

Genomic Approaches in Marine Biodiversity and Aquaculture

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SUMMARY

Recent advances in genomic and post-genomic technologies have now established the new standard in medical and biotechnological research. The introduction of next-generation sequencing, NGS, has resulted in the generation of thousands of genomes from all domains of life, including the genomes of complex uncultured microbial communities revealed through metagenomics. Although the application of genomics to marine biodiversity remains poorly developed overall, some noteworthy progress has been made in recent years. The genomes of various model marine organisms have been published and a few more are underway. In addition, the recent large-scale analysis of marine microbes, along with transcriptomic and proteomic approaches to the study of teleost fishes, mollusks and crustaceans, to mention a few, has provided a better understanding of phenotypic variability and functional genomics. The past few years have also seen advances in applications relevant to marine aquaculture and fisheries. In this review we introduce several examples of recent discoveries and progress made towards engendering genomic resources aimed at enhancing our understanding of marine biodiversity and promoting the development of aquaculture. Finally, we discuss the need for auspicious science policies to address challenges confronting smaller nations in the appropriate oversight of this growing domain as they strive to guarantee food security and conservation of their natural resources.

Key words: aquaculture; genomics; marine biodiversity; next-generation sequencing, proteomics.

INTRODUCTION

Marine life is adversely affected by non point source pollution caused by human activities as well as by natural causes. Decreases in ocean productivity and resource availability will have a domino effect, leading to potentially catastrophic transformations in both the abundance and composition of marine species. In the face of widespread and rapid deterioration of the diversity of sea life, there is an increasing need to engineer the successful, sustainable use of the seas for food through fisheries and aquaculture. Effective measures for halting this loss of biodiversity will require a better understanding of the impact of climate change on natural systems as well as documenting the fundamental causes and consequences.

Genomics and modern biotechnology are examples of modern technologies which are expanding our understanding of the underlying patterns and processes influencing the diversity of life in the oceans. Since the development of the first gene sequencing methods by Frederick Sanger in the 1970's, genetic research has seen considerable and rapid expansion in many different areas of biology and biotechnology applications. Genomic sequencing has now become a standard tool in biodiversity* research.

Although most genomics research has focused on the biodiversity of terrestrial species, the application of genomics to the study of marine species and ecosystems is gradually gaining ground. The recent sequencing of a wide range of fish genomes is providing important clues into vertebrate genome evolution, including –unexpectedly– relevant information regarding the function of genes involved in human diseases (Roest & Weissenbach, 2005). Furthermore, by pairing

the latest developments in genomics with post-genomics technologies, it is now possible to gain new insights into the evolutionary history of marine species and their responses to environmental stress, as well as key features of global marine biodiversity and ecosystem dynamics. This paper provides examples of recent discoveries and advances in the generation of genomic, transcriptomic and proteomic resources to understand marine biodiversity better and to further the development of aquaculture. We also discuss the need for science policies to address the challenges confronting smaller nations in the appropriate supervision of this growing field as they attempt to guarantee food security and conservation of their natural resources.

GENOMIC APPLICATIONS IN MARINE BIODIVERSITY

Scientific advances in molecular sequencing are aiding biodiversity assessments and revealing previously unknown genetic and functional diversity, and providing methods with the capacity to transform greatly the way we think about marine life.

Next-generation sequencing. The recent introduction of next-generation sequencing (NGS) methods in marine biology is affording us tremendous insight into the genome, transcriptome and metabolome of many marine species. Genetic variation studies of model fish systems are providing crucial data on adaptation and persistence mechanisms of great relevance for biodiversity assessment and conservation biology in marine ecosystems.

The genome of the bottlenose dolphin, *Tursiops truncatus*, has shed light on the evolution of the dolphin nervous system

* In this review we use the definition of the Convention on Biological Diversity, which defines biodiversity as “the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems”. <http://www.cbd.int/convention/articles/default.shtm?a=cbd-02>

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and reveals consistencies with other large-brained mammals such as humans. As part of a larger study of mammalian genomes, the recently sequenced dolphin genome (McGowen, 2012) was used to compare more than ten thousand protein-coding genes with corresponding matches in the genomes of several other mammals, including dogs, elephants and humans. The study found perhaps 300 genes in the dolphin lineage which have undergone significant changes under positive Darwinian selection relative to other mammals. Some of those genes correlate with the nervous system, including those related to synaptic plasticity, sleep, intellectual disabilities, microcephaly, schizophrenia and Alzheimer's susceptibility in humans. One similarity dolphins share with humans is a slowdown in the molecular rate of change exhibited by their DNA sequences over time.

Further whole-genome-wide scans (Yan-Bo et al., 2013) using the dolphin genome and focusing on positively selected genes are providing clues to the genetic bases underlying species diversification and adaptive evolution in marine mammals. Using a Uniprot tissue annotation database to investigate the tissue specificity of more than 300 positive selection genes, PSG, the study found that these PSG were largely expressed in tissues associated with the nervous, reproductive and immune systems, suggesting possible functional roles for these tissues in processes requiring aquatic adaptation, such as body fluid equilibrium and breathing in mammals.

Similarly, the sequencing of the African coelacanth's (*Latimeria chalumnae*) genome shed light on how fish may have first adapted to life on land, giving rise to amphibians, reptiles, birds and mammals. The coelacanth, a deep-sea fish that closely resembles its fossil dating back at least 300 Myr, has four, large, lobed fins which are considered to be the predecessors of limbs. The genome sequences show a slow rate of evolution, which is consistent with the general assumption that this lineage is markedly slow evolving (Zhu et al., 2012). Using *L. chalumnae* genomic DNA, the African coelacanth genome was sequenced by Illumina technologies and assembled using the short read genome assembler program ALLPAHTS-LG (Marcador De Posición 1) (Gnerre et al., 2011). Protein-coding gene evolution was examined using a phylogenomic data set based on 251 concatenated protein and 100,583 amino acid positions, concluding that the protein-coding genes of *L. chalumnae* display a diminished substitution rate compared to those of other sequenced vertebrates (Amemiya et al., 2013).

Because of its enormous genome size, nearly 100 billion base pairs, which makes it impractical to sequence, assemble and analyze, the lungfish (*Protopterus annectens*) is lacking in large-scale sequencing data. Consequently, an exhaustive genome comparison of both coelacanth and lungfish is not currently possible. Nevertheless, it has been possible to obtain good quality data sets even if from only a few genes. Deciphering the genetic makeup of the lungfish will provide deep insights into how some vertebrates adapted to life on land and valuable clues for the study of evolution of vertebrates in general.

