustrated the importance of using a species that is readily available throughout the world, and that is amenable to functional studies. Whilst we are developing so-called post-genomics approaches to study trout, the lack of a sequenced genome is a serious drawback that needs to be addressed. The complexity of a salmonid genome is a challenge, with an ancestral genome duplication leading to two forms of many genes we study. For example, with IL-1B and TNFα these two isoforms have 85% and 92% aa identity, show differences in their splice sites, show differential expression, and degree of polymorphism. Whether these ligands have co-evolved with duplicated receptors has still to be established but is also a possibility. Studies on the promoters of these genes, and their linkage would benefit enormously from an available genome. One of the major areas of interest is whether cytokine gene duplication has occurred in fish, leading to novel cytokine genes not present in mammals. In the absence of a sequenced genome, and knowing that two genes are likely to exist from the duplication event, this can take a lot of effort to establish. Nevertheless, our work in identifying chemokine genes in the trout ESTs strongly suggests this is the case, and that some fish (trout) specific groups of duplicated genes exist that are previously unknown. Their function will be much more easily established in trout than most other species, and we are currently working to this end.

I hope the above helps to show the importance of trout, and that there would be global interest in such a genome should it become available.

Yours sincerely,
Chris Secombes.

Professor C.J.Secombes DSc, FIBiol
Head, School of Biological Sciences
Head, Scottish Fish Immunology Research Centre
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Zoology Building,
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To Caird Rexroad

Re: Support for sequencing of rainbow trout genome

Our lab is definitely in support of sequencing the rainbow trout genome. The Hagerman Fish Culture Experiment Station contains three different entities that all work on rainbow trout, the University of Idaho is researching alternative diets and optimization of feeds for rainbow trout and also works on conservation of wild salmonid populations, the Columbia River Intertribal Fish Commission also works on conservation issues surrounding salmon and trout. Both these groups use rainbow trout sequence information to characterize the difference in existing wild populations and to try and understand the genetic differences between wild and hatchery fish and resident and anadromous fish. The third group consists of the USDA-ARS whose research interests include a rainbow trout selection program that is working to develop rainbow trout strains that are improved for growth and utilization on plant-derived diets instead of standard fish meal and fish oil diets. This work includes evaluation of families with various positive traits and analysis to find genetic linkages to follow these traits. Our work also looks to evaluate the expression of genes know to be linked to growth, metabolism and nutrient utilization. Most of these pathways have been well worked out in other vertebrate systems but gene sequences, as yet, are not available for rainbow trout. Hence, a sequenced trout genome would be of tremendous assistance to our research and for the University of Idaho and the Intertribal Fish Commission. As you know we have a complete molecular biology lab and two 3100 ABI genetic analyzers, any assistance or support that we can provide to aid in getting the rainbow trout genome sequenced is at your service.

Sincerely,

Ken Overturf
February 13, 2004

Dr. Caird Rexroad III
USDA/ARS National Center for Cool & Coldwater Aquaculture
11876 Leetown Road
Kearneysville, West Virginia  25430

Dear Caird:

This letter is to confirm our support for your proposal to the Community Sequencing Program of the DOE Joint Genome Institute to obtain a draft sequence of the rainbow trout genome. More is known about the physiology and biology of rainbow trout than any other fish species. Sequencing the trout genome will combine a set of diverse advantages that are not available in any other research organism. This type of information will provide a strong platform for the detailed study of carcinogenesis, comparative immunology, toxicology, and the evolutionary process.

As an example of how this information could be used, losses due to disease in world aquaculture are estimated to be in the billions of dollars annually. Vaccination and other management strategies have been effective in preventing disease for many fish pathogens, however, the lack of information and tools for understanding the innate and acquired immune defense systems of fish has severely impeded the development of additional disease control strategies. Currently, no effective vaccines or other control strategies are available for the numerous viral disease of finfish which have very significant economic impacts on cultured fish in the United States. Infectious hematopoietic necrosis virus (IHNV) currently causes millions of dollars in lost fish production in the state of Idaho which is responsible for 80% of the rainbow trout produced as a foodfish in the United States. IHN virus also has a significant economic impact on cultured salmon and trout in other areas of the Pacific Northwest and also impacts some wild fisheries. Hence, successful development of the information outlined in your proposal will not only be of great interest to Clear Springs Foods, the worlds largest producer of rainbow trout, but potentially all entities involved in public and private aquaculture operations.

We look forward to hearing about you being successfully awarded this project in the very near future and the collaborations that will come from it.

Sincerely,

Scott LaPatra, Ph.D.
Director of Research and Development
Clear Springs Foods, Inc.
Dear Drs Rexroad and Thorgaard,

This is to lend our strong support to your efforts to initiate a rainbow trout genome sequencing effort. Our laboratory in Stirling, UK is focused on the genomics and post-genomics of the closely related Atlantic salmon, and there is no doubt that the availability of genome sequence for the rainbow trout would considerably facilitate our research into the molecular basis of complex traits in salmonids.

The salmonids are of enormous scientific interest from an evolutionary perspective, and aspects of their life history present some almost unique opportunities among vertebrates for research into basic physiological responses to changing environments. At a time of global climate change, their role as sentinel species could be particularly valuable. In addition, of course, the salmonids are valuable commercial species, both in terms of provision of high quality and essential nutrients for the human diet and for sporting purposes.

Perhaps most importantly, if one looks at the species that are already the subjects of genome initiatives, and those that are scheduled to become so, it is clear that a rainbow trout initiative would fill an obvious gap around the more primitive teleosts.

We apologise for this last minute input, and we sincerely hope that it does not come too late to be of some value. We await the outcome of the submission with keen interest, and we wish you every success in this important endeavour.

With kind regards,

Alan Teale and John Taggart

Alan Teale MA., Vet.M.B., M.Sc., Ph.D., MRCVS
Chair, Molecular Genetics
Institute of Aquaculture
University of Stirling
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19 February, 2004

Dr. Caird Rexroad III
USDA/ARS National Center for Cool and Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430

Dear Caird:

Re: A white paper advocating draft sequencing of the genome of the rainbow trout, *Oncorhynchus mykiss*

This letter is specifically intended to fully support your application for the draft sequencing of the rainbow trout genome. This is one species that has been extensively studied in all areas of biology, including physiology, toxicology, immunology, nutritional sciences, genetics and biomedical sciences. If you look at the available literature for this species, it becomes clear that the background information available is astounding and, consequently, would be an ideal candidate for linking genes to functional traits. The draft sequencing of trout genome would be an important first step in that direction as this information can be used by researchers (a large number) in this field to annotate sequences based on functional characteristics. Also, sequence availability will play an important role in the commercial application of this important food fish. We could obtain a better understanding of the stress response characteristics, an important determinant in the growth and disease incidences of this species in aquaculture, that would be invaluable for increasing production, but at the same time reducing costs associated with their production. In addition, the sequence availability will be instrumental in the development of molecular tools/signatures for stress detection. Specifically, this will allow for identification and characterization of mechanisms of action of individual chemicals in complex mixtures (common to most type of pollution) - an area that is important for aquatic health management, but limited by the lack of sequence availability. Again, already available baseline information on various aspects of physiology and toxicology of this species is a key factor in the strength of this animal as a model for aquatic research as well as biomedical research. I cannot stress enough the significance of this proposal and I fully support this undertaking. Please do not hesitate to contact me if you need any assistance.

Yours truly,

Matt M. Vijayan PhD
Associate Professor
February 18, 2004

Dr. Caird Rexroad
USDA-ARS-NGCCWA
11876 Leetwon Road
Kearneysville, WV 25430

Dear Dr. Rexroad:

I am writing in support of the proposal to sequence the genome of rainbow trout. A well-annotated genome of this species will enable research conducted by several researchers at Washington State University to advance more rapidly. In particular, Dr. Thorgaard's work involves development of genetic maps and attempting to understand the genetic control of complex traits in trout and salmon. In these studies, Dr. Thorgaard is using clonal (genetically uniform) lines of rainbow trout which have developed here at WSU. These lines greatly facilitate genetic research with the species because they improve reproducibility of results and simplify interpreting them. These studies will facilitate better understanding of conservation issues, fish farming, and the use of these animals for fundamental research in immunology, toxicology and cancer research. Moreover, rainbow trout is closely related to a number of other species (e.g., chinook, coho and sockeye salmon) which are economically and culturally significant in this region. The availability of a complete DNA sequence for this species, as has been developed for a number of other organisms, should allow our understanding of the organization of genes and the control of gene expression in this species to increase dramatically. Genetic mapping results obtained in Dr. Thorgaard's laboratory will be integrated with the sequence information to enable better data interpretations.

A number of other researchers at WSU, including Professors Dan Rodgers, Mike Dodson, Hubert Schwabl, Ray Lee, and Pat Carter are using the trout model in their research and could also benefit from the genome sequence information.

In summary, because sequencing this genome will facilitate this research and help advance our understanding of the use and conservation of this and other species, I strongly support this effort.

Sincerely,

[Signature]

James N. Petersen
Vice Provost for Research
Battelle Distinguished Professor of Bioprocessing

JNP/Jo

PO Box 641033, Pullman, WA 99164-1033
509-335-9141 • Fax: 509-335-1949 • www.research.wsu.edu
Dear Gary,

I am writing this letter in support of the proposed project to the U.S. Department of Energy's Joint Genome Institute's Community Sequencing Program to obtain a draft sequence of the rainbow trout genome. As you know, this work would provide a critical tool for researcher studying many aspects of basic biology, genetics, immunology, fisheries management, and aquaculture. The ability to identify functional genes would significantly aid in many aspects of research being conducted on trout and other salmonids. This includes identifying genes important in immune function and disease resistance, which could lead to improved vaccines for aquaculture and/or development of disease resistant lines of trout or salmon. I strongly support this proposal and if there is anything I can do to be of further assistance, please do not hesitate to contact me.

Sincerely,

Ken Cain

Ken Cain

Dr. Ken Cain
Associate Director, Aquaculture Research Institute
Assistant Professor (Aquaculture and Fish Health)
Department of Fish and Wildlife Resources and
WSU/UI Center for Reproductive Biology
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17 February 2004

Dr. Caird Rexroad
USDA/ARS National Center for Cool and Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430

Dear Dr. Rexroad,

I am writing this letter in enthusiastic support of the rainbow trout genome sequencing proposal to the U.S. Department of Energy’s Joint Genome Institute’s Community Sequencing Program. As you are aware, rainbow trout are one of the most important sustainable aquaculture and sport fisheries species in the United States. Currently, enzootic and emerging diseases are the primary cause of loss to the U.S. trout industry. Approximately, one-third of cultured trout die before harvest from infectious disease agents and a better understanding of the trout immune system is urgently needed to improve vaccine efficacy and to map disease resistance genes.

A draft genome sequence of the double haploid clonal line (Swanson) will be of enormous benefit to immune gene identification and functional immunology. We have recently identified and sequenced over 40 chemokine and tumor necrosis factor superfamily ligand and receptor cDNA’s from in the TIGR Rainbow Trout EST database. The utility of the trout EST database will be significantly enhanced by the availability of the corresponding genomic sequence. Specifically, the genome sequence will help us: 1) map regulatory elements and flanking genes; 2) identify genes not present in the EST database; and 3) better understand immune gene evolution. This information will provide important directions for our functional immunology program as well as for the design of an immune gene microarray. The availability of a well characterized clonal trout line will be a superior laboratory model for future fish immunology research.

Please let me know if I can be of any further assistance with your project!

Sincerely,

[Signature]

Gregory D. Wiens, PhD
Research Molecular Biologist (Immunology)
Dear JGI Review Panel-

February 17, 2004

I am writing in strong support of the proposal to the JGI Community Sequencing Program for a draft genome sequence of the trout, *Oncorhynchus mykiss*. I lead a research group at the NOAA Southwest Fisheries Science Center that focuses on the molecular genetics of salmonids. A draft sequence of the trout genome would be a huge step forward for my lab and for our research community. The trout is one of the most important fish species in the world, with the widest natural range of any anadromous (sea-run) fish in North America. The non-migratory form, rainbow trout, has been introduced all over the world and is of great importance for aquaculture and recreation fishing. The anadromous form, steelhead trout, on the US West Coast has many populations that are listed as threatened or endangered under the Endangered Species Act. The availability of a genome sequence for the species would be useful in understanding many aspects of the species’ biology, including differences between the different forms, and to better plan for their management.

In my lab, we are studying reproductive and migratory behavior in trout and salmon using molecular genetic methods. We have recently started several mapping projects using both laboratory and natural hybridization between divergent strains. We are using recently developed gene maps to identify chromosomal regions that contain genes influencing these complex traits. The availability of an annotated trout genome sequence would transform our work and allow us to easily identify genes involved in traits following mapping.

I am very excited about the prospect of an *O. mykiss* genome sequence. I can not emphasize enough the importance of such a project to my research program and to all of those studying salmonids.

I urge you to approve the community sequencing proposal for the trout genome.

Sincerely,

Dr. John Carlos Garza  
NOAA Southwest Fisheries Science Center  
Santa Cruz Laboratory  
110 Shaffer Rd.  
Santa Cruz CA 95060  
Tel. 831-420-3903  
carlos.garza@noaa.gov
Dear Dr. Thorgaard,

I have read with interest the White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout (Oncorhynchus mykiss) that you and your colleges have prepared with enthusiasm.

In the last three years, I have been working with this species, particularly in sex determination research area and I have realized that the rainbow trout is an excellent model, which may provide important knowledge in animal biology and human biology.

As you describe in your proposal the available resources in this species make the rainbow trout an appropriate model fish species for selecting this organism for genomic sequencing. The first reason is the rainbow trout fishes facilitate the ability to do experiments, e.g. the production of genetic crosses of interest (OSU x Swanson mapping panel proposed in the project) and the culture and propagation of the animals in the laboratory successfully. Secondly, the availability of a large number of genomic resources and technologies that community working with rainbow trout currently has is highly valuable. It includes detailed genetic maps, a large number of genetic markers and genomic libraries available and several QTLs identified and associated with traits of interest –biomedically and economically important QTLs-. Finally, several investigations related with carcinogenesis, toxicology and immunology in rainbow trout seem to be promising to provide basic biological information to human health. For all of these reasons, I consider that sequence information in this specie will be effectively used for the research community working in this species and in others. I am writing this letter to give you all my support in this project for the sequencing of the rainbow trout genome.

Sincerely,

Alicia Felip Edo, Ph. D.

School of Biological Sciences
Washington State University
Pullman, WA 99164

afelip@mail.wsu.edu
February 18, 2004

JGI Community Sequencing Program
Scientific Advisory Committee

To members of the Committee:

I have been utilizing the rainbow trout in NIH-funded biomedical research for over 15 years. My research has focused primarily on utilization of the rainbow trout as a model for human cancer. I am very excited about the possibility of selection of the rainbow trout as a sequencing project by the Joint Genome Institute.

One of the most important applications for my research program over the next five years would be in use of the sequencing results to identify genes for inclusion on custom microarrays that we are producing. February 1, 2004, I submitted an R01 application to NIH to perform large ultra-low dose carcinogen studies in the rainbow trout. This proposal builds upon a recent successful study that utilized 42,000 trout to determine the dose of dibenzo[a,l]pyrene that produced an additional cancer incidence above background of 1 in 5000. These results are a 50-fold enhancement over the largest cancer study ever done in rodents, the mega-mouse ED01 study with 2-acetylaminofluorene. The grant proposal seeks support to conduct similar studies with aflatoxin B1 and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). An important component of this study involves microarray work. The hypothesis under test is that various clusters of genes will have expression altered with carcinogen dose in a particular pattern that will reveal mechanisms. Currently, we are utilizing the GRASP array for Atlantic salmon. Having the sequencing data available for rainbow trout will allow us to design, print and employ a large custom microarray specific for rainbow trout. Our goal is to identify potential human genes that may be important targets for these environmental carcinogens.

I thank the Committee in advance for their efforts and I would be pleased to respond with additional information if requested.

Sincerely yours,

David E. Williams, Ph.D.
Director
Feburary 16,2004

Dr. Caird E. Rexroad III  
USDA/ARS National Center for Coll and Cold Aquaculture  
11876 Leetown Road  
Kearneysville, West Virginia 25430

Dear Caird:

I’m pleased to support your proposal to the Community Sequencing Program of the DOE Joint Genome Institute for generation of a draft genome of the rainbow trout. A draft trout genome will greatly enhance the broad scope of biological research of trout and other economically important fish species.

At TIGR, our group has been clustering and assembling ESTs and known genes into contigs to represent the full transcriptome. The EST contigs (TCs) have been widely used for microarray oligo design, SNP detection, genome annotation and comparative genomic analyses. An efficient pipeline has been set up to assemble and annotate prokaryotic and eukaryotic genomes at TIGR. We’d like to provide all the necessary bioinformatics support for annotating the genome and further functional genomic research including the EST assembling, gene modeling by mapping the known trout genes back to genome, SNP prediction, oligo array design and comparative genomic analyses involving the Fugu, zebrafish and trout genomes etc. And all the data generated will be available for general public use as soon as possible.

We look forward to working with you on this project.

Best Wishes,

Dan Lee, PhD
15th February 2004

Dr Caird E. Rexroad III
USDA/ARS National Center for Cool and Cold Water Aquaculture
11861 Leetown Road,
Kearneysville,
West Virginia 25430
USA

Dear Dr Rexroad

A White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout, *Oncorhynchus mykiss*

I am writing in support of your joint proposal with Drs Thorgaard and Palti to develop an annotated genome sequence of rainbow trout (*Oncorhynchus mykiss*).

My own laboratory at the Aberdeen Proteome Facility has collaborated in two complementary projects using proteomics to look at rainbow trout physiology at the molecular level. Whilst it was possible to generate qualitative data on protein changes under the conditions of the experiment, subsequent protein identification to link these to the expression of specific genes was severely hampered by a lack of reliable genome sequence data. The availability of an annotated genome sequence as you propose, preferably in the public domain, would have benefited our work and allowed it to proceed at an optimal pace. Our own experience with a range of microbial, mammalian and plant proteomic studies have demonstrated the essential requirement for comprehensive and reliable genome sequence for successful application of proteomic technologies.

I support your endeavours to generate an annotated genome sequence for rainbow trout and I look forward to its successful culmination.

