Molecular Based In-Situ, Remote Sensor for Detecting Microbial Contaminants

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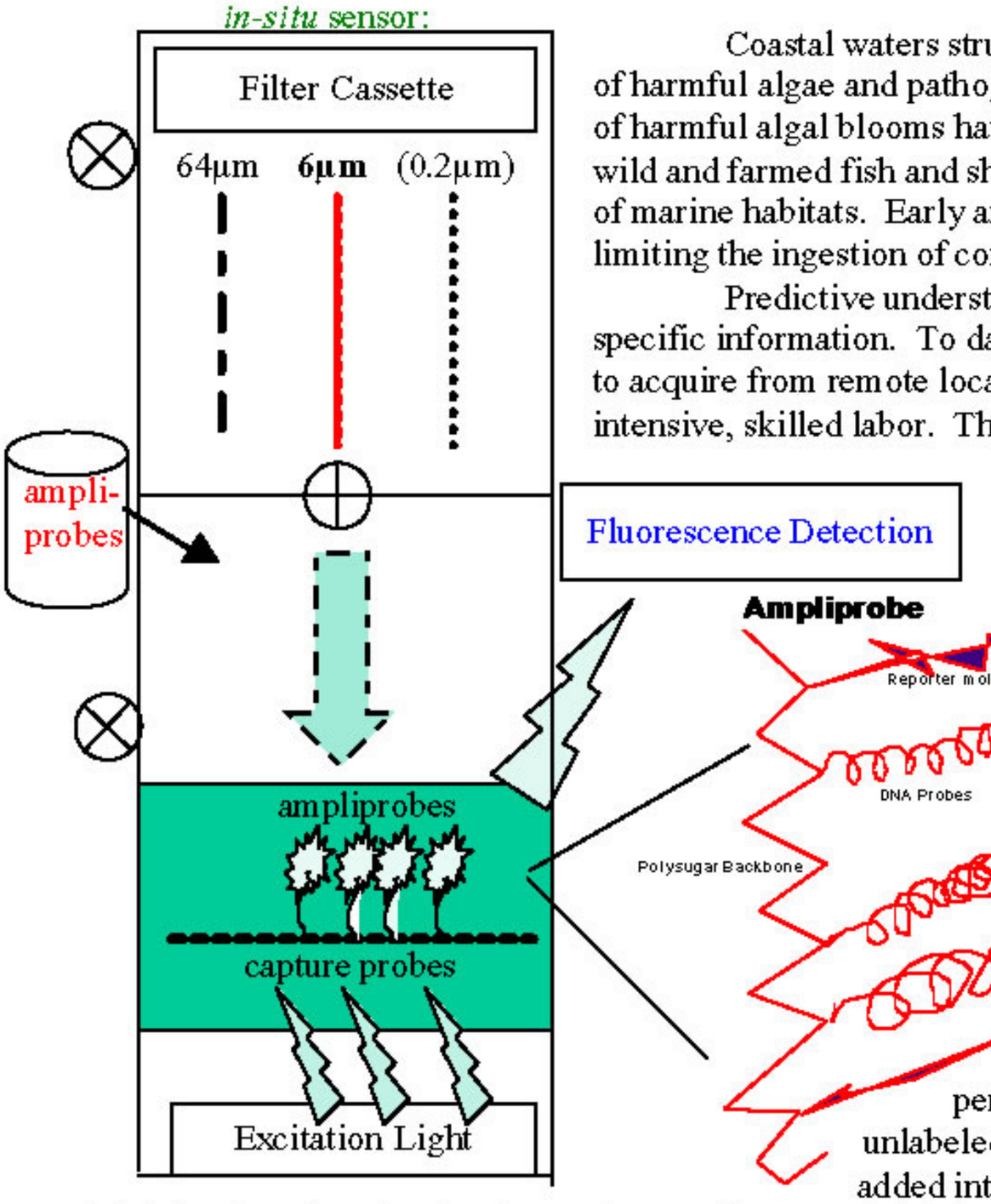

Coastal waters struggle with a number of microbial contaminants including blooms of harmful algae and pathogens derived from sewage waste. The frequency and occurrence of harmful algal blooms have increased. Consequences of such blooms include mortality of wild and farmed fish and shellfish, illness and death of humans and animals, and alteration of marine habitats. Early and accurate warnings of contamination could protect health by limiting the ingestion of contaminated fish and shellfish.