By comparing the RNA content of coelacanth with that of lungfish, also a lobed-finned species (sarcopterygian), the question was posed - Which living fish is the closest relative to the fish that first made the critical transition from water to land? - a long-debated phylogenetic question. To address this issue,

RNA-seq data from brain, gonad, kidney, gut and liver tissues from *P. annectens* were compared to gene sets from 22 jawed vertebrate species including *L. chalumnae*, and corroborated that the closest living fish to the tetrapod is not the coelacanth, but the lungfish. Also, by looking at the missing genes lost during the water-to-land transition and the newly acquired regulatory elements a series of unusual discoveries were made, including some relevant to the sense of smell for detecting chemicals in surrounding environments, immune-related regulatory changes and evolutionary development. A region of the coelacanth HOX cluster was identified that may have been involved in the evolution of extra-embryonic structures in tetrapods, including the eutherian placenta. The role of Hox genes in the regulation of limb development had been proposed in earlier work (Amemiya, 2010). It is likely that this sequence from the coelacanth was co-opted by tetrapods to help form hands and feet.

With the availability of genomes from marine species, other 'omics' technologies such as transcriptomics, proteomics and metabolomics are also providing more detailed studies into the evolutionary history of marine species.

The field of evolutionary ecology being applied to non-model organisms is benefitting from next-generation sequencing technology (NGS) and newly developed genomic tools. One of those organisms is *Streblospio benedicti*, a segmented tube-dwelling benthic polychaete which displays offspring dimorphism and is being used to study the evolutionary consequences of larval life history. 454 pyrosequencing has been used to characterize the transcriptome for embryos, larvae, and juveniles of *S. benedicti*, generating 715 reads of 400 bp and 3.08 x coverage (Zakas et al., 2012). From a total of 7,222 contigs, PipeMeta found 2,817 single nucleotide polymorphisms (SNP). Using the BeadXPress Golden Gate assay (Illumina), these SNPs were validated by genotyping individuals of different developmental stages, and 84 new SNP markers resulted effective for distinguishing between geographic and phenotypic populations of *S. benedicti*. Given the scarcity of genomic resources for marine polychaetes, this transcriptomic approach should be very suitable for standard population genetic analyses to study the molecular and regulatory mechanisms underlying their life-history variations.

Transcriptome analyses are becoming more broadly available, particularly when genome sequences are not available. A transcriptome analysis was performed recently on the Atlantic herring (*Clupea harengus*), an abundant fish found in the pelagic zone of marine waters and a critical protein source both in Europe and North America. From the biological point of view, understanding the genetic structure of *C. harengus* is of great interest because of its well-defined adaptation to salinity, being one of the few marine species capable of reproducing in both the conditions of the North Atlantic and throughout the Baltic Sea. A few studies using isozyme loci, microsatellites and SNP revealed noticeably low levels of genetic differentiation between geographically distant and morphologically distinct forms (Ryman 1984, Limborg et al. 2012, Larsson et al., 2007, Larson et al 2010, Gaggiotti 2009). This analysis was further developed through examination of the skeletal muscle mRNA to assemble a transcriptome and align genomic reads to the transcripts creating an "exome assembly" (Lamichhaney et al, 2012). Using this methodology it was possible to identify important genetic markers for phenotypic variation in *C. harengus*, allowing

further population genetic studies of adaptation and natural selection. A more comprehensive transcriptome analysis of other *C. harengus* issues along with the determination of its full genome sequence will justify using the herring as a model organism for evolutionary genetics. The full genome sequence of this large genome, on the order of 900 Mbp, may still be a major undertaking. SNP development using a combination of next-generation sequencing (NGS) and a high-throughput genotyping approach, however, has the potential to make the most of efficient use of cost and time by avoiding the costly and time-intensive laboratory stages involved in genomic work for population studies in the ocean.

The wide accessibility of NGS technologies has certainly found immediate application in model marine organisms (sea urchin, the polychaete *Platynereis dumerilii*, *Amphioxus* and others). Their application to the ecology and evolutionary studies of non-model organisms is being met with growing interest. Two sequencing platforms, the Roche 454 FLX (454) and the Illumina Genome Analyzer (GAII), have been successfully used for SNP discovery in the transcriptome of the European hake, *Merluccius merluccius* (Milano et al., 2011; Nielsen, 2006). Similarly, an integrated genomic approach that includes the construction of cDNA libraries and the generation of a large EST set for Atlantic cod (*Gadus morhua*) have been described (Bowman et al. 2010). These tools and data represent a critical resource for Atlantic cod, a valuable marine species under-represented in genomic databases.

Metagenomics and proteomics. With ground-breaking advances in metagenomic sequencing and proteomic technologies, the study and characterization of uncultured biological samples are becoming more and more practical, uncovering the compositions, functions and interactions occurring in microbial communities present in different environments. Faster sequencing technologies and the ability to sequence a broad spectrum of uncultured microbes sampled directly from their habitats are maturing and revolutionizing our view of the microbial world, along with our understanding of their effects on global processes such as carbon, nitrogen and sulfur biogeochemical cycles. Analysis of microbial communities using high throughput sequencing methods for the standard 16S ribosomal RNA gene and whole genome shotgun (WGS) sequencing is uncovering the unique organization and identity of bacterial, archaeal and viral communities in coastal and marine environments. Beyond the narrow description obtained from phylogenetic surveys generally using the ribosomal gene, fingerprinting approach to biodiversity, metagenomics provides access to the functional gene composition of whole microbial communities, resulting in more reliable and comprehensive studies (Edwards et al., 2006).

Whole genome sequencing has been used in the last decade to sequence entire coastal and marine ecosystems. Similarly, by sequencing a community of DNA from a very low diversity microbiota in acid mine drainage it was possible to apply a whole genome assembler algorithm to assemble the long capillary sequencing reads with great accuracy (Tyson et al., 2004). Applying similar technology and using a complex bioinformatics approach, the functional potential of oceanic microbial communities in the Sargasso Sea was explored (Venter et al., 2004), finding at least 1,800 different genomes with 48 unknown bacterial phylotypes and 1.2 million previously unknown genes. Subsequent metagenomic studies

(Rusch et al., 2007; Williamson et al., 2012) convincingly rendered this technology a standard application for different marine ecosystems.

Along with metagenomics, in the past few years there has been an explosion in marine environmental proteomics, the large scale characterization of proteins recovered from different coastal and marine ecosystems. While the 16S rRNA data provide essential information about the species membership of a sample and the metagenome sequences provide an account of all possible gene products in an environmental sample, proteomics is employed to determine protein expression profiles of microbial communities, greatly expanding our knowledge of marine microorganisms and their impact on the ocean.