Yours sincerely

Phillip Cash, PhD
Reader in Medical Microbiology
Co-ordinator, Aberdeen Proteome Facility

[Letter sent as email attachment]
DATE: February 14, 2004

FROM: Christopher J. Bayne, Professor, Oregon State University.

SUBJECT: Submission of a Rainbow Trout Draft Genome Sequencing Proposal to the JGI CSP.

I speak for a cadre of research personnel at Oregon State University as I write with enthusiastic support for the proposal to obtain the sequence of *Oncorhynchus mykiss*, the rainbow trout (also known as Steelhead). Growing out of an enormous body of research in many aspects of physiology and ecology, we have had an active research program with this species for the past half century. Stemming from this and supported by extensive growth in the published literature on this species, there are multiple reasons for pursuing questions with rainbow trout into the future. Yet, knowledge of the genome has begun to lag behind that of several other teleost species, and this constitutes a hurdle for us.

The Marine and Freshwater Biomedical Sciences Center at Oregon State University has initiated a project to design and construct a DNA array for the rainbow trout ([http://www.science.oregonstate.edu/mfbsc/facility/micro.htm](http://www.science.oregonstate.edu/mfbsc/facility/micro.htm)). Each month we learn of more individuals expressing the hope that they will be able to use these arrays in their developing research. It is anticipated that by the end of 2004, at least 4 research teams at OSU will be using these arrays. The features on these arrays will, of necessity, have been designed largely in ignorance of genomic sequences. Features designed on the basis of a well annotated transcriptome (as in our array) will be powerful tools. However, as researchers move to the interpretation of results and the further work up of specific genes, continuing absence of genome data would constitute a remaining hurdle to slow down progress. One illustration of this will be when dealing with gene family members that remain functional since the whole genome duplication event at the base of the salmonids.

For these are other advantages, we strongly encourage JGI to commence sequencing on this genome as soon as is feasible. If, once completed, an annotation jamboree can be scheduled, I will be keen to participate at my own expense.
Dear colleague,

Re: Submission of a Rainbow Trout Draft Genome Sequencing Proposal to the JGI CSP

We are writing to support the sequencing of rainbow trout (Oncorhynchus mykiss) genome. Our team has been studying rainbow trout pathogens, pathology and immunology for many years. We have developed a systematic study of host-pathogen interactions through systematic and differential screening methods, which resulted in the identification of several virus-induced genes. We are now involved in the development of global approaches using micro-arrays and functional genomics. A first step towards global strategies for systematic survey of pathogen-induced genes has been recently done with massive EST sequencing by the USDA (USA) and INRA (France), but genomic data are genuinely needed to develop fully integrated approaches.

We have also recently developed a dedicated T-cell receptor CDR3-spectratyping method allowing the study of rainbow trout T-cell response and T-cell repertoire diversity in rainbow trout. In this context, rainbow trout is one of the best models for the study of cellular immunity and immune repertoires in teleosts. Genomic data for TCR, and MHC locus would greatly contribute to these studies, which are of significant interest for both comparative immunology and salmonid pathology. Indeed, the study of TCR genes has been mainly performed at the transcript level, leading to the identification of a large but still partial diversity of gene segments.

Dr Abdenour BENMANSOUR
Dr Jean François BERNARDET
Dr Pierre BOUDINOT
Dr Michel DORSON
Dr Christian MICHEL

Relevant references:


To Whom It May Concern:
C/O Caird Rexroad:

I would like to offer my full and complete support for a rainbow trout sequencing project. My laboratory works on immune system cDNAs of fish and we generally use rainbow trout as a model system. We have isolated several interesting clones some of which encode chemokines - small soluble messenger molecules that attract white blood cells to sites of injury, as well as a set of three interesting clones related to the mammalian Major Histocompatibility Complex Class II Associated Invariant Chains (or just Invariant chains for short). We are continuing to characterize these genes but have come across some interesting genomic questions.

One example of this type of problem, two of the chemokine cDNA clones CK2 (Cytokine. 17(2): 71-81 2002) and CK2.1 are nearly 100% identical where they overlap – they only have a single nonsynonymous substitution – but differ in that CK2.1 has a section inserted that adds 126 nucleotides of coding sequence. We have determined that this is not an alternative splice product, but are uncertain if it is an allele or a separate gene. The products of the two transcripts appear to be differentially regulated. We wish to know if they are separate genes, but cannot rely on Southern blots as they are too similar for probes to distinguish. A rainbow trout genome project would provide an answer to this question as well as revealing the presence or absence of other chemokine genes in the genome.

The three invariant chain cDNA clones (Develop. Comp. Immunol. 27(5): 377-391 2003) include a pair that are 92% identical and resemble two different alternative splice products produced by human immune system cells. These two trout clones appear to be encoded by separate genes, but one appears to have two copies when tested by Southern blot. We think that perhaps the two different forms evolved by divergence following the duplication of the salmonid genome, but are uncertain how that would explain two copies of one of the individual genes. A map of the location and number of each of these genes would help clarify this question.

14. In addition to the above genes, we work on many more and have many questions on how the duplication of the trout genome relates to what we see – some such as CD9 (Immunogenetics 54: 604-609 2002) and CEBP-beta (Immunogenetics 55(4): 253-261 2003) appear to be single copy – have the duplicated versions been lost?

I collaborate with the other Canadian fish immunologists, there are three at the University of Alberta, and two at the National Research Council of Canada’s Institute for Marine Biosciences, as well as a genome group working on Atlantic Salmon in British Columbia, and we are very eager to see the results of a trout genome sequencing project as it would have applicability to all of our research programs. There are not enough of us in Canada to justify an
effort ourselves, even though rainbow trout aquaculture is important economically here, so we are eager to participate, contribute and collaborate with an such an effort by our American colleagues as it would advance research in this area here as well.

Sincerely,

Brian Dixon
Assistant Professor
X2665
bdixon@uwaterloo.ca
February 16, 2004

Dr. Caird Rexroad III
UDSA/ARS/NCCCWA
11876 Leetown Road
Kearneysville, WV 25430

Dear Dr. Rexroad III:

I am writing this letter to lend my 100% support on the effort of obtaining a draft sequence of the rainbow trout genome. Success of this project will bring enormous impact to scientists working on areas of physiology, endocrinology, genetic selection and breeding and disease prevention and treatment of rainbow trout.

My research programs in rainbow trout center on areas of molecular endocrinology of growth, molecular pathobiology and transgenesis. Each area of studies requires the information of genome sequence of rainbow trout. If the genome sequence of rainbow trout is available today, it will speed up my research progress several folds.

I strongly support this project, and my laboratory would like to be directly involved in this venture.

Sincerely yours,

Thomas T. Chen
Professor of Molecular and Cell Biology
Dear Caird:

It is my pleasure to support your White Paper to the Joint Genome Institute, requesting that they generate a draft sequence of the rainbow trout genome.

The point that I would emphasize (selfishly, as a comparative immunologist) in supporting your application is that great advances in understanding the immune systems of teleost fish would result from sequencing the trout genome. The fish whose genomes are being (or have been) sequenced to date (2 pufferfish, medaka, zebrafish) are not well-developed models for studies of immunity. In contrast, the trout has a very large, skilled and active community of researchers interested in its immune system, and having a draft genome sequence would be an enormous accelerant to their research.

My own research in fish immunology is in the channel catfish, and this would also benefit greatly from sequencing of the trout genome. There is no question that, following the availability of a draft genome sequence for the trout, immunologists would be able to provide many more linkages between genetics and functional immunity in teleost fish. The trout is well-poised for such advances to be made, and these advances would in turn help to illuminate the genetic basis of immunity in channel catfish. The trout is of course quite different from the catfish in terms of immune function: it is a cold-water species whose susceptibility to infection rises with environmental temperature. The catfish, in contrast, is at home in warm water of poor quality, and it is often a rapid drop in temperature that makes it sick. Thus, the ability to compare and contrast the genetics, physiology and functional immunity of trout and catfish will be very fruitful for our understanding of both species. Clearly, research on trout and catfish immune systems (and genomes) is complementary, not competitive.

I wish you every success with your application for a draft genome sequence of the rainbow trout.

Yours sincerely,

Gregory Warr
Professor
Dear Caird:

This letter is to express my support and extreme enthusiasm for your efforts to propose that the rainbow trout genome be sequenced. I have worked on this species as an experimental biologist since 1984, and it has been a mainstay of fish physiology and biochemistry for more than 40 years beginning with the work of experimental aquaculturists in Canada. It is not an exaggeration to say that this species is literally the “white rat” of fish biology, and that more is known about the functional biology of *Oncorhyncus mykiss* and other members of the family Salmonidae than any other fish group. When combined with the emerging data set on ESTs from your group and others, a full genomic sequence would enable and enhance all manner of basic and applied science. In short, this is a project whose time has come.

Thank you for your efforts on this project on behalf of the international community of fish biologists.

Best wishes,

Patrick J. Walsh
Professor and Center Director

University of Miami
Rosenstiel School of Marine and Atmospheric Science
NIEHS Marine and Freshwater Biomedical Science Center
4600 Rickenbacker Causeway
Miami, FL 33149-1098 USA
19 February 2004

Dr. Gary H. Thorgaard  
School of Biological Sciences  
Washington State University  
Pullman, WA 99164-4236

Re: Rainbow trout White Paper  
Dear Dr. Thorgaard

Thanks a lot for inviting me to support a fascinating proposal to sequence a salmonid genome. I am of course pleased to support it. It is timely for me because some interesting aspects of RAF proto-oncogene family genes derived syntenies and processing of SSTN11 insertion and preparing a couple of MS in salmonids (Atlantic salmon, Chum salmon and Rainbow trout). Several works have been conducted in collaboration with Dr. Todd Gray in New York Dept of Health, USA. Up to date, we are coming to conclusion that a "big bang" of genomic duplications at some specific genomic arrays in early evolution of vertebrates, including origin of human chromosomes such as 3, 6, 7, 22, and X. In future development of this study, I am definitely pleased to have more comprehensive analysis of genomic sequences and expression of genes in fish species. I am also interested to see multiple gene expression including those we found in fish species (Yellowtail, Red seabream) in particular physiological conditions that affect fishery production, for instance biotinylated enzymes responsible for recycling amino acids in fertile and unfertile periods of fish, some oncogenes in environmental hormone etc. When tightly linked with genomic projects on fishes, these studies not only enhance technologies in fishery, aquaculture, and environmental assessments, but also provide a new scope to human genomics and its medical applications. While some of my studies include rainbow trout and others not, the sequence of a salmonid would be quite valuable to our work.

Best Regards,

Shunnosuke Abe

Dr. Shunnosuke Abe  
Associate professor.  
Laboratory of Molecular Cell Biology  
Faculty of Agriculture, Ehime University  
3-Tarumi, Matsuyama 7908566, Japan  
Tel/fax: 81-89-946-9853 (089-946-9853)  
mailto:abe@mcb.agr.ehime-u.ac.jp
February 19, 2004

Community Sequencing Program
Joint Genome Institute
US Department of Energy

To Whom It May Concern:

We are writing this letter to express our support for the proposal to sequence the genome of the rainbow trout. We represent a core group of scientists in the Department of Biological Sciences at the University of Idaho whose research programs all focus on rainbow trout and other salmonids. Our group is comprised of 5 faculty, all of whom are endorsing this proposal. Our specific areas of research include the genetic basis of local adaptation (Robison), developmental biology and cryopreservation (Cloud), endocrine disruption and reproductive physiology (Nagler), gamete physiology (Ingermann), and the molecular endocrinology of reproduction (Young).

As detailed in the proposal before you, the scope of scientific endeavor on rainbow trout is very broad. We can think of no other fish whose genomic sequence would have such a wide ranging scientific impact. Availability of a draft sequence of the rainbow trout genome would greatly facilitate the research of many investigators in the United States and abroad, and it is our sincere hope that you consider funding this important project.

Sincerely,

Barrie D. Robison
Assistant Professor

James Nagler
Associate Professor

Joseph Cloud
Professor

Graham Young
Associate Professor

Rolf Ingermann
Professor
To whom it may concern

Re: A White Paper Advocating Draft Sequencing of the Genome of the Rainbow Trout, Oncorhynchus mykiss

I hereby fully support the above proposal to sequence the genome of rainbow trout. The availability of the complete genome of rainbow trout would benefit many areas of research. In particular, information on the genes involved in the immune system of this species would greatly enhance our ability to design appropriate therapeutics to control infectious diseases. This is imperative for a sustainable development of the aquaculture of rainbow trout in many countries around the world.

In recent years, salmonids have become the model system for immunological research in fish. The main reasons are its economical importance and the availability of a large number of immunological assays. The analyses of these assays can be boosted by the knowledge of genes involved in different immunological mechanisms.

In conclusion, elucidation of the genome of rainbow trout will contribute significantly to the area of immunology and immunogenetics with major ramifications for the rational design of strategies to control present and emerging infectious diseases.

Yours sincerely,

René J.M. Stet PhD
Associate Professor in comparative Immunology and Immunogenetics
Cell Biology and Immunology Group
Department of Animal Sciences
Wageningen University
The Netherlands
This letter is written on behalf of the rainbow trout genome sequencing proposal and I encourage the U.S. Department of Energy's Joint Genome Institute's Community Sequencing Program to fully support the proposal. Rainbow trout have been studied with great intensity within natural and laboratory environments, widely used in research as a model species, and are an economically important species (they provide revenue in both aquaculture and sport angling). Indeed, more is known about their biology and physiology than any other fish species. As such, it is imperative that the rainbow trout genome be sequenced to continue progressive research within this important species. The genome sequence of rainbow trout can provide an unlimited framework for studies in evolution, ecology, fish health and aquaculture. Furthermore, rainbow trout genome sequence information will also be extremely valuable for studies of other closely related salmonids within the genera Oncorhynchus, Salmo, and Salvelinus. In closing, I wish to stress that a well-annotated rainbow trout genome sequence is vital for maintaining progressive research of rainbow trout and other salmonids.

Sincerely,

Carl Ostberg
Fishery Biologist
US Geological Survey
Biological Resources Division
Western Fisheries Research Center
6505 NE 65th Street
Seattle, WA 98115
16 February 2004

Dr. Caird E. Rexroad III
USDA/ARS National Center
for Cool and Cold Water Aquaculture
11861 Leetown Road
Kearneysville, WV 25430

Dear Caird,

I am writing in support of your collaborative effort to obtain support for sequencing the genome of rainbow trout (Oncorhyncus mykiss). As the most intensively studied fish species, an effort to sequence this fish genome is sadly overdue. Given its wide use as a model research species, as evidenced by the volume of research publications that have and continue to appear, the genome sequence will be of immediate value to researchers across numerous biological disciplines. The evolutionary history of salmonid fishes in general and rainbow trout in particular will make the sequence of this species of great comparative value with regards to the other piscine species that have been sequenced (Zebrafish and Pufferfish). I believe that sufficient resources (BAC libraries, mapped molecular markers) are currently available to provide the necessary backbone for this undertaking and clearly both the genetic and biological resources are in place to effectively USE the information to be gained by the project.

I wish you the best in securing funding for this important scientific endeavor.

Best regards,

Kent M Reed, PhD
Assistant Professor, Genomics
February 16, 2004

Joint Genome Institute, Community Sequencing Program
U.S. Dept. of Energy
1400 Independence Avenue, SW
Washington, D.C. 20250-2241

Letter of support for

SEQUENCE OF THE RAINBOW TROUT GENOME

Gary Thorgaard, principle investigator

I am writing this letter in support of the collaborative proposal to begin sequencing the genome of rainbow trout *Oncorhynchus mykiss*. Selection of this fish is a logical choice for this project, as it is an important economic resource, a natural resource and a commonly used model for disease in other salmonids as well as other animal investigations (e.g. toxicity and carcinogenesis).

The primary investigator, Gary Thorgaard, has had a very productive collaborative research history, and the proposed research is an extension of that. He has contributed a great deal to the current knowledge of the trout genome and the associated gene products.

I fully support these efforts and hope that this project will be considered for funding.

Sincerely,

Jerri Bartholomew
Asst. Prof., Sr. Res.
Center for Fish Disease Research
Dept. of Microbiology
Oregon State University
Corvallis, OR 97331
Institute of Applied Biotechnology
University of Kuopio
P.O.B. 1627, FIN-70211 Kuopio, Finland

Dr. Caird E. Rexroad III
USDA/ARS National Center for
Cool and Cold Water Aquaculture
11861 Leetown Road Kearneysville,
West Virginia 25430, USA

Re: Project proposal

February 16, 2004

Dear Dr. Rexroad,

Your plan for sequencing of rainbow trout genome is exclusively timely and interesting. Our research team highly appreciates collaboration with you in functional genomics of salmonid fish, clones from your cDNA library were of great value for development of our cDNA microarray. We have used this platform in many experiments and a large number of genes showed differential expression in response to stress, bacterial antigens and toxic compounds. These results add to understanding of biochemistry and physiology of rainbow trout and will help to develop novel markers for environmental research and selective breeding. At present many groups are involved in similar projects and gene expression data accumulate rapidly. Obviously, studies of regulation of gene expression are essential for further development of genomic research in salmonid fish and genome sequencing will open exciting possibilities. Therefore I hope for success of your application.

Yours sincerely,

Hannu Mölsä, Lic.Ph., project leader

Aleksei Krasnov, Ph.D., principal scientist
17 February 2004

Dr. Caird Rexroad
USDA/ARS National Center for Cool and Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430

Dear Dr. Rexroad:

I strongly support your proposal on sequencing the rainbow trout genome.

The simple fact that more is known about the biology and physiology of rainbow trout than any other fish species is justification enough to give the sequencing of rainbow trout priority above any other fish. The continued intensity of research on this species and the tools currently available to exploit rainbow trout as a model species for diverse research purposes already ensures that rainbow trout research will continue to yield significant discoveries in fields including physiology, ecology, evolution, genetics, fisheries and aquaculture. The sequencing of the rainbow trout genome will thus strengthen an already vital and diverse research network that is unmatched for any other species of fish.

