BREAKING eDAWN: eDNA, Current Challenges, and the Future

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What is eDNA?

- Environmental DNA (eDNA) is DNA from organisms shed into an environmental system in various forms, such as: blood and tissue, excretions, shed epithelia, or reproductive cells
- Current research in eDNA shows promise for its application as a rapid, accurate, and ever more cost efficient tool in species monitoring efforts
- eDNA sampling is quick and allows for a broad survey area due to less time requirements in the field
- Properly designed eDNA assays can be very sensitive in the detection of small amounts of genetic material from rare or elusive species which might be missed by traditional survey methods alone
- Advances in eDNA research are allowing for better stream-lining of sample collection and processing, which translates to an overall lowering of assay cost and man-hours

Unique Challenges

- eDNA assays face many of the same challenges as ancient DNA (genetic material recovered from historic specimens) and forensic DNA (genetic material recovered from criminal investigations)
- eDNA is often low quality due to deterioration (degradation) upon exposure to environmental factors and low quantity due to dilution or degradation, making detection and analysis of the DNA difficult
- Low quality/quantity DNA is particularly prone to contamination in the field and laboratory, which can swamp out eDNA and lead to misinterpretation of results
- Inhibitors (environmental molecules preserved along with the DNA during processing) can reduce assay efficiency and sensitivity
- As with ancient and forensic DNA, properly equipped and trained eDNA labs provide reliable, actionable data

Current and Past Applications of eDNA

- Monitoring of invasive species as early stages of invasion
  - Asian carp (Jerde et al. 2011)
  - Bullfrogs (Dejean et al. 2012)
- Monitoring of threatened and endangered species (TES)
  - Frogs and salamanders (Goldberg et al. 2011; Olson et al. 2012)
  - Sturgeon (in development at ERDC)
- Monitoring species in marine ecosystems
  - Marine mammals (Foote et al. 2012)
  - Marine fish (Thomsen et al. 2012)

Efforts at ERDC

eCALS – eDNA Calibration Study, a large multi-year collaborative project with the USFWS and USGS, includes efforts to better understand eDNA "loading" from Asian carp, degradation of eDNA, movement of eDNA within a aquatic system, and introduction of carp eDNA into aquatic systems by vectors other than live fish, to develop additional eDNA markers and baseline genetic information, and to develop a Bayesian Network model to assist stakeholders using Asian carp eDNA data in making management decisions. Project concludes September 2014. Updates available at http://www.asiancarp.us/ecals.htm.

Dreissenid mussels – Study designed to apply Asian Carp eDNA methodology to the detection of invasive zebra and quagga mussels, and observe the potential use of peristaltic pumping and sieve filtration for field sampling, eliminating laboratory filtration. Results indicated detection of Dreissenid species with sieve filtration appear to have a higher degree of detection over laboratory filtration, but sample area was limited by the time required for peristaltic pumping.

Sturgeon – The application of eDNA for the detection of imperiled sturgeon species is currently being developed. Potential uses of eDNA in these systems include monitoring of seasonal movements and direction of restocking efforts. Nine generalized mitochondrial eDNA markers have been designed to detect all North American sturgeon species. Ultimately, more specific markers will be developed for monitoring individual species or populations of interest.

Desert eDNA – Study designed to investigate water resources in arid environments which may contain eDNA from organisms from across large geographic areas. Samples were collected from 15 various water sources in the desert regions of southern Arizona and assayed with highly sensitive next-generation sequencing. Recent results indicated detection of some target organisms from the bat, bear, canine, and weasel families as well as detection of many other non-aquatic mammalian, bird, reptile, arthropod, and plant species.

Fig. 1 A lesser long-nosed bat (left) and black bear (right) – two of the species targeted for detection in our on-going desert eDNA project.

Fig. 2 Several aquatic invasive and endangered species have been the focus of eDNA research at the ERDC (clockwise: sturgeon, mussel, and carp).

Interpreting eDNA Data

- Interpretation of eDNA data can be controversial, particularly when results have management implications. Typical questions include:
  - Does an eDNA detection guarantee the presence of a living organism at the sampling location?
  - Is it possible for eDNA to detect the wrong species (false positive)?
  - Can eDNA tell us how many individuals occur at a site?
- The eDNA Calibration Study is pursuing scientific answers that will address many of these issues and provide an even stronger foundation for eDNA efforts.
- Experts in the field of aquatic eDNA met during the week of July 22 for a special symposium as part of the International Conference on Conservation Biology in Baltimore, MD. These experts have agreed to collaboratively develop best-practices guidance to assist managers and researchers in designing high-quality eDNA studies.

