CHAPTER 13

Biological information for the new blue economy and the emerging role of eDNA

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Introduction

From microbes to mammals, near shore to mid-ocean, and seafloor to seabirds, humans want and need to know about ocean life. Obvious benefits have derived from more accurate means of locating high-value wild fish for food or for protection in the interests of recreation and conservation. Fishers and dive shop operators may use the same information for opposite purposes. Surveyors have traditionally monitored sea life by observing seafood markets and trawl nets, by diving with goggles and clipboards, and more recently by deploying sonars and cameras, and sieving bits of extracellular DNA shed in seawater.

In earlier times and still today, blue life has other direct uses in addition to those as a recreational resource or as seafood for human consumption. Fish meal and oil provide feed for other forms of animal life, including carnivorous fishes raised in farms. For thousands of years, bones and shells, famously of turtles, became buttons, combs, and other household products. Shells also became ornaments and jewelry. Live animals enter the aquarium trade. Codliver oil promised good health before the word nutraceutical became popular, and other parts and forms of marine life, including algae, become pharmaceuticals and cosmetics. Accurate information about the diversity, distribution, and especially the abundance of marine life obviously has value. Humanity has misjudged abundance and exceeded sustainable exploitation of marine life many times, from abalone and cod to turtles and whales (Caswell et al., 2020).

Let's step back for a moment from catching, farming, and processing of fish and other sea life, feeding and lodging coastal tourists and visitors plus their boats, whale watching, and scuba diving. Consider five additional aspects of the new blue economy and examples of their needs for biological information (Jolly et al., 2019).

- 1. Transferring goods (surface and pipelines), power and information (cables), and people. Operators of ships want to avoid collisions with marine mammals and areas where they may disturb spawning. They want to avoid biofouling and processes that shorten the lifetime of their capital.
- 2. Building boats, piers, and wharves; dredging channels; constructing barrages and new islands; reconstructing beaches. Builders of boats want to operate quietly and with bow wave pressure that minimizes harm. Port operators want to dredge at times of acceptable environmental impact. Builders of new islands and beaches want to create favorable habitat and not destroy it.
- **3.** Pumping oil and gas, extracting wind and wave energy, and mining minerals; desalinating sea water. Operators of extractive industries want timely knowledge of their environmental impact, including spills and accidents. They want to design over a life cycle so that aged structures may be welcomed as artificial reefs.
- **4.** Disposing of wastes (sewage, brines, plastics, and radioactive and other hazardous materials; decommissioning platforms). Individuals, companies, and municipalities want to create safe and harmless waste streams that will not accumulate dangerously in the tissues of marine animals. They want to monitor microbes, including viruses, in aquatic environments that can give early warning of threats to the health of humans and other animals.
- 5. Exploring and monitoring for safety, research, and prediction; mapping and hydrography. Researchers studying changes in ocean temperature or the geology of the seafloor may use active acoustics, pinging and listening for echoes and attenuations; they should conduct their experiments at frequencies and intensities that do not cause acute or chronic harm to marine life. Mappers and explorers should search for uncharted or poorly charted ecologically and biologically significant areas. Researchers who monitor the oceans for algal blooms that may be part of natural cycles also want to warn of harm to other marine life and to spur timely beach closures. Researchers exploring new or unusual species may supply valuable model organisms for science, as the squid axon has been for more than a century.

Clearly, needs for blue biological information already range widely, and we have dreams about new value from biomedical prospecting in the

ocean. Medical and biological reasons to prospect for chemicals in the ocean do not hide in darkness. Animals such as starfish can regenerate limbs. Marine species ranging from sturgeon and whales to clams and corals live for centuries. Some animals, including eels, can generate strong electric currents. Obviously, all the marine animals, as well as algae (seaweeds) and the marine plants, thrive in salty water. Surely chemicals, compounds, and structures abound in the oceans that could make humans healthier, more youthful, and more beautiful. Regeneration, longevity, resistance to cancers, tolerance to heat and cold and to salinity and alkalinity, absence of seasonal affective disorder, and resistance to fatigue entice bioprospecting.

So far, the outcomes of bioprospecting are meager, a relatively small number of large-molecule drugs for blood cancers. Currently, US regulators have approved about a dozen compounds, and four more have entered Phase III trials, 13 Phase II, 7 Phase I (Midwestern University, 2020). What about the future of marine biopropecting?