Sincerely,

[Signature]

Gregory M. Weber, Ph.D.
Research Physiologist
United States Department of Agriculture
Agricultural Research Service
National Center For Cool And Cold Water Aquaculture
11876 Leetown Road
Kearneysville, West Virginia 25430
Voice: (304) 724-8340 EXT. 2131
Fax: (304) 725-0351
Email: GWEBER@NCCCWA.ARS.USDA.GOV
Dear Dr. Thorgaard,

I am very excited to hear of your proposal advocating sequencing the genome of the rainbow trout. *Onchorhyncus mykiss* is an excellent choice of salmonid, the genomic sequence of which will have commercial, biological, and evolutionary significance.

My collaborator, Dr. Abe Shunnosuke of Ehime University in Matsuyama, Japan, and I are interested in the evolution of a family of genes and their genomic relationships. These genes include members that have been implicated in cancer and development. Piecing together the evolutionary history of their genomic architecture has given us insight into their regulation and dissemination. Because of their genetic diversity, fish have proven to be information-rich targets for analysis, and we have analyzed several species already (*Danio rerio*, *Fugu ribripes*, and *Seriola quinqueradiata*). While our studies are not rainbow trout-specific, the sequence of a salmonid would be quite valuable to our work.

Sincere regards,

Todd Gray, Ph.D.
The Genomics Institute
Wadsworth Center
465 Jordan Road
Troy, NY 12180

gray@wadsworth.org
February 18, 2004

Dr. Caird Rexroad III  
USDA/ARS National Center for Cool and Cold Water Aquaculture  
11876 Leetown Road  
Kearneysville, West Virginia 25430

Dear Dr. Rexroad,

I am very excited and pleased to offer a letter of support for your proposed project to obtain a draft sequence of the rainbow trout genome.

Troutlodge, Inc. has been involved in the breeding of rainbow trout as an aquacultured animal for over 50 years and are now recognized as the world leader in sales of live embryos to this industry. As such, it is extremely important for us to retain a competitive edge by utilizing all resources available to us. We believe that this project will offer a major source of new genetic information to industry, university, and agency researchers alike. Any major gains in knowledge as would come from such a project can only be viewed as beneficial from our perspective.

We remain supportive of work at the NCCCWA by offering whatever we can in the way of gametes from specific stocks of interest or any other research materials that might come from our brood fish. Additionally, we look forward to being able to utilize some of these remarkable new tools in our ongoing breeding programs.

We wish you the best of luck in your endeavor.

Sincerely,

James E. Parsons  
Vice-President  
Research and Technical Services  
Troutlodge, Inc.
13 February 2004

Dr. Gary Thorgaard
Department of Zoology
Washington State University
Pullman, Washington 99164-4236

Dear Dr. Thorgaard:

We are writing in support of your application for funding from the Community Sequencing Program of the DOE Joint Genome Institute (JGI) for genome sequencing in rainbow trout. Among other things, a draft sequence of the rainbow trout genome provides an important means of understanding the genetics of adaptation in salmonid populations. With conservation concerns growing for many of these species, your study offers a solid foundation for investigating the architecture of life-history and selection response in these fishes. We hope that our letter will contribute in some small way to financial support of your study.

Sincerely yours,

Michael J. Ford, Ph.D.
Director, Conservation Biology Division

Jeffrey J Hard

Jeffrey J. Hard, Ph.D.
Manager, Population Biology Program
Dear Dr. Rexroad,

I am writing to support the proposal to the Community Sequencing Program of the DOE Joint Genome Institute to obtain a draft sequence of the rainbow trout genome.

We work on trout immunology. Because of the economical importance of salmonid fishes and the convenience with which rainbow trout can be handled, trout is the main model fish for functional immunology. Fish immunology is very important in regard to disease control in aquaculture, but should also contribute to the field of mammalian immunology. Because of the complexity of the immune system many subtle processes may remain unnoticed; once discovered in a species where they are more pronounced (possibly trout) they may be found throughout phylogenetic classes.

Complete knowledge of the genomic sequence is very important for functional studies. It means that functions can be efficiently predicted and that the functions of genes/molecules can be interpreted within the proper genetic context. For example, we already found a number of trout major histocompatibility complex (MHC) class I genes, but knowing them all would surely help to understand their functions.

Although we are aware of the tremendous effort it takes to determine a full-length genome sequence, that effort is probably small in regard to the improved efficiency of trout research worldwide.

The sequencing of the trout genome will also help the discussion if whole genome duplications did contribute to evolution. That issue is heavily disputed. Salmonid fishes are among the few species in which such whole genome duplication probably has occurred relatively recently. That recent nature should allow conclusive evidence at the sequence level.

I am grateful to the researchers willing to undertake this effort.

I am happy to have my name added as a co-author.

Sincerely,

Johannes Martinus Dijkstra, Ph.D.
Institute for Comprehensive Medical Science,
Fujita Health University,
1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi-Ken 470-1192, Japan
E-mail: dijkstra@fra.affrc.go.jp
Thursday, February 19\textsuperscript{th}, 2004

Dr. Caird Rexroad III  
USDA/ARS National Center for Cool and Cold Water Aquaculture  
Kearneysville, West Virginia, USA

Dear Caird,

I would like to take this opportunity to express my support for the generation of a draft sequence for the rainbow trout genome as part of the Community Sequencing Program of the Joint Genome Institute.

I have been involved with rainbow trout genome research as a PhD student and now as a postdoctoral fellow. As such, I strongly believe that the availability of a draft sequence for the genome of this species would represent a major breakthrough for our community. In the particular focus of my field research, I anticipate considerable benefits for the analysis of the genetic basis of quantitative traits, the investigation of evolutionary processes in ancestrally polyploid organisms, and the study of vertebrate evolution. In addition, the opportunities for comparative genomics with closely related salmonid species (such as Atlantic salmon) would extend the benefits of a draft sequence to an even larger community.

Sincerely,

Karim Gharbi, Ph.D.  
Postdoctoral Fellow  
University of Guelph  
Ontario, Canada
February 16, 2004

Dear Dr. Rexroad:

I am writing to support the proposal to the Community Sequencing Program of the DOE Joint Genome Institute to obtain a draft sequence of the rainbow trout genome. We are currently working on MHC, cytokines and cell-mediated immunity in rainbow trout and are trying to isolate immune relevant genes from rainbow trout using genome data base of Fugu and zebrafish. However, we have great difficulties and limitations to utilize the genome data base of different species due to the great diversity in teleost fishes.

I am quite sure that information on genome sequence in rainbow trout will tremendously advance biology as well as molecular approaches in this species which is one of the most important fish in aquaculture world wide and a nice model for comparative immunology or biology.

I am happy to have my name added as a Co-author. I really hope we are successful in this exciting project.

Sincerely,

Teruyuki Nakanishi, Ph.D.
Laboratory of Fish Pathology, Department of Veterinary Medicine, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510 Japan. Tel: +81-466-84-3632, Fax: +81-466-84-3633 E-mail: tnakanis@brs.nihon-u.ac.jp
Dear Dr. Rexroad:

Thank you for sharing with me the proposal that you are going to submit to JGI for sequencing the rainbow trout genome. I strongly support this proposal. As you know, as an active member of the Aquaculture Genome community, I think that this is the way to go! Soon, the genome community will be divided by the genomes with genome sequences and those that do not. Trout, as one of the major aquaculture species, have been well studied. Sufficient genomic resources have been created that will be able to support a successful genome sequencing project and sequence assemblies. A large research community exist both in the US and around the world. All these elements should support the genome sequencing project in this species. The sequence information will undoubtedly positively impact the research community in a way otherwise not possible by any other research to date.

This project, if funded, will impact my research fundamentally. As you know, I am conducting much comparative genomics research in catfish that needs gene linkage information (NOT marker linkage information) from other fish species. The sequencing project will provide not only more tools to conduct efficient comparative genome analysis, but also would allow us to conduct better annotations by phylogenetic analysis, as well as gene discovery efforts.

As the Aquaculture Coordinator under the National Animal Genome Project, I strongly support this effort also from the perspectives of aquaculture. Please let me know how I can be helpful and do not hesitate to call on me.

Sincerely,

John

Zhanjiang (John) Liu, Ph.D.  
Aquaculture Genomics Coordinator  
Alumni Professor in Genomics and Director  
Aquatic Genomics Unit  
Department of Fisheries and Allied Aquacultures  
Program of Cell and Molecular Biosciences  
Auburn University  
Auburn, AL 36849
To: Community Sequencing Program of the DOE Joint Genome Institute  
From: Kenneth P. Blemings, Ph.D.  
Date: February 18, 2004  
Re: Submission of a Rainbow Trout Draft Genome Sequencing Proposal

Greetings. I am writing in support of the request for JGI to participate in obtaining a draft sequence of the rainbow trout (RBT) genome. I am a junior faculty member at West Virginia University and have begun to collaborate with researchers at several entities working with RBT. My work focuses on efficiency of nutrient use. Increasing the efficiency with which rainbow trout use their ingested nutrients for growth will decrease production cost as well as decrease nutrient effluent from fish farming operations. Access to a well-annotated genome sequence is critical to continued progress in my research program and I am certain this is true of many others. I do strongly urge you to participate in developing a well-annotated genome sequence for RBT.

Best regards,

Kenneth P. Blemings, Ph.D.  
Assistant Professor of Nutritional Biochemistry  
and of Genetics and Developmental Biology  

West Virginia University  
Division of Animal and Veterinary Sciences  
P.O. Box 6108  
Morgantown, WV 26506

(P) (304)-293-2631 X4315  
(F) (304)-293-2232  
email kbleming@wvu.edu
2/18/2004

Caird Rexroad III  
USDA/ARS National Center for Cool and Cold Water Aquaculture  
11876 Leetown Road  
Kearneysville, West Virginia 25430

Dear Caird:

It’s hard to imagine how far the field of fish genetics has come since I began in 1973! Salmonid fishes have had more genetics work done on them than any other fish species, primarily because of their cultural significance but also because of the genomic duplication that led to the origin of the group. What we lack today is the complete sequence of the genome. My work on QTLs for IHN virus resistance will be greatly enhanced by the genomic sequence, as well as my work on evolution of the Oncorhynchus mykiss subspecies.

Please add my name to the list of those who strongly support the initiative to sequence the rainbow trout genome.

Sincerely,

Bernie May, PhD, GVL Director
Dear Gary Thorgaard,

Thank you for sending me the information concerning your proposal regarding the sequencing of the rainbow trout genome.

I wholeheartedly support this venture. At present my research covers both fish toxicology and endocrinology, with the rainbow trout as one of the chosen model species (as outlined in the proposal). We are currently generating differential expressed trout cDNA libraries for various test scenario we use. This research would be greatly enhanced with knowledge of the rainbow trout genome.

Good luck with your application and please keep me informed of any progress.

Kind Regards

Nic Bury

Dr. Nic Bury
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Rennes, le 18/02/04

As a research worker (i) involved in the biology of the trout (more especially muscle development) and (ii) using genetic tools in relation with this fish species, I quite support the proposal of obtaining a draft sequence of the rainbow trout genome. The realisation of such a program would obviously help the achievement of our work as well as that of our colleagues interested in fish biology.

Dr. P.Y. Rescan
INRA/SCRIBE
Campus de beaulieu
35042 Rennes
FRANCE
APPENDIX E

STATUS AND OPPORTUNITIES FOR GENOMICS RESEARCH WITH RAINBOW TROUT

(REVIEW 2002)
Status and opportunities for genomics research with rainbow trout

Gary H. Thorgaard\textsuperscript{a,*}, George S. Bailey\textsuperscript{b}, David Williams\textsuperscript{b}, Donald R. Buhler\textsuperscript{b}, Stephen L. Kaattari\textsuperscript{c}, Sandra S. Ristow\textsuperscript{d}, John D. Hansen\textsuperscript{e}, James R. Winton\textsuperscript{f}, Jerri L. Bartholomew\textsuperscript{g}, James J. Nagler\textsuperscript{h}, Patrick J. Walsh\textsuperscript{i}, Matt M. Vijayan\textsuperscript{j}, Robert H. Devlin\textsuperscript{k}, Ronald W. Hardy\textsuperscript{l}, Kenneth E. Overturf\textsuperscript{m}, William P. Young\textsuperscript{n}, Barrie D. Robison\textsuperscript{o}, Caird Rexroad\textsuperscript{p}, Yniv Palti

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Abstract

The rainbow trout (\textit{Oncorhyncus mykiss}) is one of the most widely studied of model fish species. Extensive basic biological information has been collected for this species, which because of their large size relative to other model fish species are particularly suitable for studies requiring ample quantities of specific cells and tissue types. Rainbow trout have been widely utilized for research in carcinogenesis, toxicology, comparative immunology, disease ecology, physiology and nutrition. They are distinctive in having evolved from a relatively recent tetraploid event, resulting in a high incidence of duplicated genes. Natural populations are available and have been well characterized for chromosomal, protein, molecular and quantitative genetic variation. Their ease of culture, and experimental and aquacultural significance

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has led to the development of clonal lines and the widespread application of transgenic technology to this species. Numerous microsatellites have been isolated and two relatively detailed genetic maps have been developed. Extensive sequencing of expressed sequence tags has begun and four BAC libraries have been developed. The development and analysis of additional genomic sequence data will provide distinctive opportunities to address problems in areas such as evolution of the immune system and duplicate genes.

Keywords: Carcinogenesis; Fish; Genetics; Genomics; Immunology; Nutrition; Oncorhynchus mykiss; Physiology; Rainbow trout; Toxicology

1. Introduction

The rainbow trout (Oncorhynchus mykiss) is one of the most intensively studied fishes in a wide range of research areas. They are a member of the family Salmonidae, native to the Pacific coast of North America and Russia, and have been widely introduced around the world in regions with cool water temperatures (MacCrimmon, 1971; Behnke, 1992). Considerable basic biological knowledge has developed about this species as an outgrowth of their widespread cultivation as a food and sport fish (Wolf and Rumsey, 1985; Gall, 1992). Without question, more is known about the physiology and biology of rainbow trout than any other fish species. They are the most experimentally tractable of the salmonid fishes and can thus serve as a surrogate for research needed on the economically important Atlantic salmon, and on Pacific salmon and char species. These closely related species within the Oncorhynchus, Salmo and Salvelinus genera have also been studied extensively, such that in the past 20 years these species have been associated with over 40,000 reports on their ecology, behavior, physiology and genetics, with rainbow trout specifically being used in half of these studies. The utility of acquired structural genomic information to understanding the physiology and life history of an organism will depend in part on the applicability of that organism as a model species for experimentation. Rainbow trout have been used as a model research species for over a century. Detailed knowledge of their genetics, physiology, and ecology may be correlated with genomic information.

Rainbow trout excel as a physiological and genetic model organism. Although they are large in size relative to some other model fish species, and have fairly long reproductive cycles (2–3 years), they are easy and inexpensive to culture. Trout are more amenable to surgical manipulation than smaller species, and their size allows large amounts of specific tissues and cell types to be isolated for biochemical, immunological, and molecular biological analysis. Rainbow trout reproduction is well understood, both under natural and culture conditions, allowing collection of large numbers of gametes on a year-round basis. Eggs can be fertilized in vitro and cultured in relatively simple hatchery environments. In contrast to other fish models, much is known about the natural populations of this species that can serve as a resource for addressing evolutionary questions (Behnke, 1992; Hershberger, 1992).

The resources for effectively utilizing rainbow trout for genomic studies are in place or being developed. Five clonal lines of rainbow trout have recently been established using the chromosome set manipulation methods of androgenesis and gynogenesis (Young et al., 1996), techniques which can be readily applied to rainbow trout (reviewed in Thorgaard, 1992). These clonal lines provide valuable experimental uniformity (well-established as an advantage by research with inbred lines of mice) and provide opportunities for analysis and genetic dissection of traits as the differences among the lines are identified (Ristow et al., 1995; Robison et al., 1999) and genetically characterized (Robison et al., 2001). Four BAC libraries, including libraries based on two of the clonal lines, have also been prepared. Detailed genetic linkage maps are being developed for this species (Young et al., 1998; Sakamoto et al., 2000), with one of the maps (Young et al., 1998) is based on a cross of two of the clonal lines.

This review identifies highlights of laboratory research conducted with rainbow trout and the current research and future opportunities for genomic studies with this species.

2. Carcinogenesis and Toxicology

Rainbow trout are excellent animals for carcinogenesis and toxicology research, being extreme-
ly sensitive to a variety of agents and providing ample quantities of tissue for biochemical analyses. Their cytochrome P450 enzyme systems have also been well-characterized.

2.1. Carcinogenesis

An epizootic of liver cancer in Pacific Northwest trout hatcheries in the early 1960s contributed to the discovery of aflatoxin B1 (AFB1) as a potential human hepatocarcinogen, and led to the development of the rainbow trout as a sensitive alternative model for cancer research (reviewed in Bailey et al., 1996). The attributes of this model for cancer research include its non-mammalian comparative status, well-established husbandry and nutritional requirements, availability of multiple exposure routes, a well-defined tumor pathology, externalized gametes and embryos for experimental manipulation, tissue accessibility from animals in the milligram to kilogram size range, low spontaneous tumor background, and high sensitivity. For instance, a single microinjection of as little as 0.5 ng of AFB1 per embryo yields a 40% incidence of hepatocellular/cholangiocellular carcinoma nine months later (Dashwood et al., 1994). This is one billion-fold less aflatoxin than was required to elicit the same incidence in the monkey.

