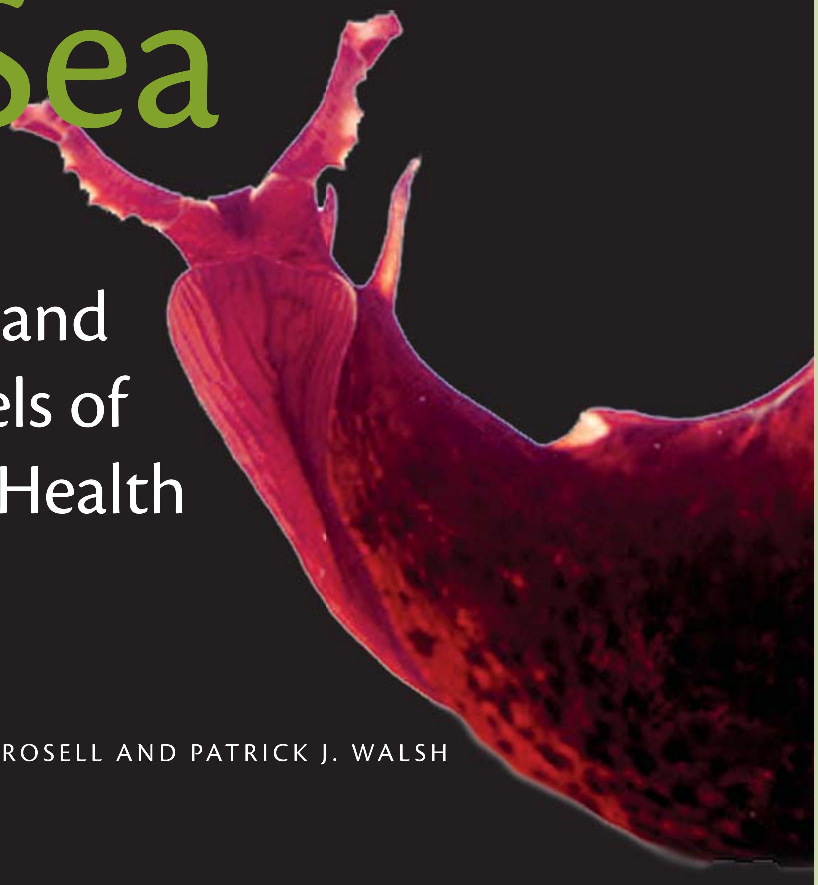
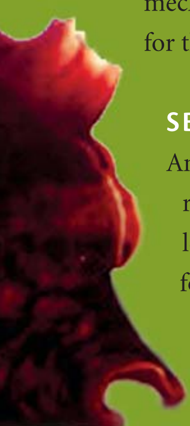
Benefits from the Sea

Sentinel Species and Animal Models of Human Health



BY MARTIN GROSELL AND PATRICK J. WALSH

Much of the current interest in the impact of the oceans on human health can be traced historically back to the earliest days of the fields of Experimental Marine Biology and Comparative Physiology. In Experimental Marine Biology, understanding of how anthropogenic pollutants impact aquatic organisms (aquatic toxicology) can be seen as a natural extension of early efforts to understand how variations in so-called "natural" environmental factors (e.g., oxygen, salinity) affected the physiology, reproductive capacity, and survivorship of marine organisms. Likewise, Comparative Physiology seeks to unify understanding of common physiological processes (e.g., osmoregulation, respiration) across many taxa; in this unification, it is not surprising that knowledge arises that is relevant to understanding human health. In this quest for unification, Comparative Physiology also gave birth to an experimental approach that has come to be known as the "August Krogh Principle," which states: "For many problems, there is an animal on which it can be most conveniently studied." While the above quote is attributed to the Danish physiologist August Krogh, it is clear that physiologists preceding Krogh applied the principle even earlier (Jørgensen, 2001). Regardless of its exact origin, we believe that the approach applies today both in the choice of sentinel species for tracking marine pollutants and understanding the mechanisms of their actions, and in the use of marine models for the efficient and insightful study of human diseases.



