

DIVING DEEP: REVEALING THE MYSTERIES OF MARINE LIFE WITH SMRT SEQUENCING

SEQUENCE WITH CONFIDENCE



Exploring the Depths Through Genetics

Science may, at times, seem like a fishing expedition. But for those who are charting the depths of the 321 trillion gallons of water that make up Earth's seas and oceans, the endeavor to collect genetic information is particularly difficult. The magnitude of global marine species diversity is immense, with thousands of species yet to be discovered and described¹.

Luckily, the process of comprehensively characterizing the genetics of marine species is well underway. Many scientists are using PacBio® Single Molecule, Real-Time (SMRT®) Sequencing to explore the genomes and transcriptomes of a wide variety of marine species and ecosystems. These studies are already adding to our understanding of how marine species adapt and evolve, contributing to conservation efforts, and informing how we can optimize food production through efficient aquaculture (Figure 1).



Figure 1. Highly accurate long-read sequencing of genomes and transcriptomes informs all areas of marine biology.

Understanding the Genetics of Adaptation

Adaptation among marine species occurs at many scales, from longer-term evolutionary changes to shorter-term adaptations. Investigation of the molecular mechanisms associated with these changes using SMRT Sequencing is producing novel insights into the genetics of adaptation (Figure 2).

The blackfin icefish (*Chaenocephalus aceratus*) exemplifies a marine animal well adapted to extremes. This fish lives in the Antarctic and can tolerate extremely low temperatures, greatly fluctuating day lengths, and high oxygen levels.

Sequencing of the blackfin icefish genome provided information on a wide range of evolutionary, ecological, metabolic, developmental and biochemical changes that the species has undergone in its 77-million-year history². Specifically, the high-quality genome assembly showed an expansion in genes encoding enzymes that help to control cellular redox state, a massive expansion of the number of genes encoding the antifreeze glycoprotein, and the absence of crucial regulators of circadian homeostasis, all evolutionary adaptations to life in the Antarctic.

Another species well adapted to its environment is the common long-arm octopus (*Octopus minor*). Unlike its many aquatic-based relatives, *O. minor* has the ability to tolerate the rapidly changing, harsh environmental conditions of the mudflats of Northeast Asia, which undergo large fluctuations in temperature, salinity, pH, oxygen availability, wave action, and tides.

Assembly of the large *O. minor* genome revealed nearly half of its genes are composed of repeats³. Comparison of transposable elements between *O. minor* and its relative, *O. bimaculoides*, further uncovered that the mechanisms enabling *O. minor* to transition from an aquatic habitat to mudflats occurred recently and independent of the octopus's lineage. Together, these findings are helping reveal the molecular mechanisms of *O. minor*'s adaptations and providing novel insights into Cephalopoda evolution.

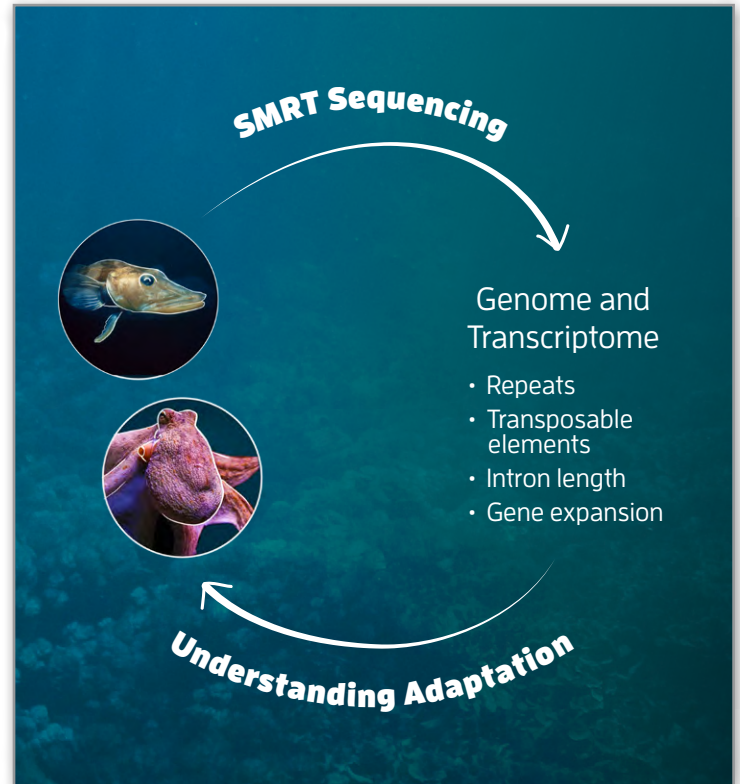


Figure 2. Sequencing the genome and transcriptome of *O. minor* and *C. aceratus* improved understanding of the mechanisms underlying their adaptation to environmental stressors.