Ground-breaking research in environmental proteomics has revealed important associations between protein diversity and ecological function in microbial communities. Proteomics can be a useful tool in determining phylogenetic relationships and resolving important questions in metapopulation biology and adaptation (review in Biron et al. 2006). High-resolution capillary liquid chromatography coupled to an LTQ mass spectrometer was used to build an accurate mass and time (AMT) tag library as a tool to analyze protein profiles of the marine alpha-proteobacterium *Candidatus pelagibacter ubique*, and to identify adaptation mechanisms and differentially expressed proteins from the exponential and stationary growth phases (Sowell et al., 2008). Most proteins found in greater abundance in the stationary phase were those involved in stress response, protein refolding, transcription regulation and mitigation of oxidative damage, suggesting that these adaptive responses are important for this alphabacterium's enduring survival under conditions of limited nutrients. The determination of proteomic variations of microorganisms in response to growth conditions has become a standard approach for discovering metabolic and regulatory processes (Sowell et al., 2009; Schwalbach et al., 2009; Smith et al., 2010).

Metabolomics. The study of all the metabolites present within cells, tissues and organs, as well as at the population level, has become an indispensable tool that goes beyond simply cataloguing bioactive compounds. Metabolomics is yielding progress in studying variations in the abundance of proteins and their subsequent post-translational modifications; and asks broader biological questions such as how metabolites reflect and affect cell function and how the environment affects the biology of marine organisms. Both nuclear magnetic resonance (NMR) and mass spectrometry (MS)-based or a combination of the two are increasingly more widely used to perform high throughput profiling of metabolites, as well as to investigate interactions of marine organisms with their environment, or marine metabolomics (Miller, 2007). Recently published advances in this field include studies on marine fish (Zhang et al., 2011; Kim et al., 2011) and other ocean vertebrates. Targeted metabolite profiling has also been described in harbor seals and porpoises to determine contamination by chlorinated and brominated compounds (Weijs et al., 2009). Metabolite profiling by MS has also recently been used on a mussel (*Mytilus californianus*) to study its metabolism in both the aerobic and anaerobic phases of its life cycle (Connor & Gracey, 2012). Similarly, NMR-based metabolic profiles have been employed to determine the metabolites responsible for the variation in the metabolic

profile of pathogenic *Vibrio coralliilyticus* under extreme natural environments (Boroujerdi et al., 2012), including variable temperatures (Boroujerdi et al., 2009).

Metabolomic applications show great potential for identifying physiological responses of marine species to their changing biotic and abiotic environments. They also provide a unique opportunity to gain insight into the mechanisms behind differential effects of closely related species as well as of individuals within the same species. In addition, metabolomics along with various modern 'omics' technologies and their applications to marine biology research are providing helpful information for understanding microbial ecosystems, along with biogeography and species diversity in the ocean.

Barcoding. While most genomic and post-genomic applications to marine biodiversity research are still at an early stage, exciting and innovative methodologies are emerging to discover and comprehend life history stages and new species that would otherwise be difficult to identify with traditional taxonomic methods. One such technique is fast large scale DNA barcoding (Hebert et al., 2003; Hebert & Gregory, 2005). Although of unquestionable value for species identification, taxonomic approaches based on phenotypic characters have limited efficiency for ambiguous taxonomic groups or for the identification of closely related species with subtle morphological differences. DNA barcoding uses mitochondrial cytochrome *c* oxidase subunit I sequences to identify any species unequivocally. For more challenging taxonomic groups, it is clear that DNA barcoding needs to be used alongside morphology-based identification systems and include a range of molecular identification strategies based on the analysis of homologous gene regions such as ribosomal RNA molecules (16S and 18S) and the internal transcribed spacer region, ITS. To date, the efficacy of molecular barcoding is finding practical applications in many taxonomic groups, including marine species, giving additional evidence for the consistency and accessibility of DNA barcoding for identifying marine organisms. This is a fascinating technique that is likely to find many uses in ecological studies.

The user-friendliness of DNA barcodes is also finding many practical applications in developing countries (Cawthorn et al., 2011; Lakra et al., 2011; Zhang & Hanner, 2012; Asgharian et al., 2011; Wang et al., 2012; de Carvalho et al., 2011; Weigt et al., 2012), which generally lack sufficient means (financial support, research materials, scientific proficiency and experienced personnel) to conduct their own research and surveillance activities in marine biodiversity, fisheries management and conservation.

GENOMIC APPLICATIONS IN AQUACULTURE

Modern biotechnology and genomics have been used to reveal unexpected features of biodiversity in the oceans. The application of these technologies promises far-reaching social and economic benefits in worldwide aquaculture production as well.

As it applies to aquaculture and fisheries, genomics is rapidly making headway with thousands of publications appearing every year, covering cutting edge topics related to aquaculture biotechnology, genomics, epigenomics and post-genomics applications in aquaculture nutrition, stress, health

and reproduction, and many other areas. Choosing selected examples, we will illustrate the impact of genomics on our current understanding of genome evolution and its potential implications directly related to key traits and infectious diseases in aquaculture.

Studies of adaptive evolution in population genetics of marine fish and other invertebrates are providing valuable insights into local adaptation, response to climate change and ecological impact of selective harvesting and world fisheries. This kind of research, for example transcriptomics of clams (Coppe et al., 2012) and sea cucumbers (Du et al., 2012), is vital for both improving our knowledge of natural evolutionary processes and for establishing coastal and marine conservation priorities. The availability of detailed clam transcriptomics has provided new perspectives in establishing the physiological role of various biochemical processes and of population response to environmental stress. Distinctive genes involved in development or adaptation to temperature changes could be used to acquire valuable information for fishery management surveys of clam beds.

Salmon (*Salmosalar*) is one of the most extensively studied fish and an important model species for fish genetics and evolution. Genomic data produced by the International Collaboration to Sequence the Atlantic Salmon Genome (ICSASG) since 2010 (Davidson et al., 2010) is serving as a reference sequence for other salmonids (salmon, whitefish, trout and char), increasing the number of salmon-specific molecular markers applicable in population genetics to distinguish and monitor various geographic populations (Ozerov et al., 2013). A number of genomic tools have been developed for the cost-efficient generation of SNP and genome-wide markers of relevance for breeding programs (repetitive elements, intron/exons, gene duplication). For instance, recent applications of restriction site-associated DNA sequencing (RAD-Seq) have facilitated large-scale marker discovery and genotyping (Houston et al., 2012). RAD markers are valuable for association and QTL mapping as well as for population genetics and evolutionary research. They present considerable advantages over other methods and are rapidly finding application in SNP discovery and genotyping studies in non-model organisms, where they can be used in genotype-phenotype association mapping, population genetics and scaffolding genome assemblies through linkage mapping (Baxter et al., 2011).