Perhaps the attribute of the trout most extensively exploited in the past decade has been the very low husbandry/per diem cost. This feature allows fundamental dose-response issues in carcinogenesis and chemoprevention to be addressed, using statistically rigorous study designs unaffordable or impossible with traditional rodent models. It is practical, for example, to employ study designs including 10 000 animals to investigate quantitative interrelationships between increasing carcinogen dose, increasing anti-carcinogen dose, level of target organ DNA adduction, and eventual tumor outcome (Dashwood et al., 1989; Breinholt et al., 1995). The latter study was important not only in providing the first demonstration of chlorophyllin (CHL) as an effective blocking agent against AFB1 exposure, but also in demonstrating the conditions under which CHL-mediated reductions in AFB1–DNA adducts were quantitatively predictive biomarkers of eventual reduction in tumor risk. These studies in rainbow trout were pivotal to the planning and design of a recent human clinical intervention trial, in which CHL proved equally effective in reducing biomarkers of effective AFB1 uptake and damage in human volunteers in Daxin, China (Egner et al., 2001). Similar trout studies showed that post-initiation exposure to the dietary supplement indole-3-carbinol provided dose-response promotion of hepatocarcinogenesis, at doses relevant to human exposure, and of a magnitude at least equal to its efficacy as a chemopreventive blocking agent (Dashwood et al., 1991; Oganesian et al., 1999). These findings were eventually proven equally applicable to the rat (Stoner et al., 2002), and continue to influence National Cancer Institute policy regarding human clinical trials with this promising but problematic agent. Perhaps the ultimate application of the low-cost trout model is the recent study utilizing 42 000 trout to establish the relationships between dose of carcinogen (dibenzo[a,l]pyrene, DBP), quantity and spectrum of target organ DNA adducts, cell proliferation, oncogene activation, and tumor response down to an above-background incidence of 0.02% (reviewed in Bailey, 2000; not yet published in detail). The latter study is the largest-ever cancer dose-response study and provides the most extensive low-dose tumorigenesis data yet generated. The central findings of this study are that: (1) DBP-DNA adducts decreased linearly over the 500-fold DBP dose range; (2) PCNA-based liver cell proliferation did not decrease with dose; (3) the percentage of neoplasms bearing oncogenic Ki-ras mutations was high and invariable with dose; and that (4) above-background tumor incidence showed a strong, statistically significant negative departure from the default EPA LED10 linear extrapolation model for risk assessment.

The rainbow trout model has made, and will continue to make, important contributions in the study of cancer and its prevention. At present, there are sufficient gene sequences available to contemplate gene expression profiling using targeted genes in limited macroarray analysis; however, data-rich exploration of unsuspected interactions within and among, and various developmental and regulatory pathways must await sequencing of the rainbow trout genome.

2.2. Environmental toxicology

The rainbow trout has also proven to be a very useful model for biomonitoring of numerous chemicals in the aquatic environment. Many of these chemicals are highly lipophilic and exhibit bioac-
cumulation and biomagnification (Kleinow et al., 1987). Examples of such chemical contaminants include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (e.g. PCBs), dioxins (e.g. TCDD) and dibenzo furans (TCDF) and organochlorine pesticides (e.g. DDT). Other toxic chemicals in fish and other animals. The metabolic products from P450 metabolism are relatively hydrophilic, generally less toxic and can be excreted. However, some carcinogens and other toxic chemicals can be converted by P450s to more polar metabolites (bioactivation).

Multiple cytochrome P450 forms have been identified in the rainbow trout, predominately...
localized in the liver, but also found in lower concentrations in the kidney, intestine, and other organs. Since the rainbow trout is in common use as an experimental animal to investigate physiological processes (Govoroun et al., 2001) or chemical toxicities (Bailey et al., 1996) involving P450 metabolism, the central roles of these enzymes in these important pathways has sparked interest in learning more about their properties. As a result, a number of rainbow trout P450 enzymes have been purified and their biochemical and catalytic properties determined (Miranda et al., 1989). In addition, 16 different rainbow trout cytochrome P450 genes now have been cloned from liver, kidney and gonadal tissues of untreated or chemically exposed rainbow trout (Buhler and Wang-Buhler, 1998).

The availability of a large number of purified rainbow trout P450s together with antibodies raised against these enzymes has prompted a variety of further investigations. For example, the relative contributions of various trout P450 forms to the metabolism of endogenous (Buhler et al., 1997) and exogenous (Yang et al., 2000) chemicals has been studied. Other experiments have determined the effect of various physiological, chemical or environmental factors on the expression of trout P450 forms and their roles in the metabolism and toxicity of environmental contaminants (Williams et al., 1998; Oikari et al., 2002). Using antibodies raised against purified rainbow trout P450s, significant insights also have been obtained on the normal physiological functions of these enzymes based on their developmental, tissue and cellular localizations (Wang-Buhler et al., 1997).

Additional genome sequencing information is certain to have substantial benefits for the use of rainbow trout as a carcinogenesis and toxicology model. Applications of genomics and proteomics approaches could facilitate the extrapolation of cancer dose–response and chemoprevention studies to humans and other mammalian species. More mechanistic work at the molecular level could also allow results from the trout model to be interpreted with respect to human health risk from exposure to environmental chemicals. In addition, full genome sequencing should make it possible to identify the approximately 20 additional cytochrome P450 genes present in the trout genome and shed light on their genetic chromosomal organization and relationship to orthologs in the human genome.

3. Comparative immunology

Immunogenetic studies of rainbow trout have served two very important functions. The comparable design and function of human and salmonid immune systems make the trout an excellent biomedical model while, as an important agricultural commodity, the same information becomes of direct and immediate importance to the aquaculture industry. Immunologically, being poised with such advantageous tools as clonal/syngeneic lines as well as an ample repertoire of immunoreagents and probes, investigators may begin extensive genomic analysis of immune system structure and function. The organization of the immunoglobulin, T cell receptor, major histocompatibility, and non-specific cytotoxic cell (NCC) gene complexes/loci will enable us to precisely envision the basic repertoires by which an individual recognizes and responds to the gamut of pathogens in its environment. This knowledge of immune capabilities will be required to exploit the unique capabilities of this less complex immune system.

3.1. The antibody gene complex

Genes essential for the production of antibodies include those encoding the antigen binding sites. In the trout, these genes are arrayed in complexes not unlike those found in the mouse and human (Warr, 1995; Wilson et al., 1996; Hordvik, 1998; Lansford et al., 1996). The largest of these complexes is the VH (variable, heavy chain). Its members are clustered into related families and, as in the mouse (Brodeur et al., 1988; Meek et al., 1990), are arranged in a tandem fashion. The production of a complete coding sequence for each antibody specificity involves a process of somatic recombination within the B lymphocyte (Warr, 1995; Lansford et al., 1996). In this process one member of each of the VH, DJ (diversity, heavy chain) and JH (joining, heavy chain) complexes are randomly selected and fused with the CH gene to yield a mature coding sequence for the antibody’s heavy chain. A similar process, involving the VL (variable, light chain), JL (joining, light chain) and CL (constant, light chain) complexes yields the light chain component. The VH is the major contributor to the antibody’s binding activity.
including two antigen contact points and a portion of the third providing for much of the antibody’s binding activity (Allen et al., 1988; Kirkham et al., 1992). These contact points are referred to as the complementarity determining regions (CDRs) 1, 2 and 3, respectively. Due to this pivotal role in determining antibody specificity, immunogeneticists working with mammals have focused considerable effort on resolving the composition, structure, and specificities encoded within the V<sub>H</sub> complex (Minoprio et al., 1989; Miller et al., 1996; Clemons et al., 1998).

As with research on the major histocompatibility complex (Jewell and Wilson, 1996), identification of specific V<sub>H</sub> genes has been instrumental in the elucidation of the requirements for prophylactic immune responses (Chung et al., 1995; Sun et al., 1999; Toran et al., 1999), the mechanisms of antibody-mediated immunopathology (Hoch and Schwaber, 1996) and the ontological acquisition of antibody specificities (Alt et al., 1987).

Particularly germane to studies on the potential for vaccine development in trout are the associations with prophylactic immunity found in man. For example, the employment of a single human germline V<sub>H</sub> gene (DP50) appears to be responsible for generating the highest affinity antibodies to a critical antigen of HIV, gp 120 (Toran et al., 1999). These antibodies are thought to be responsible for long-term survival and protection against this virulent pathogen. Another important feature of V<sub>H</sub> genes are the roles different allelic forms can play in determining resistance or susceptibility to disease. Leclerq et al. (1997) demonstrated, through the use of isogenic and congenic mice, that the ability to produce a neutralizing antibody to the poliovirus capsid protein epitope (C3) is linked to loci within the V<sub>H</sub> complex. In much the same manner, use of the clonal/syngeneic trout strains (Young et al., 1996; Robison et al., 1999) can provide similar avenues for the analysis of anti-viral immunity. Tantalizing results have already suggested differential susceptibility of clonal strains to IHNV, a major pathogen of salmonid fish (S. LaPatra, personal communication). Thus, specific alleles expressed at this locus can result in superior, prophylactic antibodies, while others are ineffectual in dealing with the same pathogen. The trout can be exploited in the identical fashion as the mouse, with the added value of being an agriculturally important species. This genetic advantage is likely to be even more important for teleost species such as the trout, which are known to express a more limited repertoire of antibodies (Wetzel and Charlemagne, 1985; Cossarini-Dunier et al., 1986; Desvaux et al., 1987; Hastings and Ellis, 1988). However, as with mammals, trout exhibit the potential to undergo affinity maturation in response to antigen (Khor, 1996; Zhang, 2000; Lewis, 2001; Kaattari et al., 2002). In this process, antigen-specific B cells of the highest affinity for antigen are selected over time and eventually comprise the majority of the responding B cells. This process can occur even if a limited repertoire of antibody genes are available. Kaattari et al. (2002) observed that new, high affinity antibodies arise during a response and eventually become dominant, a scenario consistent with the emergence of somatic hypermutations.

Lewis (2001) also demonstrated that the increase in affinity to a specific antigen, trinitrophenylated keyhole limpet hemocyanin (TNP-KLH), was commensurate with the development of newly-emergent nucleotide substitutions in the CDR 1 and 2 regions of the V<sub>H</sub>. These same sequences were not retrievable from unstimulated cDNA nor germline V<sub>H</sub> sequences, indicating that they likely were somatic mutations. Complete analysis of the process of somatic hypermutation will require complete knowledge of germline antibody genes.

In many cases the relative size and complexity of the gene complex can be a complicating factor in exploring issues of V<sub>H</sub> gene specificity; thus, the limited expressed repertoire of trout may facilitate investigation of V<sub>H</sub> specificity. Such simplicity has been amply exploited in a singular murine V<sub>H</sub> system, the S107 gene family. The primary epitope recognized by antibodies employing this V<sub>H</sub> gene is phosphorylcholine (PC), which is found in the polysaccharide antigens of a wide variety of pathogens including Streptococci (Claffin and Berry, 1988), Proteus (Claffin et al., 1987), and various fungi and parasitic nematodes (Pery et al., 1979; Choy et al., 1991). Interestingly, the murine antibody to this critical epitope initially employs only one gene family (S107), which only contains one V<sub>H</sub> gene (Crews et al., 1981). This restriction of expressed antibodies is comparable with the restriction found in teleost responses to a variety of antigens (Wetzel and Charlemagne, 1985; Cossarini-Dunier et al., 1986; Desvaux et al., 1987; Hastings and Ellis, 1988). Although this S107 repertoire would appear limited, these anti-PC antibodies suffice to protect the host against...
diseases, such as pneumonia (Briles et al., 1982). However, the process of somatic mutation can expand this antibody repertoire, often using the single gene as the substrate for the mutational process. As this is a genetically simple system, it has provided deep insights the power of somatic mutation in modeling the murine immune response (Chen et al., 1995). In a species where somatic mutation may not be routinely exploited the resulting greater dependence upon the original germline sequences would serve to accentuate the impact of selective pressure on the evolution of these genes.

To date there have been no reported studies that attempted to determine the V<sub>H</sub> genes utilized in the production of specific antibodies in fish. All cloning and sequencing efforts, thus far, have employed primers derived from various consensus sequences, without regard to their probable specificity, which are often based on sequences from phylogenetically distant species (Roman and Charlemagne, 1994; Andersson and Matsunaga, 1995; Roman et al., 1996). Specific gene families are employed in the production of specific antibodies to TNP (Family V) and the G protein of Infectious Hematopoietic Necrosis Virus (Family IV) in rainbow trout (Lewis, 2001). Family V was very small (approx. three to five members), while Family IV was much larger ~30 members, suggesting that intense selection by a virulent virus may promote the expansion of antibody gene families. Thus, the complete structure of these gene complexes can facilitate understanding the evolutionary processes that mold the capacity of an individual’s immune response.

3.2. Innate immunity and the nonspecific cytotoxic cell (NCC)

Innate immunity has taken on greater significance within the immunological community because not only are innate mechanisms the primary modes of immunity in invertebrate phyla, but they are also important in the formation of adaptive immune responses within vertebrates. One common innate defense system is that of the natural killer cell (NK) in mammals or the non-specific cytotoxic cell (NCC) in fish. This non-specific cytotoxic cell is thought to be analogous to the precursor of the mammalian NK cell. The first line of evidence that supports this assertion is that NCCs kill many of the same targets as those lysed by human, mouse and rat effectors (Evans et al., 1984a,b; Evans and Jaso-Friedmann, 1993). A second interesting parallel lies in the target cell recognition patterns of both NCC and adherent lymphokine activated killer (ALAK) cells of mammals, both of which are promiscuous killers of various targets such as protozoan parasites, allogeneic and xenogeneic tumor cells, and virus infected cells. (Graves et al., 1985a,b). Interestingly, NCCs of both salt and freshwater fish are not the large granular lymphocytes identified in the mammalian system as NK cells, but are small (<5 μm) agranular lymphocytes. Although porcine and avian species have the expected large granular lymphocytes which effect natural killing, their repertoires also contain small lymphocytes that effect natural killing (Evans and Jaso-Friedmann, 1993). A monoclonal antibody developed to the NCC receptor on the catfish cell is reported to identify an analogous receptor on the cells of birds and mammals (Evans et al., 1988). These cells are now the subjects of intense investigation because of their importance in the innate immune system.

Although it was known over a decade ago that NK cells were able to lyse particular tumor cells without benefit of immunization (Trinchieri, 1989), the elaborate mechanisms of action and control of these cells were not obvious. Now it is apparent that not only are NK cells important in surveillance against tumor cell targets, but they have great importance in the autoimmune state, maternal/fetal tolerance, tolerance to transplanted bone marrow, control of replication of certain viruses, and elaboration of important cytokines and chemokines (Biron et al., 1999; Lanier, 1997a,b, 1999; Lanier et al., 1997; Trinchieri, 1995; Yokoyama, 1995, 1997, 1998).

The NCC of the rainbow trout appear to be small agranular lymphocytes that are capable of lysing various xenogeneic targets including YAC-1, K-562, and IM-9 by both apoptotic and necrotic mechanisms (Greenlee and Ristow, 1991). Since that original study, Ristow and co-workers have incorporated homozygous clones of rainbow trout into these studies, revealing that there are strains of rainbow trout possessing both high and low levels of NCC activity in the peripheral blood (Ristow et al., 1995). Current work has revealed that a single gene may be responsible for this phenomenon in trout (Zimmerman and Ristow, unpublished results). This capability is now being mapped on a genetic linkage groups of the rainbow trout.
We are now well poised with the trout system to dissect one of the most critical elements of innate immunity. Further, much that can be modeled within the murine system can be modeled with this lower vertebrate. Such an alternate model, with a potentially simpler immune system and a slower rate of response could provide fundamental insights into the innate immune response of man and other vertebrate species.

3.3. Major histocompatibility complex

The major histocompatibility complex is clearly one of the most important regions of the vertebrate genome due to the direct role that its encoded genes play in antigen processing, transport and ultimate presentation to T-cells (Trowsdale, 2001). Antibody production by B-cell and T-cell mediated immune responses are initiated through genes found within the MHC (York and Rock, 1996; Pieters, 2000). Therefore, this region is the gateway to immune responses against invading pathogens.

Major histocompatibility class I and II molecules are membrane-associated glycoproteins that play critical roles in managing and eliminating pathogens by presenting small peptides derived from the invader to the cellular arm (T-cells) of the hosts immune system. Two types of MHC class I genes are present in vertebrates, classical (Ia) and non-classical (Ib) (Klein and O’Huigin, 1994).

MHC class Ia genes are highly polymorphic and expressed on most cell types. During infection, their transcription is modulated by specific promoter elements (interferon-mediated responses) and they are responsible for presenting endogenously-derived antigens to cytotoxic killer T-cells (CTL). CTLs recognize the MHC Ia presented antigens via their T-cell antigen receptors and co-receptors (CD4 or CD8). Humans express three different class Ia genes (HLA A, B and C) on virtually every cell, forming the basis of immunosurveillance against viral infection and cancer. MHC class Ib genes display limited polymorphism, restricted expression and are not modulated at the transcription level during infection. The function of MHC class Ib molecules is still a matter of debate, but current research suggests that they are involved in activating natural killer cells (see discussion on NK/NCC cells, this section). Several allelic aspects of the class I antigen presentation machinery should be mentioned for their roles in determining the scope of viral epitopes (processed peptides) that are ultimately presented to T-cells. First, different allelic variants of the proteasome genes are known to affect the type of peptides produced by the immunoproteasome. Therefore, different MHC haplotypes can influence the scope of antigenic peptides that can be displayed to CTLs (Kuckelkorn et al., 1995).

Fish differ from all other vertebrates in having a fourth inducible immunoproteasome subunit, PSMB9L (Hansen et al., 1999). A second important feature of the bony fish MHC class I region is that all four immunoproteasome genes are physically linked, differing from all other vertebrates where PMSB10 is not linked to the MHC (Murray et al., 1999; Clark et al., 2001a; Matsuo et al., 2002; Hansen, unpublished results). Studies involving the rat MHC have shown that the antigen transporters (ABCBs) display functional allelic polymorphisms, resulting in permissive and restrictive antigen transport (Marusina et al., 1997; Deverson et al., 1998). Again, different allelic combinations of the trout proteasome and transporter genes could influence the types of peptides that are ultimately presented by MHC class I molecules. Finally, the PSMB, ABCB and MHC class Ia and II molecules have been shown to be polymorphic in trout (Hansen et al., 1996, 1999; Ristow et al., 1999; Grimholt et al., 2000). The tight physical linkage of genes involved in the MHC class I antigen presentation pathway in fish suggests that selective pressures have acted to keep the MHC Ia haplotypes under linkage disequilibrium. This lowers recombination frequencies among alleles, favors selection of specific haplotypes, and likely results from pathogen pressures.

There have only been a few studies examining the association of the MHC with salmonid health issues, mainly due to the lack of haplotype information. In rainbow trout, a correlation was observed between the MHC class IIB region and resistance to infectious hematopoietic necrosis virus (Palti et al., 2001). The same locus was found to be associated with resistance to Aeromonas salmonicida infections in Atlantic salmon (Langefors et al., 2001). Therefore, strict definition of MHC haplotypes in rainbow trout will allow future researchers to make correlations with disease resistance and to then use the MHC loci as molecular markers in selective breeding programs.