SENTINEL SPECIES

Anthropogenic activities continue to degrade aquatic environments, threatening a very important resource for excellent model organisms (as discussed below) and a significant food source. Harmful activities include, but are not restricted to, the release of man-made chemicals, enhanced release of naturally occurring elements (nutrients and trace metals), alterations in water flow and salinity, and increased erosion of lake and river banks and coastal zones. The dominant human interaction with the aquatic environment occurs in freshwaters and along coastal zones where urbanization is most prevalent. Urbanization in many cases directly impacts the land-to-water interface and brings the problem of solid-waste disposal (in some cases combustion) and wastewater release.

A number of marine organisms have been explored as sentinel species indicative of environmental health. A comprehensive summary of the utility of these organisms is beyond the scope of this text; however, the following discussion includes some of the more prominent indicator organisms.

Bivalve Mollusks

Bivalve mollusks are found worldwide. They are sedentary and, in many cases, filter-feeding. Bivalves make excellent sentinels because they integrate exposure over time and large water-mass volumes. In addition, many bivalves are resilient to natural and anthropogenic environmental fluctuations and display very high bioaccumulation of pollutants. A wide range of trace metals readily accumulate in oyster tissues to concentrations far exceeding those of most other organisms. The phenomenon "green oysters" was first documented in the late 18th century (O'Shaughnessy, 1866); the intense green coloration was later demonstrated to be correlated with highly elevated copper concentrations (Boyce and Herdman, 1898; Orton, 1923). Oysters and other bivalves detoxify accumulated copper and other metals by inducible metal-binding proteins (metallothioneins) and by sequestration in metal-rich granules, possibly accounting for the often high tolerance to elevated environmental metal concentrations and high bioaccumulation (Viarengo et al., 1980, 1981; George and Viarengo, 1985; Langston et al., 1998).

As major components of crude oil and derivatives of fossil-fuel combustion, polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. Many PAHs are carcinogenic, mutagenic, and immunosuppresive (Ladics and White, 1996; Wootton et al., 2003). Compared to fish and other vertebrates, bivalves and other marine mollusks have very limited ability to metabolize PAHs and thus also often display high bioaccumulation of these compounds (Boehm and Farrington, 1984; James, 1989; Neff, 2002; Oros and Ross, 2005). Polychlorinated biphenyls (PCBs), used principally in insulating fluids although banned in western countries, are of constant environmental and human-health concern due to their ubiquity, persistence, bioaccumulation, and toxicity (Otchere, 2005). Like PAHs, PCBs are poorly metabolized by bivalves, making these invertebrates strong sentinels.

Bivalve mollusks thus offer utility as sentinels for the assessment of environmental degradation due to many classes of toxicants and have for this reason been used extensively, as exemplified by the U.S. Mussel Watch Project (Lauenstein and Daskalakis, 1998; O'Connor, 1998). However, substantial bioaccumulation of many compounds also presents an inherent risk of exposure to higher trophic levels, including to humans. Indeed, paralytic shellfish poisoning (PSP), which arguably is the most serious public health natural toxin threat from shellfish consumption (Chen and Chou, 1998; Ammons et al., 2001; Morono et al., 2001), is directly related to filter feeding and bioaccumulation of the toxins in bivalves. Saxitoxins, the cause of PSP, are produced by dinoflagellates and cyanobacteria (Onodera et al., 1997; Lagos et al., 1999; Velzeboer et al., 2000; Ferreira et al., 2001; Papageorgiou et al., 2005), and bioaccumulate in many bivalves feeding on these phytoplanktonic organisms.

Fish as Indicators of Endocrine Disruption

Endocrine-disrupting chemicals (EDCs) have received major worldwide attention and may be affecting the reproductive output of wildlife populations (Nash et al., 2004) and possibly humans (Colborn and Clement, 1992; Ohtake et al., 2003). A long list of compounds potentially disrupts steroidogenesis, but perhaps of greatest concern are the steroidal estrogens released primarily from wastewater treatment plants (Nash et al., 2004). These potent estrogens include the natural estradiol and estrone, and the synthetic estrogen EE2, a component of contraceptive pills (Desbrow et al., 1998; Tyler and Routledge, 1998). Oviparous vertebrates, especially fish, are very useful sentinels for xenoestrogens and estrogens in the environment. The normally female-specific yolk protein precursor, vitellogenin (Vtg), occurs at abnormally high levels in male fish exposed to estrogenic compounds (Figure 1), and it is perhaps the single most convincing biomarker examined for any contaminant to date. Reported correlations between Vtg or Vtg mRNA skewed sex ratios, prevalence of intersexuality, and reduced fertilizing capacity of gametes in fish attest to the utility of Vtg and fish as a biomarker and sentinels, respectively (Jobling et al., 1998; Larsson et al., 2000; Jobling et al., 2002). A recent study using the well-established developmental model organism, the zebrafish, demonstrated that these biochemical effects and individual-level effects are likely to also have population-level impacts on wildlife during chronic exposure to low levels of estrogenic compounds (Nash et al., 2004).