SNP arrays have been successfully used in the assessment of population genetic structure in salmon, facilitating a better understanding of the molecular basis of adaptation to a wide range of geographical environments (Bourret et al., 2010). Furthermore, some karyotype differences between European and North American Atlantic salmon via chromosomal rearrangements have been identified (Brenna-Hansen et al., 2012). These applications may have a major impact on the aquaculture industry by providing genomic information on salmon production traits relevant to industrial development as well as for improved assessments of the sustainability of wild fish populations.

Without a doubt, SNPs are becoming key markers of choice for genome research and in genetic improvement programs. Because of their practical use in the analysis of trait-genotype associations, their application in aquaculture is becoming central. Similarly, as more sequence information becomes available for marine species, other markers are increasingly

being applied in aquaculture genome research. Having well-established genetic linkage maps at hand is the basis for further genome scans for Quantitative Trait Loci, QTL, to study traits of great relevance in aquaculture.

Genome-wide association studies (GWAS) have contributed significantly to the field in the past decade. Genotypic associations with phenotypic traits can be investigated employing GWAS and QTL loci mapping. GWAS examine the association between widespread genetic variants and specific traits (phenotypes), usually quantitative, in the genome, transcriptome or proteome. Currently SNPs are frequently the first choice because of their considerable genome coverage, throughput of assay and quality assurance. Although the ideas behind the use of GWAS and QTL analysis are very similar, it is important to note that GWAS tests at marker positions, whereas QTL analysis examines between markers.

Recent progress in SNP technology for marine species now facilitates genome-wide association studies (GWAS) in aquaculture to detect and pin down candidate genes for quantitative traits in various marine species. However, due to the shortage of SNP genotyping arrays, the first GWAS for quantitative traits in fish, for example, have only recently been published. This approach has been used to identify numerous genetic loci that are associated with diseases and important for marker-assisted selection programs (Rodríguez-Ramilo et al., 2007; Ribas et al., 2013). Nevertheless, the number of genetic and biochemical processes that have been identified to explain diseases and immunity has not increased much.

While QTL mapping is considered a powerful method to identify regions of the genome that co-segregate with a given trait (F2 populations), a limitation is that only allelic diversity that segregates between the parents of the particular F2 cross can be assayed. In GWAS, on the other hand, after identifying the phenotypes of interest, it provides insights into the genetic architecture of the trait, allowing informed choice for QTL analysis and suggesting candidates for mutagenesis and transgenics. Given the most recent advances in high throughput genotyping technologies, GWAS may also stimulate the efficiency of breeding and selection in aquaculture. Moreover, epigenetic features (such as methyl at ion and histone deacetylation) will soon be expanded in GWAS.

The potentially negative impact on the gene pool of wild fish resulting from the unintended introduction of farmed fish can be addressed through the use of SNPs and microarrays. SNPs are now the principal tools for monitoring the interactions between natural and cultured fish populations. Providing expression information for thousands of genes simultaneously, DNA microarrays are having a profound impact on functional genomics research in marine organisms, including Atlantic salmon and common carp (Rise et al., 2004). More recently NGS methods are being used to identify markers useful for population screening of variations in wild and farmed fishes (Vera et al., 2013). Similarly, emerging NGS methods have been employed to detect mislabeling and prevent species substitution on the commercial market in a variety of settings and in a range of products. For instance, pyrosequencing has been successfully used in high-throughput identification of seafood species in different products and in species-specific PCR assays or microarrays. Furthermore, coupling DNA barcoding with high-throughput NGS

technologies is currently being implemented in biomonitoring programs and conservation studies (Hajibabaei et al., 2012).

Advances in high-throughput proteomics now make it possible to compare protein content between complex biological systems. Host-pathogen interactions, including pathogenicity and virulence, can now be explored through a global proteomic approach leading to the identification of new vaccine targets and novel therapeutics in marine aquaculture. In shrimp (*Penaeus vannamei*), a two-dimensional gel electrophoresis (2-DE) proteomics approach was used to examine crustacean molecular responses and variation of protein content in hemocytes during Taura syndrome virus (TSV) infection (Chongsatja et al., 2007). The same technique was applied in another shrimp, *Penaeus monodon*, to investigate shrimp immunity and to detect differentially expressed proteins when infected with the luminous bacterium *Vibrio harveyi*. The 2-DE patterns of the hemocyte proteins from the unchallenged and *V. harveyi*-challenged shrimps revealed the up-regulation of the expression of hemocyanin and arginine kinase, whereas proteins such as alpha-2-macroglobulin, calmodulin and 14-3-3 protein epsilon were down-regulated (Somboonwiwat et al., 2010). Using RNA interference (RNAi), the role of *Penaeus monodon* alpha-2-macroglobulin in response to bacterial infection was further characterized, concluding that this protein plays a role in the blood coagulation system, enhancing bacterial sequestering by protecting blood clots against fibrinolysis (Chaikerasitak et al., 2012). Altogether, the availability of high throughput techniques to examine responses of shrimp to bacterial infection at the transcriptional and translational level, as well as the functional expression analysis, are important to understand better immunity-related mechanisms in crustaceans and for disease control in shrimp aquaculture.

Aquaculture is a major source of food and employment worldwide; the application of genomics to this area has facilitated a systematic improvement in the management of wild stocks. If the implementation of genomic approaches is to grow in conservation genetics/genomics for aquaculture, a number of challenges must be effectively addressed.

The first major research challenge is utilizing new tools to process the massive amounts of data generated daily from full genome sequencing projects, and their integration with phenotypic data and data from other related sciences. The paucity of efficient platforms and methods to successfully mine, retrieve, process and investigate enormous amounts of genomic data and integrate them with additional information from various sources is a key enterprise for the whole scientific community. Second, considering the relevance of local populations of marine organisms due to their exceptional contribution to genetic diversity, population genomics studies on those local species need to be pursued. Because of over exploitation and damage in local environments, many native populations of fish may already be extinct; there is a pressing need to investigate the central population structure of many species of commercial interest. Third, the genomes of other less known and non-model organisms may also be critical for efficient recognition of key genes, biochemical processes and polymorphisms that may or may not become economically important.

Additionally, the use of genomics in small-scale aquaculture, which is the most prevalent dimension of aquaculture around the world, presents specific challenges

that are different from those of industrial scale aquaculture. Consequently, to favor this model which provides substantial local economic and employment benefits, governments must pursue public financing schemes and adopt enabling policies in support of small-scale farms for the sake of employment in poor rural areas.