Class I and II sequences (mainly cDNAs) have been cloned from a variety of bony fish species...
including zebrafish (Takeuchi et al., 1995), salmonids (Grimholt et al., 1993; Hansen et al., 1996; Shum et al., 1999), cod (Persson et al., 1999), catfish (Godwin et al., 2000; Antao et al., 2001) and pufferfish (Timon et al., 1998). Fine mapping experiments for bony fish MHC are limited, but this is an active area of research. The most unexpected discovery for MHC architectural arrangements came from studies in bony fish in which the MHC class I and II loci do not co-segregate and in fact reside on different chromosomes altogether (Takami et al., 1997; Hansen et al., 1999). This differs from all other vertebrate classes including cartilaginous fish (sharks, rays and chimeras) (Flajnik and Kasahara, 2001; Ohta et al., 2002). The take home message for bony fish MHC is that the MHC class Ia antigen processing and presentation machinery have been maintained as a solid genetic linkage group during the course of vertebrate evolution. The tight linkage of members of the MHC Ia antigen presentation pathway and the lack of class II linkage in fish present an ideal model for selecting class I and II haplotypic combinations possessing enhanced disease resistance qualities. In support of this idea, zebrafish and fugu fish show conserved synteny (gene content but not strict order) between the human and bony fish MHC class I region for more than thirty different loci, not including duplicate genes (Michalova et al., 2000; Clark et al., 2001a,b). Clearly, our understanding of the MHC of rainbow trout and other fishes will advance dramatically with detailed sequence information in those regions.

4. Disease ecology

Disease ecology includes the study of factors controlling the geographic distribution, host range, seasonality, severity, and other characteristics of a given infectious disease. For diseases of fish, these may include features of the pathogen such as virulence determinants, features of the host such as mechanisms of innate resistance, and features of the environment such as temperature that are especially important for poikilothermic vertebrates in biasing the host–pathogen relationship in favor of either health or disease. Other factors in the ecology of infectious diseases of fish include the mechanisms controlling the persistence and spread of disease such as mode of transmission, the presence of reservoirs of infection (e.g. subclinical carriers), the role of vectors or alternate hosts in the life cycle of the pathogen, and the survival of the pathogen outside the host. While a substantial body of information exists about the biology of viral, bacterial, fungal and protozoan pathogens of fish and the role of environment factors in affecting the severity of fish disease, host factors controlling susceptibility or resistance to disease in fish are relatively poorly understood. This is due largely to a lack of fish model systems that are the equivalent of highly characterized strains of laboratory mice used for mammalian research and the absence of genomic information from which to identify genes involved with disease resistance.

4.1. Model systems for studies of disease resistance in fish

Due to its worldwide distribution and commercial importance, the rainbow trout offers an attractive model for studies on the ecology of fish diseases. In addition, the long history of intensive rainbow trout aquaculture in many areas of the world has resulted in recognition of a variety of viral, bacterial, parasitic, and fungal pathogens affecting this species, making it one of the best aquatic models for disease research. Economic loss to disease has resulted in considerable research focused on interventions in the form of sanitation, chemotherapy, and vaccination. However, each approach toward disease intervention has limitations and, in aquaculture as in other forms of intensive agriculture, interest has turned to the utilization of intrinsic genetic and immunological disease resistance mechanisms for reducing losses to pathogens (Price, 1985; Chevassus and Dorson, 1990; Fjalestad et al., 1993; Jiang, 1993; Wiegerjtes et al., 1996; Gjedrem, 2000).

Knowledge of the rainbow trout genome coupled with reproducible challenge models for selected pathogens of importance to commercial rainbow trout aquaculture would open the way for a new generation of research tools and studies leading to improvements in the health of many cultured aquatic animal species and lowered disease losses by the commercial aquaculture sector. Such tools will also permit comparative genomic studies yielding information on how the presence or expression of various genes can affect the innate resistance of rainbow trout to certain pathogens, modulate the specific immune response following infection or vaccination, and alter the course of disease. Finally, these studies will provide critical
information that can be used by the commercial rainbow trout industry to assist in marker-assisted selection of broodstocks having higher innate resistance to certain pathogens or more robust immune responses to vaccination.

4.2. Genetic basis of disease resistance in rainbow trout

Heritable differences in disease resistance within rainbow trout populations have been reported in a number of studies. The most convincing criterion for determining disease resistance is survival following pathogen challenge. Pathogen challenge models have been established to investigate relationships between pathogen dose and final disease outcome and several highly reproducible challenge models utilizing viral, bacterial, and protozoan pathogens of rainbow trout have been developed.

Rainbow trout are generally considered resistant to furunculosis, which is caused by the bacterium *Aeromonas salmonicida*. However, Cipriano (1983) challenged 11 different hatchery strains of rainbow trout and reported total mortalities among the strains ranging from 0 to 83%. Similarly, differences in resistance among strains of rainbow trout to the viral disease infectious pancreatic necrosis (IPN) were reported by Hill (1982) and Okamoto et al. (1987, 1993). Identification of rainbow trout with resistance to viral hemorrhagic septicemia (VHS) was reported by Slierendrecht et al. (1996, 2001).

Differences among populations of rainbow trout in their resistance to certain parasites have also been demonstrated. Substantial resistance to the myxozoan parasite *Ceratomyxa shasta* has been shown in rainbow trout native to areas where the parasite is endemic (Ibarra et al., 1992, 1994; Bartholomew, 1998; Bartholomew et al., 2001). Recently, differences in *C. shasta* resistance have been identified among clonal lines of rainbow trout (Nichols, Bartholomew and Thorgaard, unpublished results). Resistance to whirling disease, caused by another myxozoan, *Myxobolus cerebralis*, was demonstrated more recently in a strain of rainbow trout that had been reared for many generations in an area of Europe where the disease is endemic (El-Matbouli and Hedrick, unpublished results).

4.3. Mechanisms of disease resistance

Differences in various components of the innate and specific immune systems of fish are often associated with differences in the innate disease resistance of different species or stocks of salmonids. Attempts have been made to exploit these traits as indirect measures in studies of disease resistance; however, correlations are often difficult to demonstrate. Cipriano (1983) reported that the toxin-neutralizing activity of trout serum could partly account for resistance to furunculosis, but that levels of serum agglutinins against *A. salmonicida* did not correlate with mortality data. Hollebecq et al. (1995) reported a correlation between resistance to furunculosis and serum bactericidal activity but were unable to demonstrate a clear relationship between resistance and complement hemolytic activity. In studies of the relationships between immune parameters and viral pathogenesis, Slierendrecht et al. (1996, 2001) were unable to demonstrate a relationship between differences in resistance to VHSV and the complement component C3, or MHC class II genotype. Trobridge et al. (2000) used a restriction fragment length polymorphism (RFLP) assay to show that differences in RFLP patterns of the Mx locus in rainbow trout were correlated with differences in susceptibility to infectious hematopoietic necrosis virus. The availability of the complete sequence of the trout genome will greatly enhance the search for these and other important features of innate disease resistance and provide new approaches to enhance the health of fish in culture systems.

Because the physiological and/or biochemical mechanisms responsible for resistance can have a strong genetic basis and show significant heritability (Kaastrup et al., 1991; Yamamoto et al., 1991), the ability to associate major genes with these traits would permit marker-assisted genetic selection for increased disease resistance in cultured fish (Lande and Thompson, 1990; Kono et al., 2000; Quillet et al., 2001). In an extension of the characterization of resistance to IPNV by Okamoto et al. (1987, 1993), Ozaki et al. (2001) reported the first mapping of quantitative trait loci associated with disease resistance in rainbow trout. In that study, microsatellite markers were used to identify two chromosomal regions associated with IPNV resistance/susceptibility. Their results suggest that, by using marker-assisted selection, it should be possible to improve resistance to the virus in cultured rainbow trout populations. This study also demonstrates the suitability of this model for the genetic characterization of complex traits like disease resistance. Such tools and infor-
4.4. Other host factors in disease ecology

In addition to mechanisms of innate disease resistance, research on other factors of the ecology of various infectious diseases would benefit from an understanding of the rainbow trout genome. These include studies on factors controlling the tissue distribution within a host, the mode of transmission between hosts, and the establishment of subclinical carriers that serve as long-term reservoirs for infectious agents. Such studies will allow fish health specialists to better understand the host component of the host–pathogen relationship. Finally, information on how environmental factors (e.g., temperature or contaminants) modulate the expression of specific host genes (e.g., stress response elements) will provide a more complete understanding of the role of ecological factors in altering the host–pathogen relationship.

5. Physiology

The rainbow trout is the best-studied fish across a wide range of physiological systems and is advantageous for many of these studies because of its large size and ease of culture. Due to the importance of rainbow trout in aquaculture and as a model for related salmonid fisheries there has been considerable research on many aspects of their physiology to enhance these endeavors.

5.1. Sensory systems

The primary focus of research on the sensory system of rainbow trout has been on their visual and olfactory systems. There are several specific features of salmonids that make them attractive models for study of the visual system. Firstly, salmonids mature rapidly, and ongoing retinal developmental events have been shown to occur more rapidly as well (Julian et al., 1998). Secondly, the salmonid visual system changes structurally and functionally during migration from fresh to salt water. The study of the changes, and control mechanisms for these changes, will reveal important adaptive sensory strategies (Browman and Hawryshyn, 1994). The olfactory system has also been explored using rainbow trout as a study animal (Laberge and Hara, 2001). Recently, efforts have been made to characterize the olfactory receptors in other salmonids (Wickens et al., 2001). Sequence information from the rainbow trout genome would greatly facilitate all of these lines of study. Genomic data would allow the study of gene expression of the visual and olfactory systems in the rainbow trout model during development and growth, as well as provide the basis for functional genomic comparisons with other fishes. Most importantly, comparative functional genomic studies can be done within a single organism, to further elucidate the adaptive changes needed for sensory system function in different environments.

5.2. Reproduction

At this time there is more information on the reproductive biology of salmonids than any other group of fishes. Much of this information has been drawn from studies on rainbow trout or can be extrapolated from closely related genera (i.e., Salmo, Salvelinus). As a gonochoristic, iteroparous fish model for the study of reproductive biology the rainbow trout ranks at the top of the list. One of the major contributing factors to this level of understanding is that basic husbandry and breeding of rainbow trout has been historically widespread and intensive in freshwaters (Bromage et al., 1992). The accessibility to the fish during all life history periods has provided opportunities to investigate this fish from the gamete to the sexually mature adult. A few outstanding areas of reproductive investigation include gamete physiology, sex determination, vitellogenesis, and reproductive endocrinology.

The widespread breeding of rainbow trout in captivity has lead to a broad and detailed understanding of gamete physiology (Cosson et al., 1999) and early development that includes successful cryopreservation of spermatozoa. The ability to manipulate large quantities of gametes in controlled in vitro fertilization procedures permits breeding experiments not possible with small fish models. Development is slow enough that embryonic changes can be studied thoroughly. The application of genomics to study early developmental processes in the rainbow trout is a logical step that could be easily undertaken.

One of the most profound contributions that salmonids could make to basic questions in biology is that of sex determination. The presence of various sex determination systems among fishes...
results in one of the most varied taxonomic groups for the study of sex determination and sex chromosome evolution (Bull, 1983). Cytological analyses (Thorgaard, 1977) and ploidy manipulations (Thorgaard and Gall, 1979) suggest that rainbow trout have a genetically determined X/Y sex determination system that, like mammals, involves a dominant gene(s) on the Y that initiates male development. This is in contrast to the popular zebrafish model in which the sex determination process is still unknown. Sex determination genes are being currently sought in the rainbow trout (see later section on Evolutionary and Quantitative Genetics) and the completed genomic sequence for this fish would be useful to identify the gene(s) responsible. Segregation analyses (Young et al., 1998) and gene-centromere mapping (Allendorf et al., 1994) reveal that the sex-determining locus is located distally on the short arm of a chromosome pair that may or may not be heteromorphic, depending on the population (Thorgaard, 1983).

In the medaka a recently reported Y-chromosome specific DM-domain gene has been identified that is required for male development in this species (Matsuda et al., 2002; Nanda et al., 2002). An interesting comparative study will be to look for a homologue of this DM-domain gene in the rainbow trout, which is known to have multiple DM-domain genes (Marchand et al., 2000).

The important process of vitellogenesis in oviparous animals has been well characterized in the rainbow trout (Tyler, 1991). Vitellogenesis is the synthesis by the liver of high molecular weight glycolipophosphoproteins (i.e. VTG) upon estrogen signaling from the ovary. Vitellogenin is released into the blood and taken up by the developing ovary via an endocytic mechanism. Characterization of VTG receptors on the oocyte surface (Tyler and Lancaster, 1993), and a yolk-processing enzyme (i.e. cathepsin D) in rainbow trout have been described (Brooks et al., 1997). Numerous quantitative methods to measure rainbow trout VTG are available (i.e. radioimmunoassay, ELISA), and an extensive literature exists for concentrations and kinetics under different experimental conditions (Schultz et al., 2001).

The endocrine hypothalmo–pituitary–gonadal axis is well established in rainbow trout and continues to be built upon. Our understanding of gonadotropin releasing hormones in the hypothalamus and the control of pituitary gonadotropin synthesis and release rivals most other fish species. Interest in this area continues to produce new and improved methods for measuring some of these hormones (Prat et al., 1996; Santos et al., 2001). The production of sex steroids by the gonads and their levels in the peripheral circulation during the reproductive cycle have been thoroughly studied in rainbow trout (Kime, 1993), which was the first fish for which sex steroid receptors were characterized at the molecular level (Le Drean et al., 1994). It is now possible to track the expression of estrogen receptor mRNAs in this species with the advent of quantitative RT-PCR technology and a method to measure estrogen receptor-α (Nagler et al., 2000). Since the DNA sequences for many of the important endocrine factors involved in rainbow trout reproduction are known, this area would benefit greatly from genomic information to determine tissue and cell localization, regulation, and provide comparisons with mammalian systems.

5.3. Circulation/respiration

While many facets of the circulation and respiratory systems of fish have been elucidated in studies of rainbow trout, perhaps the most comprehensive and exciting during the past decade has been the focus on the adrenergic coordination of oxygen delivery to tissues during periods of hypoxia. The catecholamine hormones (epinephrine and norepinephrine) coordinate a multitude of physiological processes impacted by hypoxia and their release leads to stabilization of red blood cell intracellular pH and thus stabilization of hemoglobin function (reviewed in Perry and Tufts, 1998). Progress has been made in cloning several of the genes associated with this response (e.g. Na⁺/H⁺ exchanger). An exciting, unfolding avenue of research is the genomic effects of hypoxia; fish red blood cells are nucleated (unlike mammals), allowing changes in gene expression patterns to be tracked.

5.4. Muscles/locomotion

In the past two decades, a great deal of research has been performed on the exercise physiology of fish, with the majority of this research on rainbow trout and other salmonids. While ready availability from hatcheries and laboratory suitability are part of the explanation, the long migrations undertaken by salmonids (and the growing impact of man’s
alteration of their riverine migration routes) also factor into this interest. Finally, the sport of angling generates intense interest in burst locomotion. Rainbow trout typify many fish species in having two anatomically separate muscle fiber types, red (‘aerobic’) vs. white (‘anaerobic’) allowing easy study of these two processes. This situation again provides an important experimental and functional counterpoint to mammals, where study is more difficult because fiber types are mixed in a given muscle group. Important reviews of the physiology and biochemistry of exercise in fish are Moyes and West (1995) and Kieffer (2000). Both show that the fish locomotory system is highly adaptable (for example during exercise training) and will be an excellent candidate for genomic studies.

5.5. Excretion/osmoregulation

Rainbow trout have been an important model system for the study of routes and mechanisms of excretion and osmoregulation, largely due to the longstanding interest in aquaculture of this species. In terms of nitrogenous waste, they appear to be representative of the vast majority of juvenile and adult teleost fish which excrete ammonia as their predominant waste product (as unionized ammonia diffusion across the gills), with minor contributions from urea and uric acid. The impact of a number of environmental factors on nitrogen excretion have recently been reviewed by Wood (2001). However, an important addition to this paradigm was also first discovered in rainbow trout. That is, embryonic rainbow trout excrete much of their nitrogenous waste as urea, a condition that appears to be related to the rich amount of nitrogen in the yolk, as well as to the lower permeability of egg membranes to ammonia. Thus, the full array of ornithine-urea cycle enzymes and urea transport proteins are expressed in rainbow trout embryos, cod embryos and a growing list of other teleost species (reviewed by Wright and Fyh, 2001). Rainbow trout represent an important choice to examine genomic patterns of expression of these genes, which ultimately underpin the terrestrial lifestyle of higher vertebrates.

Rainbow trout are perhaps the most widely studied fish species in research on osmoregulation and the related field of acid–base regulation. Probably due to the evolutionary history of anadromous behavior of many members of the family Salmonidae, rainbow trout easily adapt to the softest of freshwaters and to full strength seawater. Given that a popular hypothesis suggests an anadromous evolutionary origin for teleost fish (Griffith, 1994), trout represent an ideal choice for examining genomic aspects of salinity adaptation. The basic paradigm is that, faced with a continual passive loss of ions to, and a passive gain of water from, a freshwater environment that is more dilute than body fluids, trout compensate by actively taking up ions largely via the gill and by excreting copious quantities of dilute urine. Wood and Shuttleworth (1995) reviewed this process. Two noteworthy developments using the rainbow trout are the cloning of the V type H⁺-ATPase from gill (which energizes sodium uptake) and its localization within the gill anatomy (Perry et al., 2000), as well as the establishment of pure and mixed-cell cultures on membrane inserts, allowing the study of ion regulation in a polarized epithelium in vitro (Wood and Pärt, 1997).

5.6. Stress-response

Stress is a major factor responsible for decreased fish performance, including decreased growth rate, compromised food conversion efficiency, increased disease incidence and mortality (Barton et al., 2002). Rainbow trout, as an important aquaculture species, have been widely used as a model organism to characterize the physiological responses to a wide variety of natural (environmental) and anthropogenic (pollution and aquaculture operations) stressors (Iwama et al., 1997). The non-specific response to stress in this species is broadly categorized into a primary response which includes the release of catecholamines from chromaffin cells (Fabbri et al., 1998), and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis, resulting in the release of the major glucocorticoid, cortisol (Mommsen et al., 1999). The secondary responses, which may also be due to primary hormonal regulation of adrenoceptors and glucocorticoid receptor gene and protein expression in trout, include changes in plasma and tissue ion and metabolite levels and hematological parameters, all of which are important in the physiological adjustments that allow animals to cope with stress (see Iwama et al., 1997; Fabbri et al., 1998; Mommsen et al., 1999).