The R&D enterprise of the pharmaceutical industry, not to mention the cosmetic industry, is immense. The size of the global pharmaceutical industry investment in R&D, about \$180 billion USD in 2018 (Makulic, 2020), tempts a belief that onshore labs are doing everything in the search for designer drugs. However, quite possibly forms of marine life can serve as filters or prefilters. While we can prospect directly for compounds, a more important strategy may be to sequence parts of the genome of the organisms whose functions impress us or undertake other kinds of genetic and molecular studies. Then we may learn of proteins or other molecules and their shapes and other attributes that contribute to the impressive functions.

When a marine product becomes lucrative, we fear the problems of scaling up to meet large and potentially global markets, what Jouffray et al. (2020) call the "Blue Acceleration." The future wealth of the oceans cannot come from direct processing of sea cucumbers or starfish for their precious essences. The scale of life in the oceans could not sustain the sponge industry, nor the demand for tortoiseshell. Moreover, many desirable processes surely involve several genes and their products. While such polygenic products may also scale better through onshore synthesis, crucial clues live in the oceans. Science has barely begun to look at the 250,000 known species of marine life to classify them systematically by useful attributes.

The restraint or regulation of commercial activities in the ocean is premised on reducing collateral damage to marine life and other noncommercial interests (protection for the sake of protection) by shipping, dredging, drilling, waste disposal, research, and fishing itself. Regulations

mandate monitoring of pollution levels (e.g., bacterial concentrations at popular beaches, leaks at oil platforms, plastic flotsam) and consequent effects on sea life (e.g., injuries to whales, coral bleaching). While these monitoring activities restrain trade or tax the new blue economy, they are needed to avoid situations where profits are improperly privatized and high costs externalized. An industry of environmental consultancies exists to carry out these studies.

Environmental DNA: new mode of monitoring marine life

As we have suggested, means of collecting biological information are changing, and those entering the field or remaining in it may soon find themselves with a different workload and working environment. What has been a mostly manual undertaking in the salt spray of the field may increasingly resemble space physics, where engineers design and launch probes that feed data to deskbound analysts. To share the example with which we are most familiar, the processing and profitable analysis of extracellular or environmental DNA (eDNA) requires different skills than might be found aboard a typical trawl vessel. Turning deckhands and experts who gauge the age of a fish from its ear bone into crew members of teams alongside molecular biologists and data scientists will challenge the maritime community.

Monitoring the location and movement of marine wildlife has been cumbersome and costly. The advent of techniques to isolate, amplify, and analyze eDNA can reduce the cost in time and money for oil and gas operators to monitor their operations, port operators to dredge, fisheries to open and close, coastal developers to assure the public that their works operate responsibly, researchers to explore and track biological changes, and governments to nominate areas for protection and restoration and to evaluate their success (Stoeckle and Ausubel, 2019).

Typically, in the United States and many other nations, maritime operators contract out evaluations of the impact of their activities on wildlife to an environmental engineering or consulting firm. In some cases, these consultants gather original data (from aerial surveys, for instance). In others, they compile and synthesize data from public agencies. These public agencies (arms of NOAA, for example), rely on reporting from commercial fishing vessels through vessel monitoring systems (VMS) or vessel trip reports (VTR) and "fisheries-independent" data from trawl surveys by governments, universities, and other nonprofits. Trawls have been a mainstay of ocean-life monitoring since at least the 1960s; NOAA's

Northeast Fisheries Science Center began theirs in 1963. The spatial coverage of the resulting data—when extrapolated with extensive modeling—is excellent, but the spatial resolution is poor, typically displayed in blocks of 100 square miles (260 square kilometers) apiece. Genomics offer the eventual prospect of precision, accuracy, and coverage, with the information available anytime, anywhere, and fast, a trio emblematic of the new blue economy.

In the interim, significant opportunities exist to augment trawls with water sampling and eDNA analysis, including when trawls are canceled or reduced owing to storms, equipment failures, costs, or crises such as COVID-19. eDNA promises much greater temporal resolution and precision due to the short time and therefore short distance over which shed DNA is thought to degrade and due to the detection of DNA from fish that would slip through or otherwise evade a trawl net. A time series for 11 species off the coast of New Jersey monitored simply by collecting 1 liter of water from the shore matches well with costly trawls of tens of millions of liters (Fig. 13.1).