Predictive understanding of many ecological processes often requires species-specific information. To date, species-specific information has been difficult or impossible to acquire from remote locations. Furthermore, even manual detection of species requires intensive, skilled labor. The goal of this project is to capitalize on advances in biotechnology in order to improve water quality monitoring. Specifically, we propose to develop an *in-situ* measurement system based on molecular indicators in order to relay the abundance of microbial contaminants in near real-time.

Initially, we propose to work with the dinoflagellate *Karenia brevis*. *K. brevis* is an attractive organism with which to demonstrate proof-of-concept for the proposed technology because it is unicellular, unarmored (naked), and comparatively fragile. It is therefore amenable to reproducible, remotely controlled nucleic acid extraction. Species-specific probes will be designed based on sequence of the large subunit of the rDNA.

The proposed *in situ* sensor would use molecular indicators based on ampliprobe technology (AccuDx, patent pending). The ampliprobe system consists of an immobilized, unlabeled capture probe and a fluorescent-labeled signal probe that is added into solution during hybridization. The signal probes allow direct hybridization of probe-signal complexes without steric hindrance. The signal probes are unique in that they contain 100's of fluorescent labels, greatly reducing the detection limit for the target. The structure of the signal probe consists of a polysugar backbone. The branches of the backbone contain signal chains and probe chains. The signal chain consists of polylysine labeled with fluorescent molecules. The probe chain consists of biotinylated target-specific oligomers attached to the backbone via streptaviden. The advantages of this proprietary system include the elimination of target amplification (PCR), uniform melting point temperatures for probes (which is essential for micro-arrays), and the long-term stability of the signal probes. With the advantages that ampliprobes bring, it should now be possible to harness biotechnology to document the population dynamics of microbial contaminants in near real-time.

The hybridization reaction would be performed based on the parameters found during optimization. Optimization will proceed in series of steps that will begin with well-defined laboratory samples and systematically expand to complex environmental samples. The optimization procedure will be performed using a standard 96-well plate. The well-plate assay in itself will produce a useful technology for manual detection of microbial contaminants.

The sensor will begin by filtering a seawater sample through a prefilter and onto the collection filter. For *K. brevis*, the collection filter will be a 6-µm filter. Filter material will be wound on a cassette-type transport and advanced between samples. This approach will avoid intersample contamination and cumulative clogging of the filters. The entrance valve will be closed, a small amount of detergent will be added, and the sample will be subjected to sonification. The nucleic acid and cell fragment mixture will be drawn into the 1-ml reaction chamber at the bottom of the inverted cone shaped sonification chamber using a weak electric field, and the signal probes will be added. The immobilized capture probes will be on a membrane wound on a cassette, which will be housed at the bottom of the chamber. The membrane in the cassette will be advance prior to collecting a new sample, insuring that fresh probes are available for each hybridization reaction. Unhybridized probe will be washed into a collection reservoir. The fluorescent signal will be collected using fiber optics and routed to a miniature spectrophotometer. Background environmental fluorescence will be subtracted by averaging the signals coming from the device during the time points prior to hybridization events. A quantitative reaction will be achieved through the use of a reference panel to calibrate the spectrophotometer and the use of an internal reference standard by which the signal of each hybridization event can be normalized. To conclude the sample sequence, the reaction chamber and sonification chamber will be flushed. Prior to taking a new sample, the system would be flushed again, and the cassette transports will be advanced to position clean pre-filters, sample filters, and capture probes. The operation will be controlled with one or more embedded microprocessors to oversee sampling, reagent additions, sonification, fluorescence measurement, flushing, and system reset in addition to communicating the system telemetry provided by the integrated station main electronics unit. The filter and capture probe cassettes as well as the reagent reservoirs will be easily exchangeable, and field maintenance will primarily consist of a simple, rapid exchange of plug-in modules. The sensor could be deployed on buoys or shipboard.

The *in situ* sensor would provide remote, species-specific quantification of microbial contaminants, greatly improving present assay methods and improving the ability to protect human health and economic interests.