Marine Mammals

Marine mammals are not often used as sentinels or biomarkers, possibly due to scarce abundance and the fact that many are threatened or endangered. However, as long-lived organisms and top predators in complex and diverse ecosystems, they offer an ecological integration over

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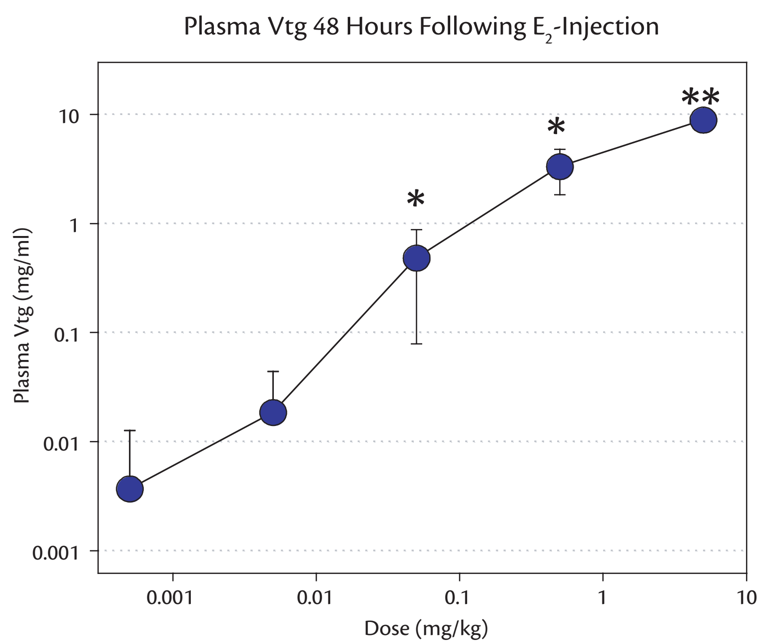

Figure 1. Characteristic response of circulating plasma egg yolk protein precursor (vitellogenin = Vtg) levels determined by ELISA in male largemouth bass 48 hours after injection with various doses of estradiol (E2). Vtg is an excellent biomarker for excess steroidal estrogens in wastewater. Chronic exposure to estrogens can have an adverse impact on fish populations. (From Bowman et al., 2002, with permission.)

time, making them potentially valuable sentinels for environmental health (see case study by Bossart, this issue).

The Exxon Valdez oil spill in 1989 in the Prince William Sound (Alaska) offers an example of the usefulness of a marine mammal, the sea otter (Figure 2), in assessment of environmental health. This tragic event resulted in acute mass mortality, and perhaps even more concerning, in retarded rates of sea otter recovery with suppressed population numbers more than ten years after the oil spill (Peterson et al., 2003). Survival in years after the spill was generally lower and included elevated mortality in animals born after the spill, demonstrating contributions from chronic exposure (Monson et al., 2000). Indeed, consistent PAH exposure ten years after the spill has been confirmed by the detection of higher levels of the detoxifying enzyme CYP1A in individuals from affected areas (Bodkin et al., 2002). Population recovery rates in northern Prince William Sound were not retarded by the abundance of sea otter prey (mainly bivalve molluscs), but have been attributed to PAH bioaccumulation in the bivalves (Peterson, 2001). Recovery of oiled mussel beds in the area is estimated to require up to three decades (Carls et al., 2001). Otters feeding on the PAH-containing bivalves continue to experience chronic exposure, probably accounting at least in part for the retarded population recovery.

Figure 2. Photograph of a sea otter, a top-level carnivore and potential environmental sentinel (photo by Daniel P. Costa, University of California, Santa Cruz, with permission).