An Omics Approach to Coral Conservation

Corals form the basis for complex ecosystems and are among the marine life most readily affected by climate change. From coral bleaching to the spread of infectious disease, scientists are using various SMRT Sequencing approaches to understand the effects of rising ocean temperatures on coral ecosystems (Figure 3).

Rice coral (*Montipora capitata*) is an environmentally robust species native to Hawaii. The generation of a high-quality nuclear genome assembly and transcriptome analysis provided information on the architecture of the coral genome, population variation, and transcriptional pathways⁴. Specifically, DNA sequencing of *M. capitata* has helped identify the major genes involved in the coral's stress response, including their expression during development, and the evolutionary history of the lineage. RNA sequencing has further uncovered the basis for its low sensitivity to ocean acidification and thermal stressors relative to other corals.

A reef system in Thailand has been studied to shed light on the role that coral-associated microbiomes play in the health of coral reefs. Scientists used full-length 16S rRNA sequencing to assess microbial diversity of *Porites lutea* coral between samples from the Gulf of Thailand and the Andaman Sea. In the first metagenomic study of its kind, they characterized geographically distinct microbial profiles between the two locations as well as a conserved community of bacteria present at both reef habitats⁵. The findings showed that in response to a natural thermal bleaching event at one location, the algal communities previously thought to be important for coping with rising temperatures were unchanged, whereas a shift in the composition of symbiotic bacterial communities was observed⁶.

Additional sequencing of the *P. lutea* transcriptome under normal and heat stress conditions led to the identification of novel genes and isoforms and the detection of alternative splice variants, which are known to be overrepresented as part of the coral stress response⁷.

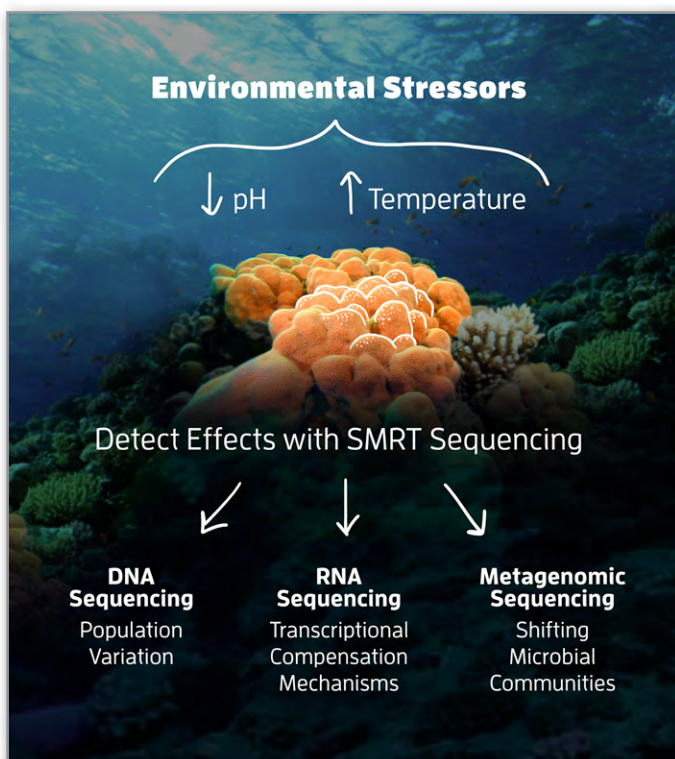


Figure 3. Investigation of coral genomes, transcriptomes, and associated microbiomes provide novel insights into coral stress response.

Together, these studies of coral and its associated microbiomes are opening up new avenues of research and providing genetic tools to assist in combatting the effects of climate change on corals worldwide.

Efficient Aquaculture – Starting with the Genes

Efficient aquaculture practices are critical to the survival of many marine species as well as to meet the demand of growing human populations. Scientists are turning to PacBio sequencing to better understand immune response, disease resistance, and sex determination.