At the cellular level, stress-dependent gene(s) induction followed by translational activation is crucial for stress tolerance and commonly used as
indicators of cellular stress response. The most widely studied stress-inducible genes belong to the family of heat shock proteins (HSPs), and elevated HSP induction provides tolerance to stressors (Iwama et al., 1998; Feder and Hofmann, 1999). Studies in fish suggest the feasibility of using titers of 70 kDa HSPs (HSP70) as indicators of stress, especially because HSP70 mRNA accumulation occurs within an hour after stressor exposure. Furthermore, chronic stress-induced HSP70 remain elevated for long periods of time (Iwama et al., 1998). As HSP70 induction occurs with most types of stressors studied, especially those affecting the protein machinery, its value as a stressor-specific indicator in fish is limited. The cytochrome P450 1A (CYP1A) and metallothionein mRNA and protein levels have been used as indicators of exposure to specific stressors (polychlorinated biphenyls and heavy metals respectively), but the results are not unequivocal (Adams, 2002). However, we know that no single gene or protein is involved in the stress response process, but instead a whole gamut of genes and proteins (involved in cell physiology, metabolism, growth, immune responses and reproduction) orchestrate the cellular responses to regain homeostasis. While the identification and/or regulation of stress-responsive genes in rainbow trout is still very much in its infancy, the fact that trout can tolerate and acclimate to a wide range of environmental stressors and the extensive characterization of the physiological responses, makes this species an ideal candidate for elucidating the functional genomics of the stress response process. Although the field of gene profiling is still relatively new in fish (Gracey et al., 2001), DNA microarrays hold promise to successfully identify novel stress-responsive genes as is evident from model organisms, including yeast, Arabidopsis, mice and humans. Sequencing the rainbow trout genome would provide fundamental insights into the regulation of the stress-responsive genes as well as allow the discovery of novel genes that are regulated either in a stressor-specific or non-specific fashion.

6. Transgenic technologies for functional analysis of the trout genome

To understand the relevance of genomic information for an organism, functional analysis of specific genes, gene families, and gene regulatory hierarchies will be required to couple structural and functional information (Schwerin, 2001). In this regard, the transfer of new genetic information into species has been and will continue to be a powerful tool for studying the physiological, phenotypic, and fitness consequences of specific genes (Pereira, 2000; Tyagi and Mohanty, 2000). Gene transfer technologies for mice, Drosophila, nematodes, and other models were well-established in the early 1980s, and similar research for fish and shellfish followed closely with the first transgenic fish being reported in 1985 (Zhu et al., 1985). Currently, over 3000 reports of transgenic aquatic organisms exist in the literature, testimony to the large academic and commercial interest in this field. A great deal of research has been undertaken with model fish species, including zebrafish and medaka, as well as with salmonids, carps, catfish, tilapia, loach, and shellfish (Bachere et al., 1997; Sin, 1997; Ivetac et al., 2000; Maclean and Laight, 2000; Hew and Fletcher, 2001).

6.1. Rainbow trout as useful tool for transgenesis

Germ-line transgenic rainbow trout have been produced by microinjection of gene constructs into the fertilized egg shortly after fertilization (Table 1). Trout eggs can be easily incubated, and the process of fertilization can be separated from initiation of development by using appropriate ionic strength solution which prevent egg activation which simplifies the collection, storage and microinjection of large numbers of eggs. Approximately $10^6$ to $10^7$ copies of linear DNA are microinjected in a volume of 1–2 nl into the germinal disc of the egg using ground glass needles of approximately 5 μm in diameter.

For microinjection, DNA is injected cytoplasmically, and is incorporated into nuclei following mitotic division and nuclear membrane dissolution and reforming. The reported frequency of DNA integration into the trout genome varies widely (see Devlin, 1997), in part due to different criteria used to define a transgenic organism. Although mosaic integration in founder animals is common, germ line integration events occur at reasonable frequencies, which allow individual families to be readily formed for subsequent analysis of defined transgene inserts. DNA is integrated into the genome as single-copy or concatemerized arrays of the gene construct (Tewari et al., 1992), and the site of integration appears to be random.
Table 1
Transgenic rainbow trout

<table>
<thead>
<tr>
<th>Research area</th>
<th>Gene promoter</th>
<th>Coding region</th>
<th>Phenotypic effect</th>
<th>Reference</th>
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</thead>
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<tr>
<td>Endocrine control of growth</td>
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<td>Human or bovine GH</td>
<td>No effect</td>
<td>(Chourrout et al., 1986a; Tewari et al., 1992)</td>
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<tr>
<td></td>
<td>mouse metallothionein, mammalian viral,</td>
<td>Human, bovine or rat GH</td>
<td>No effect</td>
<td>(Guyomard et al., 1989; Rokkones et al., 1989; Penman et al., 1990; Chourrout and Perrot, 1992)</td>
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<tr>
<td></td>
<td>MHC</td>
<td></td>
<td></td>
<td>(Maclean et al., 1987; Penman et al., 1991; Maclean et al., 1992)</td>
</tr>
<tr>
<td></td>
<td>Mouse metallothionein</td>
<td>Rat GH</td>
<td>No effect</td>
<td>(Inoue et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>RSV/chicken β-actin</td>
<td>Trout GH</td>
<td>Growth enhancement</td>
<td>(Devlin et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Ocean pout antifreeze</td>
<td>Chinook salmon GH</td>
<td>Growth enhancement</td>
<td></td>
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<td></td>
<td>Atlantic salmon GH</td>
<td>Atlantic salmon GH</td>
<td>no effect</td>
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</tr>
<tr>
<td></td>
<td>Sockeye salmon metallothionein-B</td>
<td>Sockeye salmon GH</td>
<td>Growth enhancement in some strains</td>
<td>(Devlin et al., 2001a)</td>
</tr>
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<td>Altered physiology and</td>
<td>Carp α-globin</td>
<td>Carp α-globinGlutonolactone oxidase</td>
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<td>No expression</td>
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<td>Expression, altered metabolism</td>
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<tr>
<td>Immune function</td>
<td>mouse Ig</td>
<td>Reporter</td>
<td>Lymphocyte-specific expression</td>
<td>(Michard-Vanhee et al., 1994)</td>
</tr>
<tr>
<td>Controlled reproduction</td>
<td>GnRH (Pab)</td>
<td>Antisense GnRH</td>
<td>Impaired maturation</td>
<td>(Uzbekova et al., 2000)</td>
</tr>
<tr>
<td>Developmental biology</td>
<td>Vasa</td>
<td>GFP reporter</td>
<td>Germ-line specific expression</td>
<td>(Yoshizaki et al., 2000)</td>
</tr>
<tr>
<td>Promoter analysis</td>
<td>prolactin</td>
<td>Reporter</td>
<td>Reporter expression analyzed</td>
<td>(Amoros et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>Drosophila hsp 70</td>
<td>Reporter</td>
<td>Reporter expression</td>
<td>(Gibbs et al., 1988)</td>
</tr>
<tr>
<td></td>
<td>carp β-actin</td>
<td>reporter</td>
<td>Reporter expression</td>
<td>(Iyengar and Maclean, 1995)</td>
</tr>
<tr>
<td></td>
<td>CMV, Xenopus EF1</td>
<td>GFP reporter</td>
<td>Reporter expression</td>
<td>(Takeuchi et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>RSV, CMV</td>
<td>Reporter</td>
<td>Reporter expression</td>
<td>(Inoue, 1991; Tewari et al., 1992; Yoshizaki et al., 1992; Michard-Vanhee et al., 1994)</td>
</tr>
</tbody>
</table>

Consequently, expression levels and patterns can vary widely among different founder lines for a particular construct. In many cases, this variability provides very useful differences for examining the effect of gene expression level on phenotype, but in other cases (i.e. when promoter function analysis is being studied), more stable expression patterns are desired. To achieve this, border elements have been isolated from fish for the purpose of buffering local chromosome domain effects and stabilizing expression in transgenic fish (Caldovic and Hackett, 1995).

Alternatives to microinjection utilizing sperm-mediated incubations, biolistic (gene gun), or electroporation methods have been explored in fish. However, these have produced equivocal or nega-
tive results (Chourrout and Perrot, 1992; Sin, 1997), and no confirmed cases of germ-line transformation of rainbow trout to date.

The ability to transfer large chromosomal blocks of genes to produce transgenic trout has been demonstrated (Disney et al., 1988), allowing the possibility of examining expression of genes from large syntenic chromosomal regions. This technology may be particularly important for large-scale genomic studies examining the influence of chromosomal architecture on gene function, and can be coupled with the analysis of transgenic animals containing artificial chromosomes (PACs, YACs and BACs) for more detailed structural and functional evaluation of specific chromosome regions (McCormick and Nielsen, 1998; Giraldo and Montoliu, 2001).

Other approaches including targeted integration (Shashikant et al., 1998; Copeland et al., 2001) and gene knockouts (Harris, 2001) are possible in other germline transgenic vertebrates, and are being experimentally explored, but not yet achieved, in fish. Recent progress in the development of embryonic stem (ES) cells and blastomere transplantation in rainbow trout and other fish species (Nilsson and Cloud, 1992; Ma et al., 2001; Takeuchi et al., 2001) suggests that targeted gene replacement in the genome of trout will be available soon and provide a powerful technology for functional genomic analysis.

The production of any germ-line transgenic vertebrate can require significant resources, and alternate strategies of investigation should be simultaneously explored. One alternative is to explore gene expression in transgenic animals that have had DNA introduced only into somatic cells. Such an approach allows more rapid evaluation of gene construct functionality and, in some cases, could be used to evaluate phenotypic effects. In rainbow trout (and several other fish), somatic expression of transfected genes has been demonstrated for several tissues (primarily muscle) using different modes of DNA administration (Traxler et al., 1999; Corbeil et al., 2000; Lee et al., 2000; Lorenzen et al., 2001; Sudha et al., 2001).

6.2. Trout transgenesis as a tool for analysis of vertebrate genomes

Genomic technologies will provide a wealth of data on relationships among species and among genes within a genome. The expression patterns of those genes under different physiological or environmental conditions will help to reveal mechanisms controlling the behavioral, physiological and morphological phenotype of the organism. Such phenotypes are what the organism uses to interface with the environment (physical and biological) and variance in these phenotypes determines fitness and potential for evolutionary change. Transgenic approaches will allow detailed examination of gene function in many areas, including endocrinology, physiology, neurobiology, metabolism, nutrition, immunology, and developmental biology (Abe et al., 1998; Weller et al., 1998; Ko, 2001; Trethewey, 2001; Fiehn, 2002). To date, transgenic trout have already been produced in several of these research areas (Table 1), and a great deal of information has been gathered regarding methodology and the function of a few well-studied gene systems. Historically, however, such activities have been hampered by the availability of genes to study, relying on those isolated by traditional molecular biology approaches. The emergence of genomic technology will remove this limitation, and will make a plethora of gene sequences available for study.

Genetic maps developed for rainbow trout using DNA markers (Young et al., 1998; Sakamoto et al., 2000; Danzmann and Gharbi, 2001) now allow identification and isolation of chromosomal regions associated with quantitative trait loci (QTLs) (Sakamoto et al., 1999; Perry et al., 2001). While the chromosomal regions associated with such QTLs are still quite large (assuming 1 Mb of DNA per meiotic map unit (cM); Lander et al., 2001), physical maps constructed by BAC contig analysis and anchored with simple sequence repeat markers (Marra et al., 1999; Cai et al., 2001) will allow more refined isolation of specific chromosomal regions. These regions can be subjected to medium-scale sequence analysis from which coding sequences can be identified and candidate genes selected. Transgenesis will provide a useful adjunct for determining or verifying the suspected functions of genes identified by QTL (Schwerin, 2001).

Similar to physical mapping approaches, microarray technology will allow identification of candidate genes involved in controlling phenotype by monitoring tissue mRNA levels for altered gene expression under different intrinsic and extrinsic conditions. Large-scale cDNA isolation and characterization from fish species is being undertaken...
Transgenic approaches have the ability to test gene function and alter phenotypes in several ways, analogous to other forms of mutagenesis. In mice and other model organisms, expression can be altered to enhance expression (hypermorphs), reduce expression (hypomorphs), block function (antimorphs), and provide new function (neomorphs). Although some of these approaches have already been demonstrated for rainbow trout, most effort has been directed to overexpression of protein-coding regions. Overexpression of gene products is useful for examining the function of gene products that are rate-limiting in their normal function, traditionally achieved by producing gene constructs containing alternate regulatory sequences controlling the expression of coding regions. The simplest approach to achieve overexpression from a gene which is widely developmentally or spatially expressed is to fuse a promoter region just upstream of the start codon of the gene of interest. The resulting gene construct is capable of driving the expression of the coding region in a novel fashion. The most common of these constructs used in transgenic fish to date (Table 1) are designed to overexpress growth hormone (GH). Such gene constructs allow the extraputatory expression of GH gene coding regions in many tissues, are not controlled by the normal seasonal influences that modulate normal GH gene expression, and result in dramatic alteration in phenotype in some cases.

Reduction in gene products can theoretically be achieved in several ways, but to date only antisense technology has been experimentally examined. Antisense approaches (Dean, 2001; Halloran et al., 2001) will provide hypomorphic mutation in most cases by reducing but not eliminating transcripts encoding. For rainbow trout, expression of antisense mRNA for gonadotropin release hormone has been successfully used to influence the reproductive development of male trout (Uzbekova et al., 2000).

Coupling transgenesis with cloning (Di, 2001) has the capacity to produce large numbers of genetically-identical transgenic organisms from which stable gene expression patterns can be anticipated and environmental effects can be studied. Similarly, testing different gene constructs in the same host genetic background would facilitate analysis of phenotypic effects due to reduced background genetic variance among individuals. Thus, this approach will have important implications for future genomic analyses of phenotype in many organisms (Di, 2001), and rainbow trout are represented among the few vertebrate species to date where fertile isogenic lines have been successfully produced (Young et al., 1996).

6.3. Testing gene interactions and evolutionary hypotheses

Traditionally, genes have been studied and shown to primarily influence single phenotypic traits. However, it is now understood that genes function as part of a large physiological complex and that each has many functions and interactions (epistatic, synergistic, additive, and neomorphic) with other genes. This complexity is important for understanding how the genome can orchestrate complex developmental events, and provides an exciting framework from which evolution can direct the fitness of the organism. However, when phenotypic variance derived from nature is being examined, in many cases, the interactions among genes may be very subtle and beyond the capability of the research design to experimentally decipher. Transgenic approaches may alter the expression of genes significantly beyond that normally observed in nature, and gene interactions may become exacerbated and more easily identified. Indeed, a remarkable feature of transgenic research in fishes and other organisms is the degree to which pleiotropic effects are identified. For example, in fish, over-expression of growth hormone can alter growth rate significantly, but many
other effects have also been observed including effects on muscle structure, swimming performance, feeding behavior, metabolism, organ size and development, and skeletal structure (Farrell et al., 1997; Ostenfeld et al., 1998; Devlin et al., 1999; Hill et al., 2000). While many of the effects can be rationalized after they are observed, these novel phenotypes provide direct experimental evidence that the gene being studied is able to directly or indirectly influence a variety of pathways and may be subject to selection and influence the phenotypic variance of the trait in an evolutionary context in nature.

Much of the information derived from genomics will involve comparisons of gene and protein structures among species. Hypotheses will arise from these comparisons regarding differences in function that may account for known differences in physiology and life history. Transgenesis, by transferring genes between species, provides a mechanism to directly test some of these hypotheses. One such example with rainbow trout is the transfer of the α-globin gene from carp to rainbow trout (Yoshizaki et al., 1991) to determine whether differences in anoxia sensitivity between these species is derived from differences in hemoglobin structure. Analogous tests of gene function can be envisaged for many other phenotypic differences among species.

6.4. Caution in the application of transgenic technology

In addition to the tremendous potential that transgenesis provides for studying gene function, there may be advantages to the use of genetically-modified organisms in commercial settings. The trout aquaculture industry is very large and very competitive worldwide, requiring high-efficiency production and very high product quality standards. Transgenic technologies have now and will continue in the future to yield strains that possess enhanced production characteristics (e.g. enhanced growth and feed conversion efficiency, enhanced flesh nutritional quality) that could provide a market advantage (Hew and Fletcher, 2001). Significant scientific and public controversy currently exists regarding the use of genetically engineered animals for food production, and thus this approach is not currently a focus of commercial producers. However, this situation is likely to change in the future as society becomes accustomed to the benefits of the technology and as science provides more answers regarding food safety and environmental risks.

A concern regarding the use of transgenic strains on large commercial scales is the potential for accidental release of these fish into natural ecosystems. At present, we have a very poor understanding of the relationship of how genes influence physiology and phenotype, and even less capability to predict how such phenotypic effects may influence the long-term fitness of the organism in culture and natural environments (Devlin, 1998). While resolving this shortcoming is a large part of the impetus for utilizing transgenesis in genomic analysis, it also makes appropriate caution regarding the use of this technology prudent. Escapes of transgenic fish to natural aquatic ecosystems cannot practicably be recovered, and hence the impact they may have is a function of their fitness relative to natural conspecifics and other organisms in the environment (Kapuscinski and Hallerman, 1991; Devlin and Donaldson, 1992; Maclean and Laitg, 2000; Muir and Howard, 2001). Thus, to prevent escape or release, it is necessary to culture transgenic strains under physical containment systems and to implement backup measures of reproductive containment in the form of sterility (Devlin and Donaldson, 1992; Thorgaard, 1992). Currently, the most viable method for producing sterility on a large scale in fish is by induced triploidy, since the polyploid condition results in the production of aneuploid sperm in males and essentially obliterates gonadal development in females (Benfey, 1999). All-female triploid populations are synthesized by a combination of chromosome manipulation and sex reversal techniques (Donaldson, 1986a). Chromosome manipulation techniques for directly inducing triploidy in trout by pressure or temperature-shocking eggs shortly after fertilization have been described (Thorgaard et al., 1981; Chourrout and Quillet, 1982), as has the synthesis of tetraploid strains (Reftie, 1981; Chourrout, 1984) that produce diploid gametes which yield only triploid offspring when combined with natural haploid gametes (Chourrout et al., 1986b; Blanc et al., 1993). Similarly, production of sex-reversed XX males to allow production of all-female populations is also well-established for rainbow trout (Donaldson, 1986a,b; Thorgaard, 1992). Thus, a great deal of previous research focusing on reproductive containment methods for rainbow trout provides the prerequisite for allowing genetically-
engineered strains of this species to be controlled to minimize reproductive interaction between escaped transgenic fish and natural conspecifics, and thus minimize potential environmental interactions. However, the efficacy of such techniques in large-scale applications will need to be critically evaluated on a case-by-case basis.