AQUATIC ANIMAL MODELS FOR HUMAN HEALTH

There are numerous instances where aquatic animals have contributed to our basic understanding of cell biology, physiology, biochemistry, and molecular biology, as well as directly to better insights into human-disease states. A review can be found in the 1999 National Academy of Sciences report, *From Monsoons to Microbes: Understanding the Ocean's Role in Human Health* (Fenical et al., 1999). We have selected a few examples from this review and subsequent/additional literature to both illustrate the August Krogh Principle and to show the direct benefits to humans. Before turning to specific examples, however, there are a number of attributes that apply generally to aquatic organisms, or at least to many aquatic species, making them suitable subjects for experimental study (Table 1). Importantly, in addition to specific experimental advantages of individual species, two key overall advantages are generally lower cost and lower social concerns compared to mammalian counterparts.

The Contributions of Squid and Sea Hares to the Neurosciences

It is not an overstatement to say that much of what we know today about how the human nervous system functions is based on the fundamental discoveries regarding the action potential in squid (*Loligo sp.*) neurons (Hodgkin and Huxley, 1939), and on how cells learn in the ganglia of sea hares (*Aplysia californica*) (Kandel, 2004). Why were these species chosen as experimental models? In these cases, the Krogh Principle is applied primarily by virtue of size and numbers. For squid studies, the glass microelectrodes of the time (in the 1930s) were rather cumbersome by today's standards, and therefore the large diameter (0.5 to 1 mm) squid giant axon was simply a larger target for intracellular recording. The evolutionary rationale for the large diameter of the squid giant axon is to be

Table 1. General attributes of many aquatic species making them attractive experimental models and sentinel species.

Trait	Advantage
High Species Diversity	Limitless choice of native environments and susceptibility or resistance to toxicants/environmental variables.
High Fecundity/ Rapid Development	Ultimately amenable to large scale breeding in captivity to potentially lower costs compared to mammalian models, and to reduce environmental impact of scientific collecting.
Large Egg Size	Facilitates genetic manipulation and production of transgenics.
Non-keratinized Skin, Gills	Simple natural intensive exposure system where organism can be bathed directly in toxicants or other test solutions.
Non-Mammalian	Greater Social Acceptance; in the case of invertebrates in the USA, no current Internal Animal Care and Use Committee (IACUC) issues.
Variable Body Temperature	Allows use of temperature as a realistic experimental variable.

able to rapidly signal mantle contraction for propulsion, and the non-myelinated (non-"insulated") invertebrate neurons can achieve rapid conduction by the enhanced conductive properties of the large diameter; by comparison, mammalian neurons achieve rapid conductance with smaller-diameter neurons by myelination and saltatory conductance (Withers, 1992).

Three main factors contributed to our understanding of memory and learning using *Aplysia*: (1) the small number of ganglia (ten) and the relatively small number of neurons in them (~2000), (2) the ability to re-identify neurons enabled the linkage of particular neurons to specific simple behaviors, and (3) the large diameter of the neurons (up to 1 mm) enabled easy access for microelectrodes as well as the dissection of specific single cells for cell cultures (Figure 3). With this species, Kandel and others were able to link specific behaviors (e.g., gill withdrawal) to specific neurons, to condition these behaviors, and to further study cell-cell communication in culture, ultimately establishing that neurons learn by changes in gene expression.

Notably, there are National Institutes of Health-sponsored culture facilities for selected species of squid and for *Aplysia californica* (for more information see http://www.utmb.edu/nrcc/LiveAnimRes.htm and http://www.rsmas.miami.edu/groups/sea-hares/) that minimize the environmental impact of taking wild animals from ecosystems for research. Furthermore, defined culture conditions for experimental marine organisms offer researchers animals that are consistent in their backgrounds with respect to diet, temperature, photoperiod, and even ultimately genetic background, and provide representatives of all life stages (Figure 3).

There are numerous similar examples of how marine organisms have contributed to similar *basic* understanding of cell biology and systems physiology that arguably underpin much of our mechanistic understanding of human physiology and health. Yet, there are even more direct examples of impacts on human health.