POLICY ISSUES FOR MARINE BIOTECHNOLOGY AND GENOMICS

The prohibitive cost of equipment and training for exploration, along with insufficient policies for promoting research and innovation hamper research and development in marine genomics and post-genomics in developing countries, including those in Central America. A recently developed research and training program, the "Central American Marine Genomic Initiative", was launched to study and preserve local marine biological diversity and ecosystems in the Central American region (Huete-Perez et al., 2012). Through this initiative the researchers involved are establishing a dynamic genomic archive to study and preserve the diminishing biological diversity in the oceans along the coasts of Central America. Building the archive involves the collection, preservation, identification, documentation and storage of genome resource samples, recording GPS coordinates and sample images, designing, creating and managing databases and establishing norms for sharing data with the international community for educational and research purposes. Through the creation of the genome archive, a baseline registry of the current state of the region's marine biodiversity is being established (Huete-Pérez et al., 2012), setting the stage for new discoveries and conservation practices. Collected organisms are identified by standard taxonomic methods as well as by molecular barcoding. Because of the excessive cost of genome sequencing, transcriptome analysis may be a more immediate route to obtain ecological information. These efforts along with future genomic sequencing of the newly discovered organisms may provide new insights on fundamental features of their biology and functional ecology.

Scientific and technical initiatives such as this facilitate local knowledge sharing and are designed to advance the field of marine biotechnology in the region. However, a number of policy-level issues and challenges relating to the development of marine biotechnology and genomics for food security and biodiversity conservation need to be addressed. Policies for promoting marine biotechnology and genomics could encompass support for public education regarding these technologies, streamline the permit process, develop the scientific research base in universities, research institutes and government laboratories, provide grants for applied research, improve access to financing, ensure intellectual property protection, facilitate access to marine resources and develop a technical work force. Policies for regulation and public safety include issuance of permits, performance standards, specifying good management practices, worker safety, and environmental protection (Halvorson et al., 2009).

The timely articulation of policies relevant to marine biotechnology and genomics serves to establish commitments on research funding, intellectual property protection, regulatory framework, rules of access to marine resources and more. National policies on marine biotechnology can

also create a climate for facilitating technology transfer and commercialization of marine biotechnology products, as well as other emerging technology applications designed to help preserve and maintain the marine environment. Because of the multidisciplinary nature of marine biotechnology and genomic applications and related activities in aquaculture, bioprospecting, environmental monitoring and biodiversity research, overseeing this field may involve a wide range of government agencies and an equally diverse range of policies. While the primary goals of these policies are to protect public health and safety by minimizing risks and promoting the benefits of the technologies, policy considerations are also important in managing current or potential land use conflicts in coastal zones.

It is important to recognize both national and international efforts to establish and maintain Marine Protected Areas (MPAs), dealing with the specific challenges of developing countries. Hundreds of such areas have been established in ecologically sensitive zones with varying levels of restrictions affecting naval transportation routes, commercial fishing, resource extraction and other activities. MPAs present a unique opportunity for long-term field research on marine organisms and related habitats. For these efforts to be effective, local policy provisions must be in place to provide continuity of enforcement and objective criteria for institutional access.

CONCLUDING REMARKS

The growing availability of many marine species' genomes, along with transcriptomics and proteomics resources, have prompted a flood of research in diverse fields including ecology, biochemistry, molecular phylogenetics and biotechnology. In this article we have discussed recent practical advances in some areas of marine biology such as genetic structure and phenotypic variation, species diversification and adaptation, and how incorporating data from genomics and some post-genomics applications will benefit future work on biodiversity research and aquaculture.

Better integration of molecular and "omics"-derived tools on both academic and industrial levels needs to be achieved to strengthen selective breeding schemes in aquaculture and to design advanced monitoring tools for fisheries management. Genomics has been applied to study the structure, function and evolution of genomes of relevance to aquaculture and is laying the foundation for future large-scale studies of gene expression, protein metabolism and adaptive traits. Some examples outlined in this paper illustrate the great potential of genomics to improve aquaculture. In the case of small-scale aquaculture, however, several challenges must be overcome if genomics is to be successfully implemented. Particularly, there are significant knowledge gaps in understanding the genetics of commercially important traits in many local populations of coastal fish, as well as a lack of relevant information for improving growth, survival and disease resistance.

The importance of marine biodiversity for human societies can be appreciated both at the economic and ecological levels. At the ecological level, an astonishing variety of organisms drive Earth's biogeochemical cycles, affecting environmental conditions on local and global scales. Unfortunately, as a result of human exploitation of natural resources, environmental degradation, pollution and the spread of pathogenic and invasive species, marine ecosystems all over the world

are rapidly losing taxonomic, phylogenetic, genetic, and functional diversity (Naeem et al., 2012). Marine biodiversity contributes food, biofuels, biomaterials, genetic resources and many other cultural and economic benefits. Reduction of ocean productivity and resource availability, as well as threats to food security and degrading marine environments will have profound effects not only on the incomes of discrete communities around the globe but also on the world economy as a whole. An increasing number of studies show that protecting marine biodiversity through a variety of ecosystem services schemes brings about many economic benefits (McCook et al., 2010).

Marine organisms are of great value also for pharmaceutical, biomedical and biotechnology industries. Although the field of marine biotechnology has gradually been providing new sources of proteins and other molecules, it is through genomic techniques that it is now possible to access and understand a broader range of marine resources and associated environments.

Genome sequencing has been increasing rapidly worldwide and has been promoted through international collaborations. These programs provide high-throughput genomic approaches and bioinformatics platforms for the analysis and curation of data, seeking a better understanding of the biology of marine species and ecosystems. Two important sequencing research centers are the Joint Genome Institute of the Department of Energy and the Broad Institute, both in the USA. An ongoing initiative on functional genomics of marine species, *The Marine Genomics Project*, was developed as a clearing house to provide a pipeline to upload, preprocess, cross-reference, annotate, submit and store Expressed Sequence Tags (ESTs) and gene expression micro array data (Mckillen et al., 2005). Microbes Online (www.microbesonline.org) is a valuable resource for microbial genomes.

The new marine biodiversity initiative mentioned earlier in this article is preserving and studying the genomes of marine life in Central America. It is also contributing to capacity building and research on coastal marine biodiversity and water quality in under explored developing countries of Central America. Vouchers are stored and data is freely accessible for research in the region, while tissues and extracted DNA are permanently stored in the Central American archive for future use. DNA barcoding, a key tool employed in this initiative, provides several major assets, facilitating comparisons between data sets obtained in different laboratories, helping in cases of cryptic species and in determining genetically distinct populations. Despite known limitations of DNA barcoding, specifically for recently diverged species and for slowly evolving groups, it is considered an effective tool for biodiversity research, particularly in resource-limited laboratories.