7. Nutrition

Rainbow trout are one of the best-understood fish species in terms of nutritional requirements. Over 140 species of fish are reared as food fish and for stocking purposes, plus another 200 or more ornamental fish species are reared in captivity for hobbyists. Of these, less than 20 have been the subject of serious study to determine their nutritional requirements, and even for these 20, the amount of information is thin compared with that known about the dietary needs of terrestrial domesticated animals and poultry. Given that various species of fish run the gamut from cows to cats in terms of digestive physiology, natural diet, and nutritional needs, fish nutrition is decades away from reaching the sophistication and knowledge base that exists for other farmed animals. However, the basic rules of intermediary metabolism apply to fish and, in those species studied in depth such as rainbow trout, approximately 40 nutrients, including 10 amino acids, 14 vitamins, essential fatty acids, macro minerals and trace elements and, in salmonids, the carotenoid pigment, astaxanthin, are required (NRC, 1993; Halver and Hardy, 2002; Hardy, 2002; Webster and Lin, 2000). Except for several essential minerals that fish can obtain directly from their rearing water, essential nutrients must be supplied by the diet.

Research on the nutritional requirements of fish lagged behind that of rats, poultry, and other animals for decades until the development of a suitable semi-purified diet to which individual nutrients could be deleted and added back accurately. The first such diet was developed for salmon and trout approximately 50 years ago, and progress since then has been rapid, especially for salmonid species (Halver, 2002). Quantitative nutritional requirements have been estimated for several commercially important fish species, including rainbow trout, channel catfish, Atlantic salmon, European sea bass, red sea bream, gilt-head sea bream, hybrid striped bass, tilapia and red drum. However, many species of fish are reared in great numbers despite the lack of information on their nutritional requirements. For fish used in medical research, e.g. zebrafish, medaka, virtually nothing is known despite the obvious importance that they be maintained in optimum health and reproductive status during research studies. Rainbow trout have become the standard research fish used as a surrogate for other carnivorous fish species because of the relatively complete information on their dietary requirements and also because they are relatively easy to rear (Hardy, 2002). Channel catfish serve a similar role for omnivorous fish species.

Fish nutritional research is complicated by the aquatic existence of fish. Ammonia resulting from amino acid catabolism in adult bony fish, including trout, is excreted as ammonia rather than urea or uric acid, as is the case with mammals and birds, respectively. They also excrete most nitrogenous wastes directly via the gills. Elasmobranch fishes concentrate urea in their tissues to osmoregulate and thus differ from bony fishes. These mechanisms increase the amount of metabolic energy that fish derive from protein, compared with mammals and birds. Furthermore, fish have evolved in a food chain system that, for the most part, does not include the leaves and seeds of plants, the main energy source of terrestrial animals. Most fish exploit plankton, both phytoplankton and zooplankton, either directly in the case of forage fish, or indirectly in the case of omnivorous and carnivorous fish species. Nearly all of the fish species raised by man, even omnivorous species, are carnivorous at some stage in their life cycle. Thus, most species studied to date are more prone to use dietary protein as a source of metabolic energy than are birds and mammals (Halver and Hardy, 2002). This is certainly the case with rainbow trout. For this reason, establishing a proper balance of digestible protein to dietary energy is critical for efficient conversion of dietary protein to tissue protein, and appreciating the limitations of utilizing carbohydrates as sources of dietary energy is essential when formulating salmonid diets.

Feeding levels are also a complicated issue with fish. Many temperate water species undergo seasonal fluctuations in food abundance in nature and are thus prone to accumulating large lipid reserves, especially when fed to excess or fed high-energy diets. In captivity, feed is available year-round, and feeds and feeding levels must be adjusted to account for this. In the case of rainbow trout,
declining photoperiod induces a period of low growth, know as winter dormancy, when protein synthesis is reduced regardless of feeding level or diet formulation (Overturf and Hardy, 2001). Many other fish species from northern latitudes presumably react similarly. Water temperature also has a profound effect on growth and appetite, but not on essential nutrient requirements. Feeding levels must be adjusted accordingly to achieve optimum feed conversion ratios.

With respect to specific nutritional requirements, fish require essential amino acids, and analysis of the whole body amino acid composition of rainbow trout provides a surprisingly accurate estimate of their dietary requirements (Wilson, 2002). This observation has been extended to other fish species. For rainbow trout, the quantitative requirements for essential amino acids in the diet are well documented (Hardy, 2002). Non-essential amino acids are also generally provided in the diet for optimum growth. Recent work with rainbow trout demonstrates that highest protein (nitrogen) retention occurs at a ratio of essential to non-essential amino acids of between 3:2 and 1:1 (Green, 2002).

Reasonably complete estimates of the quantitative vitamin and mineral requirements have been made for rainbow trout (Hardy, 2002). Vitamin requirements of trout and other fish are similar to those of poultry, with the exception of a requirement for ascorbic acid, and, in carnivorous species lacking active gut microflora, inositol. Requirements for calcium and phosphorus are lower than those of birds and mammals, presumably because fish are neutrally buoyant and therefore do not require a massive skeleton to oppose gravity. Phosphorus metabolism has been studied in depth in rainbow trout because of the need to reduce the amount of phosphorus in trout farm hatchery water effluents. In contrast to mammals, phosphorus homeostasis is controlled by excretion in the urine rather than by regulation of intestinal absorption. Spillage of phosphorus in the urine is a sensitive measure of the dietary requirement for phosphorus, and using this approach, Sugiura et al. (2000) found that small rainbow trout have a higher dietary phosphorus requirement than do large rainbow trout. Requirements of fish for electrolytes differ from those of birds and mammals; some fish, especially marine species obtain them from water. However, trace mineral requirements are, in general, similar. Requirements for several trace elements, e.g. copper, iron, manganese and zinc, are well-established in rainbow trout (Hardy, 2002).

Marine fish species require omega-3 fatty acids in their diets, whereas freshwater species require omega-6 fatty acids, with perhaps a small amount of omega-3s. Rainbow trout require between 0.5% and 1% omega-3 fatty acids in their diet, and can effectively elongate linolenic acid to docosahexaenoic acid (DHA). Anadromous fish species that live in both fresh and salt water during their life cycle require both omega-3 and omega-6 fatty acids. The most effective sources of omega-3 fatty acids are the polyunsaturated fatty acids (PUFAs), DHA and eicosapentanoic acid (EPA), found in lipid sources of marine origin. These fatty acids are essential components of cell membranes, facilitate adjustment to varying water temperatures and serve as precursors of eicosanoids and other metabolically active compounds. They are also critical for normal egg development and fertility.

Fish possess all of the necessary digestive and metabolic enzymes involved in carbohydrate digestion and metabolism. However, carbohydrate utilization is highly variable among fish species. Omnivores and herbivores generally have relatively long intestinal tracts (Rust, 2002). In contrast, top carnivores, such as salmon, halibut, and trout are less tolerant of high dietary levels of available carbohydrates and exhibit prolonged elevated serum glucose levels and excessive liver glycogen levels when dietary levels are too high. Warm water species tend to tolerate higher levels of dietary carbohydrate than do cool water species. Rainbow trout exhibit elevated rates of gluconeogenesis when fed diets extremely low in available carbohydrates, but gluconeogenesis is generally not a major source of glucose, even during periods of starvation (Halver and Hardy, 2002).

Nutritional requirements change with life history stage in fish, as in most animals. Young fish (fry) of all species, including grass carp, an herbivore at the post-juvenile stage, require high protein diets. Rainbow trout initiate exogenous feeding with fully formed digestive tracts, and flourish on particulate diets. This is one of the qualities of rainbow trout that make them a desirable research fish. Some fish species having larval stages appear to require free amino acids in the diet; these larvae do not possess fully functional digestive tracts at first feeding (Rust, 2002). This may explain the preference of these larvae for zooplankton, most of which maintain osmolarity by having relatively
high levels of free amino acids in their bodies. Efforts to develop microparticulate diets for larval fish are progressing, but these diets are not yet equal to live food for many larval fish species. Rotifers and Artemia are the main live foods used to rear larval fish in captivity. Once the digestive system is developed, fish can be weaned to feed particles and small pellets. Frequent feeding, as often as every 10 min, is necessary to ensure high larval and fry survival during this period. Even first-feeding trout require frequent feeding. Automatic feeders are often employed to guarantee abundant feed to young fish. As fish develop and grow, dietary protein levels can be lowered, and dietary energy levels can be increased, with energy being supplied by either lipid or carbohydrate sources, depending upon the species of fish. Generally, there is little problem with feeding higher than needed protein levels; economy, not biology, dictates reductions in dietary protein levels as fish grow.

Fish nutrition cannot be discussed without discussing the effects of feed on water quality. Rainbow trout can tolerate a range of environmental conditions, but flourish best at water temperatures of 8–12 °C, with dissolved oxygen concentrations >5 mg/l. For fish reared in static or recirculated water systems, it is critical to avoid overfeeding and subsequent water quality problems. Ammonia is toxic to fish, and levels can build up quickly in water systems lacking adequate biological filtration systems to convert ammonia to nitrite and nitrate (Wedemeyer, 2002). In flow-through water systems, care must be taken to minimize the levels of phosphorus and fecal waste in farm discharge water. Such discharges are subject to increasing regulation to preserve the quality of receiving waters, and to avoid eutrophication. With respect to phosphorus, fish, like all monogastric animals, lack the ability to digest phytate phosphorus, the storage form of phosphorus in seeds, e.g. grains and oilseeds such as soybeans. Supplementation of diets with microbial phytase greatly increases the bioavailability of phytate phosphorus, as does the use of low-phytate mutant varieties of grains and oilseeds in diets (Sugiura and Hardy, 2000). Minimizing fecal solids production involves the use of digestible feed ingredients and avoiding high-fiber ingredients. Intercepting and removing fecal solids prior to farm water release is a practice widely employed in flow-through fish farming systems.

Fish feeds are subject to several problems common to all animal feeds, e.g. the presence of anti-nutritional factors, contaminants, molds, and products of lipid oxidation. Fish are much more sensitive to these than are terrestrial animals and birds, most likely because they did not evolve in contact with them, by virtue of their aquatic existence. Anti-nutritional factors can be avoided by proper processing of plant-derived feed ingredients (Dong et al., 2000). Contamination of feed ingredients is an increasing global problem but problems can be minimized by careful sourcing and vigilant testing. Molds are the result of the use of contaminated ingredients, and excessive moisture (>12%) in finished feeds. Both problems can be easily avoided. Rainbow trout are extremely sensitive to aflatoxins, showing LD50 values of 0.81–1.90 mg/kg, depending upon the toxin. By comparison, rat LD50 values are 5.5–7.2 mg/kg. Catfish and Pacific salmon are less sensitive to aflatoxin than rainbow trout. Oxidation is another concern with rainbow trout. The use of high PUFA lipid sources in fish feeds increases the prospect of lipid oxidation. Naturally-present antioxidants offer some degree of protection, and most marine oils are fortified with added antioxidants during production. Nevertheless, antioxidants are sacrificial, and once they are used up capturing free radicals, oxidation accelerates extremely rapidly, often in feeds after they have been sold to the customer. Products of oxidation are toxic to fish, and consumption of oxidizing feeds burdens the antioxidant systems within cells, leading to clinical disease associated with vitamin E deficiency and toxicosis (Hardy and Roley, 2000). Young fish are more susceptible to oxidation, probably because their body reserves of vitamins C and E are low. In any event, care must be taken to avoid oxidizing feeds, and to dispose of feeds when they have reached their shelf-life. Testing for oxidation in feeds, and for the latent level of antioxidant protection in feeds, is relatively easy and should be a routine practice in research programs utilizing fish.

Fish nutrition has advanced significantly in the past 50 years, and although the quantitative dietary nutrient requirements for many cultured fish species are not known, requirements estimated for other species, such as rainbow trout, seem to work reasonably well when applied to similar species for which little is known. This across-the-board approach is responsible for the double-digit growth of global aquaculture production over the past 15 years.
years. Aquaculture now supplies over a quarter of all seafood in the market, and given the fact that the majority of wild fisheries stocks are either fully or over-exploited, future seafood demand will only be met through increased aquaculture production. With respect to fish nutrition research, current emphasis is on developing low-pollution diets based upon sustainable feed ingredient sources to augment finite global supplies of fish meal and fish oil. Research with rainbow trout is central to these efforts. Also of increasing interest is the development of diets that support optimum fish health. This involves re-evaluation of estimates of dietary vitamin and mineral requirements, using immune response or disease resistance to experimental challenge as response variables (Gatlin, 2002). Dietary supplements that are possible immune enhancers or stimulants in fish also require careful evaluation, and rainbow trout are the likely test fish for this work. The study of muscle growth and development is also being researched with great intensity in fish, with the evaluation of the genes involved in growth and differentiation being cloned and their expression pattern studied (Rescan et al., 2001; Delalande and Rescan, 1999; Rescan, 2001). The fact that muscle cell and fiber development in fish differs from mammalian muscle by cell number increase during rapid growth and that the size and number of muscle fibers relates to fillet texture makes this an issue of great interest (Randall and Farrell, 2000). Molecular examination of the components of muscle are now also being used to monitor dietary utilization of protein in relation to growth (Overturf and Hardy, 2001). With the advent of molecular biology being used in aquaculture research, fish nutritionists and geneticists are both in a position to gain valuable knowledge as specific genes involved with metabolic regulation are isolated and studied. Researchers have recently begun isolating genes involved with glucose metabolism (Pansera et al., 2000). A more complete understanding of these metabolic pathways and their relationship with different dietary components will be beneficial in generating diets suited for fish consumption and genetic selection programs for the production of growth enhanced fish. Research to develop defined, optimized diets for fish used in medical research is also badly needed. Finally, research to define the nutritional requirements and optimized diet formulations for new fish species and for larval stages of life history are a major priority for the future.

8. Evolutionary and quantitative genetics

Rainbow trout are native to the Pacific coast of North America from Baja California to the Alaska Peninsula, and to the Kamchatka Peninsula and nearby regions of Russia. A number of healthy native populations exist with diverse natural adaptations (Hershberger, 1992). These populations differ in traits including tendency to migrate to the ocean as juveniles (steelhead) or to remain resident in freshwater, apparent ability to tolerate high temperatures, disease resistance and juvenile behavior. Populations differ in allozyme frequencies (Allendorf and Utter, 1979) and in chromosome number resulting from chromosome rearrangements (Thorgaard, 1983; Ostberg and Thorgaard, 1999).

Rainbow trout have several distinctive attributes as research animals for evolutionary genetic studies. These include a high incidence of duplicated gene loci as a result of having a tetraploid ancestry, interesting variations in development rate associated with well-characterized and yet-to-be-identified genes, and a primitive state of sex chromosome evolution. The ease of conducting controlled matings and ample genetic variation present within the species have also made them a preferred species for quantitative genetic analyses among fishes.

8.1. Duplicate genes and post-tetraploidy evolution

Rainbow trout and other salmonid fishes are distinctive in having a relatively recent tetraploid ancestry (reviewed in Allendorf and Thorgaard, 1984). This has resulted in a large proportion of the genes in these fishes being present in multiple copies per haploid genome. The presence of duplicate genes can complicate the interpretation of genetic information because very similar sequences and gene products may in fact represent different genes. Inheritance patterns can also be complicated because ancestral homeologs resulting from the tetraploid event can still pair with each other in some instances, leading to tetrasomic inheritance patterns (e.g. Allendorf and Danzmann, 1997).

The positive aspect of this complexity of salmonid genomes is that it provides a natural laboratory for following the process of evolution by gene
duplication. This process, which is generally acknowledged to be a central one in evolution (Ohno, 1970; Force et al., 1999), is still taking place in these fishes. The salmonid genome duplication is more extensive and recent than the similar event which has been studied in the zebrafish (Amores et al., 1998). Polymorphisms exist within rainbow trout in these duplicate genes, some related to variations in tissue-specific patterns of gene expression (Allendorf et al., 1983) and some related to gene silencing (e.g. Ferguson et al., 1988). Duplicate gene copies are beginning to be studied at the DNA sequence level (e.g. Brunelli et al., 2001). Wider-scale sequence information for rainbow trout will provide an excellent and distinctive system for studying the aftermath of a genome-wide duplication event and the associated structural and regulatory gene changes. Sophisticated bioinformatics approaches will likely be needed to identify and characterize the internal genomic homologies resulting from this tetraploid ancestry and obtain full benefit from this dataset.

8.2. Development and evolution

The embryonic development of rainbow trout is well-defined (Ballard, 1973), and numerous variations in the process and their genetic basis have been studied (Thorgaard and Allendorf, 1988; Danzmann and Ferguson, 1990). An interesting regulatory gene mutation in phosphoglucomutase activity has been shown to lead to rapid embryonic development (Allendorf et al., 1983). This mutation has significant effects on the phenotype, including early sexual maturation, morphological effects and behavioral dominance (Allendorf et al., 1983; Leary et al., 1984a; Ferguson and Danzmann, 1985). A difference in development rate between two clonal lines (Robison et al., 1999) has been shown to associate primarily with one linkage group in a QTL analysis from a cross between these two lines (Robison et al., 2001). The thorough background information available and existence of natural variations in development rate in trout represent a valuable system for studying genes controlling development rate.