Rainbow Trout Model of Aflatoxin Carcinogenesis

In this next example, we broaden our definition of "seas" slightly to include freshwater/anadromous species. Beginning in the 1960s, researchers in an Oregon State University group, which was to eventually become an National Institute of Environmental Health Sciences (NIEHS) Marine and Freshwater Biomedical Sciences Center, were able to link higher levels of liver tumors in "control" rainbow trout to the carcinogen, aflatoxin B_1, produced by mold in commercial diets (Sinnhuber et al., 1968). In the ensuing years, the rainbow trout model has been used to understand the mechanisms of how aflatoxin B_1 causes tumors, as well as to establish the lowest doses where it has an effect (for a recent review, see Williams et al., 2003).

Because tumor frequency is relatively low in many studies of carcinogens, the sample size needed for statistical validity can be rather high; in these lowest-effective-dose studies with rainbow trout, the

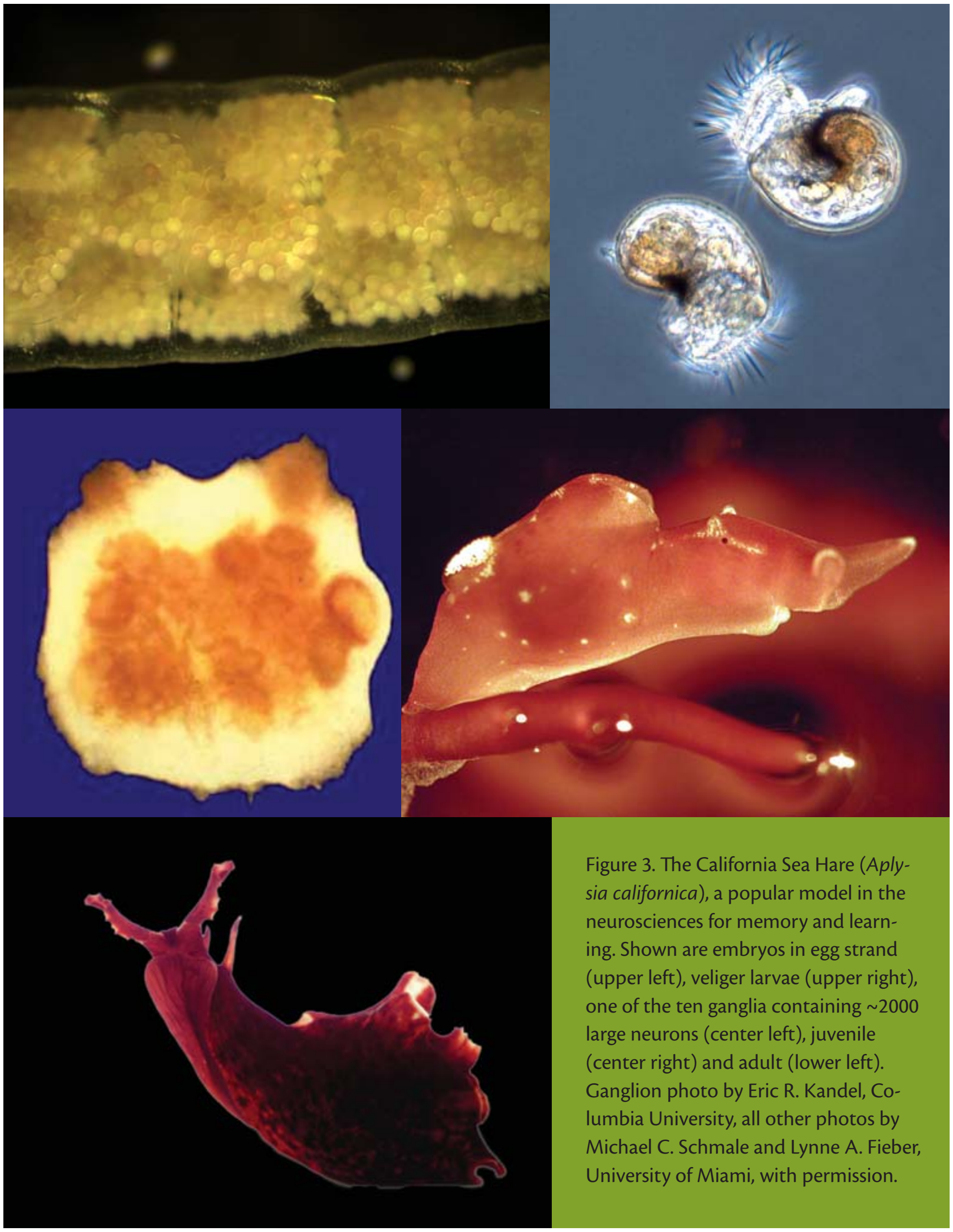

Figure 3. The California Sea Hare (*Aplysia californica*), a popular model in the neurosciences for memory and learning. Shown are embryos in egg strand (upper left), veliger larvae (upper right), one of the ten ganglia containing ~2000 large neurons (center left), juvenile (center right) and adult (lower left). Ganglion photo by Eric R. Kandel, Columbia University, all other photos by Michael C. Schmale and Lynne A. Fieber, University of Miami, with permission.

sample-size requirement is for tens of thousands of individual fish! While this is not a "cheap" experiment in terms of person hours and the husbandry costs of the fish themselves, the cost to conduct a similar experiment in rodent models would be astronomical and prohibitive. This is a clear example of the utility of aquatic test organisms.

In addition, the trout model has also been used to assay the effectiveness of dietary chemoprevention molecules. While mold/aflatoxin B_1 contamination of grain supplies destined for humans is not a tremendous problem in developed nations, often in the rural areas of less-developed nations, grain storage is less than optimal, and the occurrence of hepatocellular carcinomas is higher. Trout studies first revealed that molecules such as chlorophyllin are effective in offsetting the effects of aflatoxin B_1 (Breinholt et al., 1995). On this basis and subsequent rodent studies, chlorophyllin is being examined as a chemopreventive agent in rodent models and humans in rural areas of China (e.g., Kensler et al., 2004). Thus, the rainbow trout/aflatoxin B_1 model is a clear example of the effectiveness of aquatic species and how such studies can be used to affect human-health outcomes.

The August Krogh Principle in the Modern Genomic Era