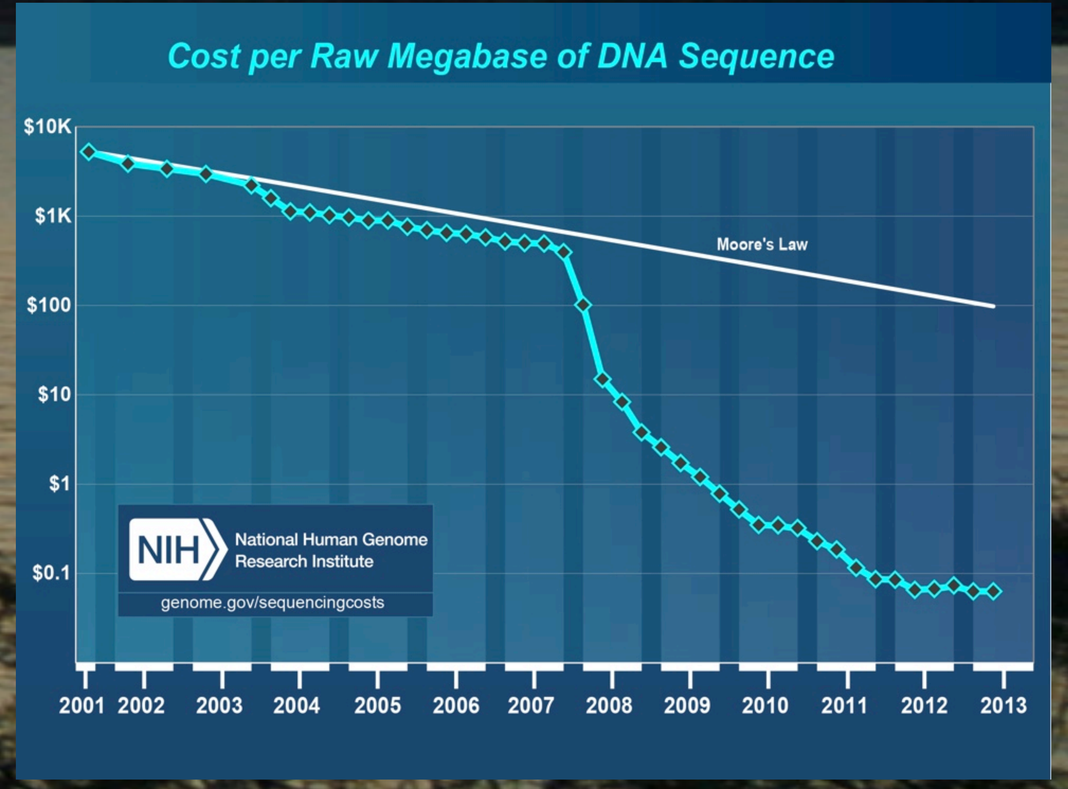
The Future of eDNA

More Data, Better Data, Less Cost, Better Inference

- Cost of obtaining DNA data, especially sequence data, has and continues to drop (Fig. 3)
- Next-generation DNA sequencers are becoming less costly and more widely available
- Increased computing power allows for more efficient processing of next-generation sequencing data
- The quality of data, such as DNA sequence read lengths, continues to improve.
- Growing capabilities to process extremely large numbers of samples in single, cost-effective sequencing or quantitative polymerase chain reactions (qPCRs).
- New technologies and increasing available genetic information will allow us to not only monitor single species, but suites of species or entire ecological communities (DNA meta-barcoding).
- What other developments beckon? Real-time automated eDNA monitoring of aquatic systems?
- At the recent eDNA symposium in Baltimore, eDNA experts discussed advances being made in the development of this tool. It is clearly an area of rapidly growing interest, and advances in the application and science are quickly developing.

Fig. 3 The power of DNA sequence data production has increased considerably over time, while data generation costs have declined -- shown here in the context of Moore's Law , which reflects the doubling of the computing power every two years. A rate of technological improvement that meets or exceeds Moore's Law is considered to be advancing at an exceptional pace.

Wetterstrand, KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP). www.genome.gov/ sequencingcosts/



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