The large and repetitive genome of the Pacific white shrimp (*Litopenaeus vannamei*) posed substantial challenges for researchers working to understand this shrimp's unusual immune system. To overcome these challenges, a targeted RNA sequencing approach was used to generate an isoform-level reference transcriptome of the immune region of the genome⁸. This allowed for identification of immune-related processes and pathways for further exploration of the innate immune and defense mechanisms of shrimp (Figure 4a). They hope to ultimately prevent and possibly treat diseases that currently cause huge losses within the shrimp industry.

Sex is an important commercial trait in farmed marine species, with males often growing much larger and more quickly than females. This has led many research groups to turn to SMRT Sequencing to decipher sex determination loci using improved genetics tools (Figure 4b).

For example, a chromosome-level genome assembly of the yellow catfish (*Pelteobagrus fulvidraco*) is enabling functional genomics studies into sex determination⁹. Similarly, an improved genome assembly of Nile tilapia (*Oreochromis niloticus*) was used to identify the long-range structure of both XY sex determination region in that species and a WZ sex determination region in a related species¹⁰.

Continued improvements to the Pacific bluefin tuna (*Thunnus orientalis*) reference genome have yielded new insights, including male-

specific variants, facilitating the development of a PCR-based assay for sex determination in this species¹¹. This assay will facilitate improved control of sex ratios in farmed bluefin tuna, as well as estimation of sex ratios in wild populations.

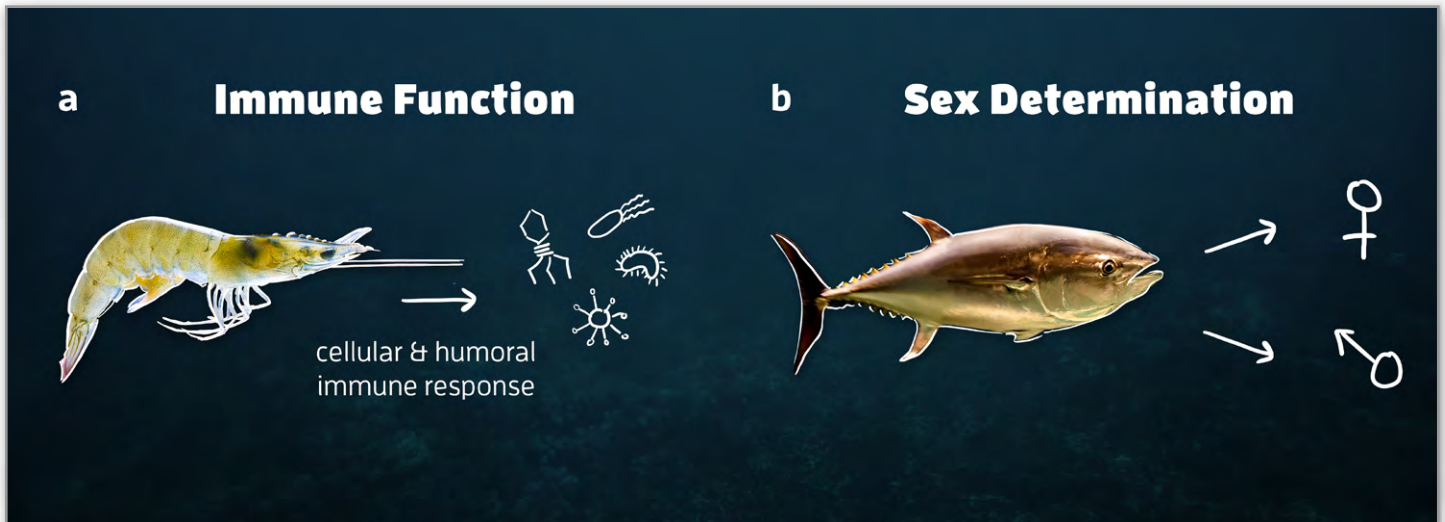


Figure 4. Exploration of the genetic basis of immune function and sex determination in aquaculture stocks.

SMRT Sequencing – A Vital Tool for Marine Biology Research

The insights gained from these and other studies using SMRT Sequencing are helping researchers to understand how marine species adapt and evolve, aid in conservation efforts, and support in the establishment of more efficient aquaculture practices. Improved understanding of the genomes and transcriptomes of marine species with highly accurate long reads is critical to addressing the most pressing challenges facing marine species and their environments.

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