Accordingly, many universities and government organizations have begun incorporating eDNA collection and analysis into their oceanographic research, and companies (Jonah Ventures, Mr. DNA, NatureMetrics, Spygen) have begun selling eDNA testing as a service. Typically, the customer mails in a filter through which a small quantity of freshwater or saltwater has been passed, DNA is then extracted from sediment on the filter, and the DNA is then analyzed in a standard next-generation-sequencing and bioinformatics pipeline. DNA identifications are usually based on existing reference sequences (such as those of the mitochondrial

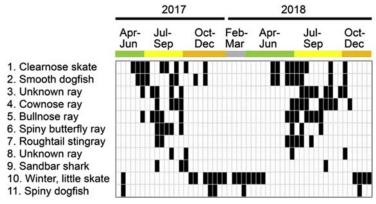


Figure 13.1 Fish species detected through water sampling (eDNA) at Barnegat Light, NJ, by month of appearance (Stoeckle et al., 2020).

12S rRNA gene) in GenBank. In the continental US exclusive economic zone (EEZ) relatively good coverage (>80%) of resident fish and marine mammals makes this process straightforward. eDNA collection can quickly identify a single species of interest (e.g., a marketable fish or an invasive species) with single-species assays (Stoeckle et al., 2018) or search for a class of life (e.g., vertebrates or molluscs) with metabarcoding (Stoeckle et al., 2017) or support certain customer tasks: to characterize completely a parcel of ocean over an appropriate period (e.g., a port over several seasons).

Actual collection and filtration of water still demand labor, including dipping liter containers at the surface, or Nansen/Niskin bottles at depth. Expansion of eDNA's use will require a streamlining of these "field services," particularly to characterize larger spatial areas or volumes of water. While eDNA produces excellent data on the presence and probably on abundance for a specific location, depth, time, and cost (an estimated \$25–50/sample), traditional means complement it with valuable information on age structure and health of sampled populations.

eDNA need not process the volumes of water currently flowing through trawl nets around the world. Work underway will clarify how near to one another in space and time these water samples must be taken to characterize accurately a given surface area or parcel of ocean—at least comparably to trawls or sufficient to satisfy reporting regulations as currently worded. Trawls have a very high throughput of water and must actually catch the fish; eDNA must simply capture the shed genetic material trailing a fish or school of fish. Currently sampling is strictly on a point-by-point basis, with more expansive extrapolations or assumptions necessary (vs. trawling) to deem an area of ocean properly "surveyed." In comparisons, eDNA captured 90% of the species netted by a trawl and detected more unique species than the paired trawls.

A New York—area energy project was the subject of the report "New York State Offshore Wind Master Plan: Charting a Course to 2400 MW of Offshore Wind Energy" (New York State Energy Research and Development Authority NYSERDA, 2017). Its fish and fisheries component, Chapter J (Energy and Environment Engineering, 2017), was prepared for the New York State Energy Research and Development Authority by Ecology and Environment Engineering (NASDAQ: EEI), a \$50 million/year consultancy based outside Buffalo. In addition, the companies who ultimately intend to operate in the six lease areas (Equinor, Orsted, Deepwater Wind, Vineyard Wind, and US Wind) will contract their own studies. At present, we are aware of only one company that has

delivered end-to-end fish stock assessment by eDNA using existing technologies: Battelle, whose team members collected and analyzed eDNA from deep water off the North Slope of Alaska for Shell (Stever, 2015).

Indeed, to advance use of biological information in the new blue economy, the oil and gas industry helped inaugurate the International Workshop on Environmental Genomics in 2016. As a member of its trade group, the International Association of Oil and Gas Producers formed an industry subgroup, the International Consortium on Environmental Genomics (ICE-G), which launched in 2019 the Environmental Genomics Research Joint Industry Program (JIP) to pool resources of energy companies (including Chevron, Eni, Equinor, ExxonMobil, Hess, Shell, and Total) to develop protocols and encourage establishment of regulations governing eDNA for environmental monitoring during 2020–23 (IOGP, 2020). The JIP considers proposals on eDNA topics applicable to all phases of the offshore energy industry, from exploration to decommissioning.