The sequencing of full genomes of aquatic species is accelerating at a heady pace, providing even greater utility for models of human health and environmental sentinel species. Can the August Krogh Principle be applied even to these new molecular experiments? The answer is undoubtedly yes. In fact, researchers who identified the Fugu (pufferfish) as one of the first vertebrates to have its genome sequenced, perhaps unknowingly, applied the August Krogh Principle. While the pufferfish genome contains many homologies to human genes, and in fact in many cases genes are arranged in a very similar order on chromosomes, the non-coding (intronic) regions of these genes are very much abbreviated. In other words, while containing the "full complement" of vertebrate genes, the pufferfish genome size is some ten-fold smaller than the human genome. Thus, when sequencing costs and effort were high during initial attempts at full genome sequencing, it made "Kroghian" sense to first pick an organism with a less-intronic sequence to begin to answer questions like: What constitutes the beginning and end of "a gene"? How many genes are there in vertebrates? What genes are on which chromosomes? The discoveries made with the Fugu genome (see for example, Edwards et al., in press and articles within that issue) enabled a more informed approach to the sequencing of the human genome, undoubtedly leading to incredible advances in human health.

Lastly, the cost for sequencing of genomes and most especially "Expressed Sequence Tags" (ESTs) (i.e., partial sequences of the mRNA/cDNA that are expressed in a given organism and tissue) are rapidly falling, and the availability of the technology to small laboratories is increasing, such that genome and EST projects, and related microarray studies of gene expression, are now underway for all manner of aquatic species, sentinels, and health models included (for review, see for example, Cossins and Crawford, 2005). It is also likely that not too far behind, proteomic approaches will proliferate for these species. Because of the unifying conceptual aspects of gene and protein sequences and mechanisms of changes in gene expression, we predict that the lines among research on environmental sentinels, aquatic animal health models, and direct human-health impacts will blur even more favorably. One can easily envision circumstances where a gene or suite of genes impacted in a sentinel species whose expression levels are quantified by use of an "environmental microarray" (perhaps even ones deployed in the field) could be used to predict target genes to examine in human studies. Alternatively, genes that are known to be affected in a particular human disease with a component of environmental susceptibility could be used to guide choices for particular sentinel and model species (and genes) for environmental monitoring and experimental biology. Indeed, we are on the cusp of an even greater appreciation of the role of aquatic organisms in affecting and improving human health.

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