Ongoing global advances in science and technology are helping to stimulate new public sector interest with regard to the marine environment. There is also a growing public recognition regarding marine biotechnology and genomics applications as tools for understanding the marine environment and harnessing its potential for societal benefit. However, to increase and sustain a vital marine biotechnology and genomics presence in developing countries such as those in Central America, a number of essential policy elements need to be considered. These include supportive government infrastructure, public acceptance through awareness and

involvement, a high quality globally competitive human resource pipeline, government grants for research and development, funding of high risk startup companies, a strong intellectual property system and a clear and consistent regulatory framework. In addition to providing government funding, public policy initiatives must encourage private investment in translational research, and market-driven applications must be considered.

Most of the studies reviewed here were published in the last 3-5 years. As new technological applications emerge, novel insights will be gained into key evolutionary features of population genomics, biodiversity and ecology of marine species, which will in turn yield fundamental information for marine ecosystem management, aquaculture industry and the sustainable management of our oceans.

REFERENCES

- AMEMIYA CT, ALFODI J, LEE AP, FAN S, AMEMIYA CA, PHILIPPE H, et al. (2013) The African coelacanth genome provides insights into tetrapod evolution. *Nature*, 496 (7445), 311-316.
- ASGHARIAN H, SAHAFI HH, ARDALAN AA, SHEKARRIZ S, ELAHI E (2011) Cytochrome c oxidase subunit 1 barcode data of fish of the Nayband National Park in the Persian Gulf and analysis using meta-data flag several cryptic species *Mol Ecol Resour.* 11(3):461-72. doi: 10.1111/j.1755-0998.2011.02989.x.
- BAXTER SW, DAVEY JW, JOHNSTON JS, SHELTON AM, HECKEL DG, et al. (2011) Linkage Mapping and Comparative Genomics Using Next-Generation RAD Sequencing of a Non-Model Organism. *PLoS ONE* 6(4): e19315. doi:10.1371/journal.pone.0019315.
- BOROUJERDI AF, JONES SS, BEARDEN DW (2012) NMR analysis of metabolic responses to extreme conditions of the temperature-dependent coral pathogen *Vibrio coralliilyticus*. *Lett Appl Microbiol.* 54(3):209-16.
- BOROUJERDI AF, VIZCAINO MI, MEYERS A, POLLOCK EC, HUYNH SL, SCHOCK TB, MORRIS PJ, BEARDEN DW (2009) NMR-based microbial metabolomics and the temperature-dependent coral pathogen *Vibrio coralliilyticus*. *Environ Sci Technol.* 43(20):7658-64.
- BOURRET V, KENT MP, PRIMMER CR, VASEMÄGI A, KARLSSON S, HINDAR K, MCGINNITY P, VERSPOOR E, BERNATCHEZ L, LIEN S (2013) SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*) *Mol Ecol* 22(3):532-51.
- BOWMAN S, HUBERT S, HIGGINS B, STONE C, KIMBALL J, BORZA T, BUSSEY JT, SIMPSON G, KOZERA C, CURTIS BA, HALL JR, HORI TS, FENG CY, RISE M, BOOMAN M, GAMPERL AK, TRIPPEL E, SYMONDS J, JOHNSON SC, RISE ML (2010) An integrated approach to gene discovery and marker development in Atlantic cod (*Gadus morhua*). *Mar Biotechnology* . doi: 10.1007/s10126-010-9285-z.
- BRENNAN-HANSEN S, LI J, KENT MP, BOULDING EG, DOMINIK S, DAVIDSON WS, LIEN S (2012) Chromosomal differences between European and North American Atlantic salmon discovered by linkage mapping and supported by fluorescence in situ hybridization analysis *BMC Genomics.* 13:432.
- CAWTHORN DM, STEINMAN HA, WITTHUHN RC (2011) Establishment of a mitochondrial DNA sequence database for the identification of fish species commercially available in South Africa. *Mol Ecol Resour.* (6):979-91. doi: 10.1111/j.1755-0998.2011.03039.x.
- CHAIKEERATISAK V, SOMBOONWIWAT K, TASSANAKAJON A (2012) Shrimp alpha-2-macroglobulin prevents the bacterial escape by inhibiting fibrinolysis of blood clots. *PLoS One.* 7(10):e47384. doi: 10.1371/journal.pone.0047384. Epub 2012 Oct 17.
- CHONGSATJA PO, BOURCHOOKARN A, LO CF, THONGBOONKERD V, KRITTANAI C (2007) Proteomic analysis of differentially expressed proteins in *Panaeus vannamei* hemocytes upon Taura syndrome virus infection *Proteomics.* 7(19):3592-601.
- CONNOR KM, GRACEY AY (2012) High-resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus* *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R103-R111.
- COPPE A, BORTOLUZZI S, MURARI G, MARINO IA, ZANE L, PAPPETTI, C (2012) Sequencing and Characterization of Striped Venus Transcriptome