Rainbow trout have presented an excellent model for studying the origins and control of fluctuating asymmetry as measured by variations in the numbers of meristic elements present on the left and right sides of the animal. Asymmetry increases as the level of genetic variation decreases in trout (Leary et al., 1984b) and is decreased in triploids, which have higher levels of genetic heterozygosity (Leary et al., 1985). This sensitive measure of developmental disturbance has not been applied to investigations of environmental toxicology with trout. However, this may represent a major research opportunity for linking organismal and biochemical investigations in toxicology.

The mitochondrial genome of rainbow trout has been fully sequenced (Zardoya et al., 1995). Trout present excellent opportunities to examine the effect of mitochondrial variation on development and physiology because using androgenesis it possible to create lines with the same nuclear but different mitochondrial genomes (Brown and Thorgaard, 2002).

The period of adaptation to seawater (smoltification) is another important developmental stage in rainbow trout. This transition has been intensively studied at the physiological level (Hoar, 1988; Dickhoff et al., 1997) but the genetic control of this trait has not yet been elucidated. There are marked differences among trout populations in tendency to undergo this transformation and an understanding of control of this developmental program represents another challenge and opportunity for future research. The related Oncorhynchus species vary in the timing of smoltification (Hoar, 1976), and genomic information in the rainbow trout model could provide fundamental information for addressing this fascinating trait in a range of related salmonid species.

8.3. Sex chromosome evolution

It is generally accepted that sex chromosomes evolved from a pair of homologous autosomes. Rainbow trout sex chromosomes exhibit variable levels of differentiation with most populations exhibiting heteromorphic sex chromosomes (Thorgaard, 1983). Isolated populations near the edge of the range appeared to maintain primitive, undifferentiated sex chromosomes. The low level of differentiation combined with intraspecific variation in sex chromosome morphology suggest that rainbow trout sex chromosomes are in the early stages of differentiation. Similar intraspecific genetic variation likely existed during the early stages of sex chromosome differentiation in other organisms, including mammals. Consequently, analysis of the genetic basis of this variation may
provide important information about the process of early sex chromosome evolution.

Genetic mapping has revealed that most of the X and Y chromosomes pair with each other and recombine during meiosis in rainbow trout (Allendorf et al., 1994; Young et al., 1998; Sakamoto et al., 2000). Although a duplicated copy of the 5S rDNA locus is located on the X (Moran et al., 1996), no sex-linked molecular marker from the non-recombinating region of the Y (Y-linked) has been identified in this species. Numerous markers have been identified that are tightly linked to the SEX locus. A sex-linked marker derived from a randomly amplified polymorphic DNA (RAPD) band has been identified (Iturra et al., 2001) however, it does not appear to be completely Y-linked in all populations, suggesting that it is not in the non-recombinating region. Another study screened over 12,000 AFLP marker polymorphisms and failed to identify a completely Y-linked marker (Young et al., unpublished results). These results suggest that the non-recombinating region near the SDL is very small and/or consists of low-complexity DNA sequences. In contrast, numerous sex-linked markers have been identified in other species of the genus *Oncorhyncus* (Devlin et al., 2001b), indicating intrageneric variation in sex chromosome organization.

In rainbow trout YY males are completely viable (Parsons and Thorgaard, 1985), suggesting that few if any genes essential for survival have been lost from the Y chromosome. This, combined with the physically small region associated with the sex-determining locus (SDL), could make it relatively easy to identify genes associated with sex determination following complete genome sequencing. Comparative analysis of the function of the SDL in a lower vertebrate would add significantly to our understanding of the process of sex determination. Mammalian genes associated with this process, such as *Sry* and *Zfy*, do not appear to be associated with sex in the fish families that have been examined (reviewed in Devlin et al., 2001a,b). The relatively high sequence homology among the species will enable comparative analysis of the SDL gene(s) among salmonid species. Characterizing a SDL and comparing it to homologous copies within the rainbow trout genome (a consequence of tetraploid ancestry) and among other salmonid species would contribute greatly to our understanding of the genetic basis of sex determination.

The characteristics discussed here make rainbow trout a good vertebrate model for the study of sex chromosome evolution. These include a genetic X/Y sex determination system, intraspecific sex chromosome morphological variation including what appear to be primitive undifferentiated forms, and the presence of a highly saturated genetic map of the sex chromosomes which will enable the sequences associated with these chromosomes to be identified, including regions within 1 cM of the SDL.

8.4. Quantitative genetics

Many of the organismal characteristics that are current targets of functional and comparative genomics research are inherited in a complex manner, involve multiple loci, and are influenced by the environment. Quantitative genetic analysis of many of these traits in rainbow trout has revealed much about their inheritance (through the study of additive and dominance effects) and the relationships among traits (through the estimation of genetic covariance). It is from these basic foundations that many of the interesting questions in salmonid comparative genomics arise.

From a quantitative genetics perspective, there are several advantages to working with rainbow trout and salmonids in general. Quantitative genetic analysis is facilitated by their high fecundity and external fertilization. This provides tremendous flexibility in breeding design, as the eggs of a single female can be split into many different groups, each group fertilized by a different male. Similarly, the sperm from a single male can be used to fertilize many different females. The combination of high fecundity and external fertilization allows the use of the optimum combination of family size and number of families, which minimizes the standard error of genetic variance estimates (Lynch and Walsh, 1998).

The breeding characteristics of rainbow trout also allow cross-classified experimental designs that are not typically available to most vertebrate systems. These designs have distinct advantages over traditional parent-offspring regression and sib analyses. Several different familial relationships can be analyzed, such as full sibs, maternal and paternal half sibs, and when clonal trout are available, reciprocal sibs and even selfed families. This array of familial relationships significantly expands the range of genetic variance components that can
be estimated. Cross-classified designs also allow increased precision in estimating quantitative genetic parameters, and some designs will allow additional parameters, such as the average degree of dominance, to be estimated (Lynch and Walsh, 1998).

Despite these advantages, traditional breeding designs are not practical in many wild populations of salmonids. Molecular marker information can be used to provide estimates of quantitative genetic parameters in these cases. For example, Mousseau et al. (1998) studied the genetic basis of weight, length, flesh color, and propensity for precocious maturation in a population of chinook salmon. In this study, relatedness among individuals was determined using VNTR DNA fingerprint markers, and a maximum likelihood method was used to estimate heritabilities and genetic correlations.

Phenotypic variation for a wide variety of morphological, behavioral, physiological, and life history traits in the Pacific salmonids has a genetic basis. In rainbow trout specifically, there are many traits of biomedical, evolutionary, and agricultural importance that have a significant genetic component and are now targets of more detailed genomic analyses. Some of the more interesting classes of quantitative characters with significant genetic components include resistance to pathogens and life history variation.

Resistance to pathogens is important from the perspective of evolutionary biology, biomedicine, and the aquaculture industry. Dissection of the genetic architecture of pathogen resistance relies on knowing to what extent variation in resistance levels is genetically determined. Several studies indicate a genetic basis to resistance to a variety of salmonid pathogens, including viral hemorrhagic septicemia (Dorson et al., 1995), infectious hematopoietic necrosis (Yamamoto et al., 1991), diptheria toxoid (Eide et al., 1994), Vibrio (Fja- lestad et al., 1996), and Ceratomyxa shasta (Bartholomew, 1998).

The physiological response to stress is another class of complex traits that are important in a variety of contexts. The stress response is important to the aquaculture industry, where the animals are exposed to high-density fish culture conditions and frequent handling. In a more general context, elucidation of the genetic architecture of stress responses in rainbow trout can serve as a model system for biomedical research. Stress-related physiological traits that have shown evidence of genetic variation within or among strains of rainbow trout include plasma cortisol levels (Fevolden et al., 1993, Pottinger and Carrick, 1999), glucose stress response (Fevolden et al., 1993), red blood cell membrane fragility (Gjedrem et al., 1991), and lysozyme levels (Fevolden et al., 1999, 2002). In addition, some of these physiological traits are genetically correlated with each other (Fevolden et al., 1999) and with growth (Fevolden et al., 2002) and behavior (Pottinger and Carrick, 2001).

The opportunity to study the genetic architecture of the wide variety of life history traits is one of the most compelling reasons to do genomics work in rainbow trout, as many life history traits in salmonids have a strong genetic basis. Body size is a highly heritable trait in rainbow trout (Gall and Huang, 1988; Sylven and Elvingson, 1992; Elvingson and Johansson, 1993; Horstgenschwark 1993; Su et al., 1996; Pante et al., 2002). The genetic architecture underlying body size and growth rate, however, is sex-specific, and is intertwined with reproductive traits such as maturation timing (Crandall and Gall, 1993a,b,c). Female reproductive performance (Gall et al., 1988; Su et al., 1997), age at maturity (Gall et al., 1988; Su et al., 1999), spawning time (Siitonen and Gall, 1989; Sakamoto et al., 1999) and development rate (Hebert et al., 1998) also show heritable genetic variation.

From the preceding examples, it is clear that the rainbow trout system provides a wide variety of opportunities for studying the genetic architecture of quantitative characters in greater detail. Future genomic analysis of complex traits in rainbow trout will provide fundamental insights into the molecular nature of quantitative trait variation. Opportunities also exist for strain improvement in the aquaculture industry through the identification of important genes for traits such as maturation timing, growth rate, and disease resistance. Finally, the vast array of locally evolved life history variation provides the opportunity to study adaptation at the genomic level.

9. Genetic improvement of rainbow trout for aquaculture

There is international recognition that the natural ocean and freshwater fisheries are being harvested to near their limit and abundance may even decline
in the future due to overfishing, habitat destruction, and pollution. Aquaculture is the only avenue for producing finfish and shellfish to meet the increasing worldwide demand. The maximum sustainable yield of natural fisheries product is between 100 and 120 million metric tons (Aquacultural Genetics and Breeding, 1988). In 1948 the world fisheries catch was approximately 19.6 million MT and has risen to almost 125 million MT in 1999, of which 33 million MT were aquaculture production. During the 1990s inland aquaculture was the only component of world fish catch that grew steadily from 11 million MT in 1994 to 21 million MT in 1999. Production per annum from marine capture, marine aquaculture and inland capture for the same period was unchanged at approximately 84 million MT, 12 million MT and 7 million MT, respectively (FAO, 2001). Aquaculture is recognized for its efficiency in producing animal protein. Genetically improved salmon and trout can achieve food conversion ratios of less than 1.5, that is 1 pound of live fish is obtained from each 1.5 pound of feed. By comparison, the average feed conversion ratio for other meat producing animals ranges from 12 in grass-fed cattle to 1.7 for highly improved lines of chicken (Aquacultural Genetics and Breeding, 1988).

The rate of response to genetic selection in aquatic species is very high compared to farmed land animals. The genetic variation for growth rate is 20–35% in fish and shellfish compared with 7–10% in land animals (Gjedrem, 1997). The recent domestication of most aquaculture species and availability of natural resource populations are major contributors to this greater variation. The high fecundity of aquatic organisms allows for strong selection pressure, which increases the rate of response. Heritability estimates for growth rate in rainbow trout older than 1 year are between 0.17 and 0.38 with an average of 0.23. For young fish the range is larger (between 0.06 and 0.52) with an average of 0.21 (Gjedrem, 1990). In a recent breeding program with a commercial strain in Idaho the growing cycle was shortened by 4–5 days per generation (James Parsons, personal communication). Improved growth rate and feed conversion also result in less metabolic waste released per unit of feed.

Rainbow trout are the model organism for cool and cold aquaculture research. The major advantages for using rainbow trout for genetic research are:

1. High fecundity—Typically, females produces 2000 eggs per kg fish and a large amount of sperm (~10 ml) is available from a single male over a breeding cycle.
2. Ease of manipulation and handling of gametes—This enables transportation of gametes before and after fertilization from a broodstock holding facility to grow out farms or research institutions, which dramatically reduces the cost for small farms and individual researchers. The hardiness of rainbow trout gametes also facilitates chromosomal set manipulations by means of heat shock, hydrostatic pressure shock, or irradiation. These methods are widely utilized by farmers and/or scientists. Gynogenesis is used for producing all-female populations and to prevent deterioration of flesh quality due to early sexual maturation, and triploidy is used for producing sterile fish to reduce genetic contamination of wild populations by farmed fish (Thorgaard, 1992; Palti et al., 1997). Androgenesis is used to produce true homozygous lines in two generations (Young et al., 1996).
3. Year round supply of gametes—Due to a wide natural variability in spawning time (Siitonen and Gall, 1989; Sakamoto et al., 1999) and the use of photoperiod control.
4. Semen cryopreservation—Well developed and widely used methods for rainbow trout semen cryopreservation (e.g. Wheeler and Thorgaard, 1991) are available, which allows for crosses between strains that spawn at different seasons and between different generations (e.g. for backcrossing mating designs). It is also used for conservation of germplasm from wild populations and from economically important strains.

Diseases are the major cause for fish losses in rainbow trout aquaculture. Thirty three million fish were lost to diseases in the farming segment of the US industry in 2001, causing a $33 million loss. Fish diseases are also a major limiting factor for aquaculture worldwide. Current methods to control infectious diseases consist of hygiene, vaccination, drug therapy and eradication of infected populations. Improving disease resistance by genetic means is an attractive alternative because of its prospects for prolonged protection. As discussed elsewhere in this review, rainbow trout have been shown to have a significant genetic variation in disease resistance which can be util-
ized in selection programs that will benefit the industry.

10. Current status of genomics resources for rainbow trout

The development of genomic tools for rainbow trout research has been progressing rapidly in recent years. Two genetic linkage maps have been published (Young et al., 1998; Sakamoto et al., 2000), and more detailed maps are being developed. Over 250 polymorphic microsatellite markers have been developed for rainbow trout at the USDA/ARS National Center for Cool and Cold Water Aquaculture (NCCCWA) in the past 2 years (e.g. Rexroad et al., 2001). Approximately half of the microsatellites developed for Atlantic salmon work for rainbow trout (Moira Ferguson, personal communication) and most microsatellites developed for Pacific salmon successfully amplify the corresponding rainbow trout sequences. This brings the total number of microsatellites currently available to over 500.

The total number of nucleotide sequences for rainbow trout, which were deposited on the NCBI databases as of April 30, 2002, was 2200, of which 632 were expressed sequence tags (ESTs). These figures are far behind other fish model organisms such as zebrafish and medaka (245,000 and 46,700 nucleotide sequences, respectively). A major effort is now focused on increasing the rainbow trout ESTs database. Forty-five thousand clones from a normalized cDNA library, which was derived from gill, liver, brain, kidney, spleen and muscle are currently being sequenced to produce additional ESTs in another effort at the NCCCWA. The INRA (Institut National de la Recherche Agronomique), France trout genome and transcriptome project is now focused on increasing the rainbow trout ESTs database. Forty-five thousand clones from a normalized cDNA library, which was derived from gill, liver, brain, kidney, spleen and muscle are currently being sequenced to produce additional ESTs in another effort at the NCCCWA. The INRA (Institut National de la Recherche Agronomique), France trout genome and transcriptome project is expected to complete a sequencing project of 120,000 ESTs (60,000 clones in the 5' and 3' directions) by mid-2003. Their cDNA library is derived from liver, interrenal, brain, muscle, blood, intestine, ovary, testis, differentiating gonads, adipose tissue, gills, pituitary and kidney. A radiation hybrid panel is being constructed by the INRA group and is expected to be completed by the end of this year. It should be useful for rapid linkage mapping of the ESTs (Rene Guyomard, personal communication).

Four BAC libraries of the rainbow trout genome have been constructed to date. Two were constructed in Japan by Katagiri et al. (2001). They contain an average insert size of 58 kb and 110 kb, and provide haploid genome coverage of 6.7-fold and 5.3-fold, respectively. Two were constructed in the US from the OSU female homozygous line and from the Swanson male homozygous line. Their average insert size is 130 kb and they provide a 4x (OSU) and 10x (Swanson) genome coverage. BACs from the OSU library have been used as probes in fluorescence in situ hybridization to anchor the genetic linkage map to specific chromosomes (Phillips, 2001). The Swanson library is now being characterized with 20 genes of interest. Both libraries will be used for mapping of type I markers (ESTs), and can be used to produce a sequence-ready BAC contig map.

A high-density genetic map and complete sequence of the genome will be extremely useful resources for rainbow trout breeding programs. To date much of the effort has been directed toward mapping quantitative trait loci (QTL) using markers from the genetic linkage maps described above (Sakamoto et al., 1999; Ozaki et al., 2001; Perry et al., 2001; Robison et al., 2001). High density maps that can be developed from a genome sequencing project are needed for fine mapping of those QTL and for identifying more economically important QTL (e.g. immune and stress response, growth rate, feed conversion, osmoregulation). Fine mapping will enable application of marker-assisted selection and gene introgression to rainbow trout breeding programs. The complete sequence of the genome is needed for positional cloning and identification of novel candidate genes for the QTL.

High-density maps can be used in gene introgression between stocks that spawn at different seasons, or alternatively, aid in changing the genetically predetermined spawning season by changing the alleles of the genes that control spawning time. Year round availability of rainbow trout gametes for production is achieved by using several broodstocks that spawn at different seasons. Spawning time is strongly controlled by genetic components and is highly polygenic in rainbow trout (Siitonen and Gall, 1989; Sakamoto et al., 1999). Selection programs for desired traits such as disease resistance or feed efficiency are typically carried out on a specific broodstock with a narrow window of spawning time. Gene introgression to transfer economically desired traits from one population to another have been used extensively in plant breeding (e.g. Young et al., 1988; Stamova and Chetelat,
in this group. Genomes were used to enhance genomic mapping of conserved synteny between mammalian duplications and gene loss create a network of the tomato and Arabidopsis genomes indicated that duplications and gene loss create a network of synteny (Ku et al., 2000). However, large regions of conserved synteny between mammalian genomes were used to enhance genomic mapping of species in this group (O’Brien et al., 1999). Due to the large evolutionary distance between trout, zebrafish and fugu fish, it is likely that comparative genomics between those species will not be very useful for fine mapping, but rather for identifying conserved regulatory elements and general genomic organization. The rainbow trout is a well-developed, experimentally tractable model. Their size, ease of controlled reproduction, and the knowledge about and availability of both natural and clonal research populations set them apart from other model fish species. As outlined above, the background information and reagents are poised for applying modern molecular methods and information to address a range of fundamental biological issues with this species.

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