While eDNA research has advanced rapidly over the past decade (Tabarlet, 2018), eDNA is in its infancy as a tool for regulators and commercial entities of the new blue economy. Its value proposition (efficiency + precision) awaits regulatory validation and broad commercial endorsement. The cost of incumbent technology such as conventional trawl surveys is somewhat obscured in the budgets of public agencies, or within the broad offerings of environmental consultants, making marketsizing and efficiency comparisons difficult. NOAA's "Fisheries Data Collections, Surveys, and Assessments' budget was \$155 million in FY18, while their fishermen's contingency fund had \$349 million set aside in FY 18 to compensate fishermen for the effects of oil and gas activity in the US EEZ. The National Marine Fisheries Service manages 474 marine and anadromous (river dwelling) fish stocks (including 230 with commercial value) within the US EEZ as well as invertebrates, sea turtles, marine mammals, and other marine and coastal species and their habitats, all of whom are assessed by what NOAA calls its ABC Method: parameters including abundance, biology, and catch are fed into a statistical model, which produces a stock assessment.

eDNA's employment in these stock assessments would require fewer personnel and less time at sea and necessitate different or retrained personnel and new kinds of equipment on shore. Firms including Biomeme, ANDE, Minion, and Illumina, and counterparts in Europe and Asia, are introducing portable DNA preparation and analysis hardware. As an emerging component of the new blue economy, we could envision a potential

commercial operator developing a strategy using ships of opportunity—akin to the VMS and VTR fisheries reporting that already exists—to collect, filter, and store seawater eDNA samples over broad ocean routes to determine cost, reliability, chain of custody, and repeatability of data gathering, analysis, transmission, and archiving.

Companies could cater to monitoring needs with relatively localized operations at sea ("genomic weather stations") and perhaps even provide basin-scale (eventually global-scale) maps and databases with near-real-time updates of the presence and abundance of key marine species of scientific, ecological, and commercial value. Successful market penetration will benefit from advances in water handling at larger scales (tens and hundreds of liters), in some ways reminiscent of the continuous plankton recorders that have been employed since the mid-20th century, or even autonomous water collection and filtration, as demonstrated by MBARI and Proactive. New regulations must explicitly allow or require eDNA monitoring (vs. trawling) or establish that the two methods are comparable in the context of environmental impact submissions. Public attitudes may also play a part, as eDNA helpfully avoids the bycatch and habitat destruction of trawling.

Eventually one could imagine an efficient, end-to-end application of eDNA, if the water collection can be automated and the isolation, amplification, sequencing, and bioinformatics performed by lower-cost, "one-touch" machines that do not require PhD-level personnel in shore-based scientific facilities. Priorities to realize the potential of eDNA for the new blue economy include:

- 1. Building up the reference library of species.
- **2.** Developing the technology of remote sampling (e.g., drone and marine robots that could collect and filter water and save the filters).
- 3. Integrating, and speeding and lowering the cost, of automated processing of samples (e.g., development of a single "machine" that could trap sediment containing DNA, extract the DNA from the sample, do the further steps such as sequencing, and then email the sequence file to the user).
- **4.** Improving bioinformatic and big data storage in relation to eDNA; e.g., further improve the relevance and ease of use of programs such as the DNA Subway of Cold Spring Harbor Laboratory (2020) and also develop sites or enterprises that would aggregate and store eDNA information and results so that these could be integrated to reveal larger patterns over space and time.

Summary

eDNA and companion developments in genomics can transform biological information for microbes to mammals, near shore to mid-ocean, and seafloor to sea birds. Combined with advances in tagging and tracking and in acoustics, we can foresee in a decade or two more reliable, more comprehensive, and more timely biological information about the new blue economy. This information will tell us about ecosystems as well as species. Concurrently we hope that researchers unlock some of the secrets of marine life for regenerative medicine and other fields where breakthroughs would bring enormous benefit. The key is not ships and other expensive platforms, but autonomy, sensors, analytics, and the other technologies that characterize the emergent new blue economy.

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Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-821431-2

For information on all Elsevier publications visit our website at https://www.elsevier.com/books-and-journals

Publisher: Candice Janco

Acquisitions Editor: Louisa Munro Editorial Project Manager: Grace Lander

Production Project Manager: Sujatha Thirugnana Sambandam

Cover Designer: Mark Rogers