- Expand Resources for Clam Fishery Genetics. *PLoS One*. 7(9):e44185. doi: 10.1371/journal.pone.0044185.
- DAVIDSON WS, KOOP BE, JONES SJ, ITURRA P, VIDAL R, MAASS A, JONASSEN I, LIEN S, OMHOLT SW (2010) Sequencing the genome of the Atlantic salmon (*Salmo salar*). *Genome Biol.*11(9):403. doi: 10.1186/gb-2010-11-9-403. Epub 2010 Sep 30.
- DE CARVALHO DC, OLIVEIRA DA, POMPEU PS, LEAL CG, OLIVEIRA C, HANNER R (2011) Deep barcode divergence in Brazilian freshwater fishes: the case of the São Francisco River basin. *Mitochondrial DNA. Suppl* 1:80-6. doi: 10.3109/19401736.2011.588214.
- DU H, BAO Z, HOU R, WANG S, SU H, YAN J, TIAN M, LI Y, WEI W, LU W, HU X, WANG S, HU J (2012) Transcriptome sequencing and characterization for the sea cucumber *Apostichopus japonicus* (Selenka, 1867). *PLoS One*. 7(3):e33311. doi: 10.1371/journal.pone.0033311.
- EDWARDS RA, RODRÍGUEZ-BRITO B, WEGLEY L, HAYNES M, BREITBART M, et al. (2006) Using pyrosequencing to shed light on deep mine microbial ecology. *BMC Genomics* 7: 57. doi: 1471-2164-7-57 .
- GAGGIOTTI OE, BEKKEVOLD D, JØRGENSEN HBH, FOLL M, CARVALHO GR, ANDRE C & RUZZANTE DE (2009) Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study. *Evolution*, 63(11), 2939-2951.
- GNERRE S, MACCALLUM I, PRZYBYLSKI D, RIBEIRO FJ, BURTON JN, WALKER BJ Y OTROS (2011) High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc. Natl Acad. Sci.*, 4 (108), 1513-1518.
- HAIJIBABAEI M, SPALL JL, SHOKRALLA S, VAN KONYNENBURG S (2012) Assessing biodiversity of a freshwater benthic macroinvertebrate community through non-destructive environmental barcoding of DNA from preservative ethanol. *BMC Ecol.* 12:28. doi: 10.1186/1472-6785-12-28.
- HALVORSON HO, JOHNSON R, BÁEZ MA, QUEZADA F (2009) Políticas Públicas para la Biotecnología Marina. Book Chapter in J.J. Paniagua Michel (ed.), Biotecnología Marina, AGT Editor, S.A.
- HEBERT PD, CYWINSKA A, BALL SL, DEWAARD JR (2003) Biological identifications through DNA barcodes. *Proc Biol Sci.* 270(1512):313-21.
- HEBERT PD, GREGORY TR (2005) The promise of DNA barcoding for taxonomy *Syst Biol.* 54(5):852-9.
- HOLMES BH, STEINKE D, WARD RD (2009) Identification of shark and fin rays using DNA barcoding. *Fisheries Research* 95: 280-288.
- HOUSTON RD, DAVEY JW, BISHOP SC, LOWE NR, MOTA-VELASCO JC, HAMILTON A, GUY DR, TINCH AE, THOMSON ML, BLAXTER ML, GHARBI K, BRON JE, TAGGART JB (2012) Characterisation of QTL-linked and genome-wide restriction site-associated DNA (RAD) markers in farmed Atlantic salmon. *BMC Genomics.* 13:244. doi: 10.1186/1471-2164-13-244.
- HUETE-PÉREZ JA, ROBERTS RJ & QUEZADA F (2012) Marine genome resource sustainability in Central America. Electronic journal of biotechnology, Vol: 15 no5. Retrieved from <http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/viewFile/v15n5-14/1524> in December 2012.
- HUETE-PÉREZ JA, MENDOZA-RAMÍREZ EN & PÁIZ-MEDINA L (2012) Genomic Biorepository of Coastal Marine Species in Estero Padre Ramos and Estero Real, Nicaragua. *Encuentro No.* 93, 6 -18.
- KIM JW, ISOBE T, RAMASWAMY BR, CHANG KH, AMANO A, MILLER TM, SIRINGAN FP, TANABE S (2011) Contamination and bioaccumulation of benzotriazole ultraviolet stabilizers in fish from Manila Bay, the Philippines using an ultra-fast liquid chromatography-tandem mass spectrometry Original. *Chemosphere.* 85, 751–758.
- LAKRA WS, VERMA MS, GOSWAMI M, LAL KK, MOHINDRA V, PUNIA P, GOPALAKRISHNAN A, SINGH KV, WARD RD, HEBERT P (2011) DNA barcoding Indian marine fishes. *Mol Ecol Resour.* (1):60-71. doi: 10.1111/j.1755-0998.2010.02894.x.
- LARSSON LC, LAIKRE L, PALM S, ANDRÉ C, CARVALHO GR AND RYMAN N (2007) Concordance of Allozyme and Microsatellite Differentiation in a Marine Fish, but Evidence of Selection at a Microsatellite Locus. *Molecular Ecology*, 16: 1135-1147. doi: 10.1111/j.1365-294X.2006.03217.
- LARSSON LC, LAIKRE L, ANDRÉ C, DAHLGREN TG, RYMAN N (2010) Temporally stable genetic structure of heavily exploited Atlantic herring (*Clupea harengus*) in Swedish waters. *Heredity* (Edinb); 104(1):40–51.
- LAMICHHANEY S, MARTÍNEZ BARRIO A, RAFATI N, SUNDSTRÖM G, RUBIN CJ, GILBERT ER, BERGLUND J, WETTERBOM A, LAIKRE L, WEBSTER MT, GRABHERR M, RYMAN N AND ANDERSSON L (2012) Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. *Proc Natl Acad Sci U S A.* Nov 20; 109(47):19345-50. doi: 10.1073/pnas.1216128109.
- LIMBORG MT, HELYAR SJ, DE BRUYN M, MARTIN TI, NIELSEN E, OGDEN R, et al. (2012) Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Mol Ecol.* 21 (15), 3686-3703.
- MCGOWEN MR, GROSSMAN, LI, WILDMAN DE Dolphin genome provides evidence for adaptive evolution of nervous system genes and a molecular rate slowdown. *Proc Biol Sci.* 279 (1743), 3643-51. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/22740643>.
- MCCOOK,LJ, AYLING T, CAPPO M, CHOAT JH, EVANS RD, et al. (2010) Adaptive management of the Great Barrier Reef: A globally significant demonstration of the benefits of networks of marine reserves. *Proceedings of the National Academy of Sciences* 107: 18278–18285.
- MCKILLEN DAVID J, CHEN YIAN A, CHEN CHUMING, MATTHEW J. JENNY, TRENT HAROLD F III, ROBALINO JAVIER, MCLEAN,DAVID C, PAUL JR, GROSS S, CHAPMAN ROBERT W, GREGORY, W WARR, ALMEIDA JONAS S (2005) Marine Genomics: A clearing-house for genomic and transcriptomics data of marine organisms. *BMC Genomics*, 6:34.
- MECKLENBURG CW, MOLLER PR, STEINKE D (2011) Biodiversity of arctic marine fishes: Taxonomy and zoogeography. *Marine Biodiversity* 41: 109-140.
- MILANO L, BABBUCCI M, PANITZ F, OGDEN R, NIELSEN RO, et al. (2011) Novel tools for conservation genomics: Comparing two high-throughput approaches for SNP discovery in the transcriptome of the European hake. *PLoS ONE* 6: e28008.
- MILLER M. (2007) Environmental metabolomics: A SWOT analysis (strengths, weaknesses, opportunities, and threats). *J. Proteome Res.*, 6, 540–545.
- NAEEM S, DUFFY JE, ZAVALETA E (2012) The functions of biological diversity in an age of extinction. *Science* 336:1401-6.
- NIELSEN EE, HANSEN MM, MELDRUP D (2006) Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): Implications for inferring population structure in non-model organisms. *Mol Ecol.*; 15:3219–3229. doi: 10.1111/j.1365-294X.2006.03025.x.
- OZEROV M, VASEMÁGI A, WENNEVIK V, NIEMELÄ E, PRUSOV S, KENT M, VÄHÄ JP (2013) Cost-effective genome-wide estimation of allele frequencies from pooled DNA in Atlantic salmon (*Salmo salar* L.). *BMC Genomics.* Jan 16;14:12. doi: 10.1186/1471-2164-14-12.
- PHELPS TJ, PALUMBO AV, BELIAEV AS (2002) Metabolomics and microarrays for improved understanding of phenotypic characteristics controlled by both genomics and environmental constraints. *Curr Opin Biotechnol* 13:20–24.
- RIBAS L, PARDO BG, FERNÁNDEZ C, ÁLVAREZ-DIÓS JA, GÓMEZ-TATO A, et al. (2013) A combined strategy involving Sanger and 454 pyrosequencing increases genomic resources to aid in the management of reproduction, disease control and genetic selection in the turbot (*Scophthalmus maximus*). *BMC Genomics.* 14:180. doi: 10.1186/1471-2164-14-180.
- RISE ML, VON SCHALBURG KR, BROWN GD, MAWER MA, DEVLIN RH, et al. (2004) Development and application of a salmonid EST database and cDNA microarray: data mining and interspecific hybridization characteristics. *Genome Research*, 14: 478-490.
- RODRÍGUEZ-RAMILO ST, FERNÁNDEZ J, TORO MA, BOUZA C, HERMIDA M, et al. (2013). Uncovering QTL for resistance and survival time to *Philasterides dicentrarchi* in turbot (*Scophthalmus maximus*). *Anim Genet.* 44(2):149-57. ROEST CH & WEISSENBAACH J (2005). Fish genomics and biology. *Genome Res* (12), 1675-82.
- RUSCH DB, HALPERN AL, SUTTON G, HEIDELBERG KB, WILLIAMSON S, SHIBU Y, et al. (2007) The sorcerer II global ocean sampling expedition: Northwest Atlantic through eastern tropical Pacific. *PLoS Biol.*; 5:e77.
- RYMAN N, LAGERCRANTZ U, ANDERSSON L, CHAKRABORTY R, & ROSENBERG R (1984) Lack of Correspondence between genetic and morphological variability patterns in Atlantic Herrin (*Clupea harengus*). *Heredity*, 21 (15), 687-704.
- SCHWALBACH MS, TRIPP HJ, STEINDLER L, SMITH DP, GIOVANNONI SJ (2009). The presence of the glycolysis operon in SAR11 genomes is positively correlated with ocean productivity. *Environ Microbiol.* Available: www.ncbi.nlm.nih.gov/pubmed/19889000.
- SINGH OV (2006) Proteomics and metabolomics: the molecular make-up of toxic aromatic pollutant bioremediation. *Proteomics* 6:5481-5492.
- SMITH DP, KITNER JB, NORBECK AD, CLAUSS TR, LIPTON MS, et al. (2010) Transcriptional and Translational Regulatory Responses to Iron Limitation in the Globally Distributed Marine Bacterium *Candidatus Pelagibacter Ubique*. *PLoS ONE* 5(5): e10487. doi:10.1371/journal.pone.0010487

- SOMBOONWIWAT K, CHAIKEERATISAK V, WANG HC, FANG LO C, TASSANAKAJON A (2010) Proteomic analysis of differentially expressed proteins in *Penaeus monodon* hemocytes after *Vibrio harveyi* infection. *Proteome Sci.* 8:39. doi: 10.1186/1477-5956-8-39.
- SOWELL SM, NORBECK AD, LIPTON MS, NICORA CD, CALLISTER SJ, et al. (2008) Proteomic analysis of stationary phase in the marine bacterium "Candidatus Pelagibacter ubique". *Appl Environ Microbiol.*; 74:4091-4100. doi: 10.1128/AEM.00599-08 .
- SOWELL SM, WILHELM LJ, NORBECK AD, LIPTON MS, NICORA CD, et al. (2009) Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. *ISME J.* 3:93-105. doi: 10.1038/ismej.2008.83 .
- TYSON GW, CHAPMAN J, HUGENHOLTZ P, ALLEN EE, RAM RJ, RICHARDSON PM, et al. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428: 37-43.
- VENTER JC, REMINGTON K, HEIDELBERG JF, et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science.*; 304:66-74.
- VERA M, ÁLVAREZ-DIOS JA, FERNÁNDEZ C, BOUZA C, VILA R, MARTÍNEZ, P (2013) Development and Validation of Single Nucleotide Polymorphisms (SNPs) Markers from Two Transcriptome 454-Runs of Turbot (*Scophthalmus maximus*) Using High-Throughput Genotyping. *Int J Mol Sci.* 14(3):5694-711. doi: 10.3390/ijms14035694.
- WANG ZD, GUO YS, LIU XM, FAN YB, LIU W (2012) DNA barcoding South China Sea fishes. *Mitochondrial DNA.* 23(5):405-10. doi: 10.3109/19401736.2012.710204.
- WEIJS L, DAS K, SIEBERT U, VAN ELK N, JAUNIAUX T, NEELS H, BLUST R, COVACI A (2009) Concentrations of chlorinated and brominated contaminants and their metabolites in serum of harbour seals and harbour porpoises. *Environ. Int.* 35, 842-850.
- WEIGT LA, BALDWIN CC, DRISKELL A, SMITH DG, ORMOS A, REYIER EA (2012) Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. *PLoS One.* 7(7):e41059.
- WILLIAMSON SJ, ALLEN LZ, LORENZI HA, FADROSH DW, BRAMI D, et al. (2012) Metagenomic Exploration of Viruses throughout the Indian Ocean. *PLoS ONE* 7(10): e42047. doi:10.1371/journal.pone.0042047.
- YAN-BO S, WEI-PING Z, HE-QUN L, DAVID IM, YONG-YI S & YA-PING Z (2013) Genome-Wide Scans for Candidate Genes Involved in the Aquatic Adaptation of Dolphins. *Genome Biol Evol.*, 5 (1), 130-139.
- ZAKAS C, SCHULT N, MCHUGH D, JONES KL & WARES JP (2012) Transcriptome Analysis and SNP Development Can Resolve Population Differentiation of *Streptosio benedicti*, a Developmentally Dimorphic Marine Annelid. *PLoS ONE* 7, doi:10.1371/journal.pone.0031613.
- ZHANG J, HANNER R (2012) Molecular approach to the identification of fish in the South China Sea. *PLoS One.* 7(2):e30621. doi: 10.1371/journal.pone.0030621
- ZHANG X, XU H, ZHANG H, GUO Y, DAI Z, CHEN X (2011) Simultaneous determination of albendazole and its metabolites in fish muscle tissue by stable isotope dilution ultra-performance liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 401, 727-734.
- ZHU M, YU X, LU J, QIAO T, ZHAO W & JIA L (2012) Earliest known coelacanth skull extends the range of anatomically modern coelacanth to the Early Devonian. *Nat Commun.* 3, 772.

