Phil. Trans. R. Soc. B **371** doi: 10.1098/rstb.2015.0207

Complementary approaches to diagnosing marine diseases: a union of the modern and the classic

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Supplemental Material

Critical types of data and techniques are needed to make a correct disease diagnosis using both classic and modern approaches. Consider the following a hypothetical situation concerning a moribund sea star found on the seashore and the steps one might take to diagnosis a potential etiology:

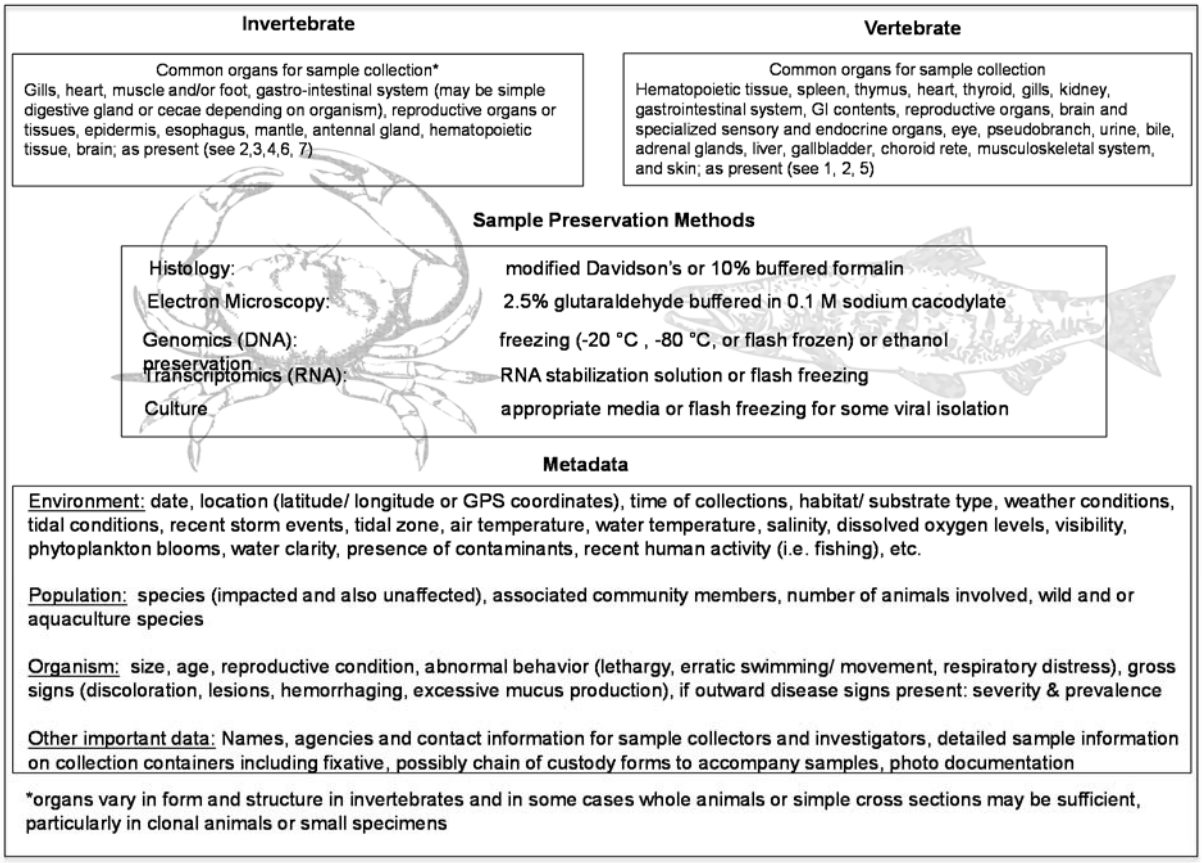
One could record: SPECIES: Pisaster ochraceus SIZE/APPEARANCE: small, at 6 cm avg. arm length, orange color morph, LOCATION: in the wild, low tide from transect B, North side of Caution Point, Friday Harbor, San Juan Island, WA USA, UTM, Zone 10 U, 499488.38 m E, 5377094.62 m N). ENVIRONMENTAL CONTEXT: Rocky intertidal with boulders and crevices, with near 100% algal cover. Site is a marine reserve. Recent seawater (13 °C) and air (26 °C) temperatures warm during daytime low tides, no recent rain. Salinity at 30.5 PSU. Several sea stars of several species observed dead or dying in the sampling location. Additional samples include samples of other species of sea star.

Disease observations should include metadata (data about data), such as information about sample collection [date, location, collection method, name of collector(s)], organisms collected [species, size, condition, tissue type(s)], processing of the collected specimens [fixation and preservation, method of processing, name of processor(s)], and infectious agent(s) (e.g., parasite abundance).

For our example sea star, in addition to the environmental and demographic data, the following notes would also be useful. Samples taken: Histopathology, EM, molecular, and culture. Case # EIMD-15-1 Cross section of sea star arm next to the leading edge of the lesion including both epidermis, tube feet, pyloric caeca, gonads, and radial canal in histology cassette # EIMD-15-1-1 and placed into Invertebrate Davidson's solution for 24 hours followed by 70% ethanol (care should be taken to use appropriate pen or pencil for marking histopathology cassettes). Additionally, tube feet and epidermis fixed and post-fixed followed by tissue embedding for Transmission Electron Microscopy. Molecular samples, tube feet, epidermis, and coelomcytes were flash frozen in liquid nitrogen. Tissue swab of sterile cut into epidermis on Marine Agar 2216 and Gram-stained. Tissue imprints of leading edge of epidermis taken. Histopathology samples also taken from normal P. ochraceus case # EIMD 15-2-1.

For each sample type, both pictures and a written description are useful to understand the context of the sample.

Collection and processing of samples from live organisms for tissue, cellular, and gene-based technologies is an important consideration. We've created a generalized flow chart of appropriate sample types to take based on typical hosts you might encounter in the field (Supplemental Figure 1). At current time, there is no centralized processing of samples for marine disease, but for certain diseases, the World Organization for Animal Health (OIE) has expert laboratories for sample diagnoses. For other diseases, there are a number of laboratories and experts in the field with diagnostic capabilities. There are a number of manuals or guides, primarily for sampling, fixation, and microscopic analysis of tissue of marine organisms (e.g. (1-7)), as well as specific guidelines for diagnoses set by the OIE for both development of diagnostics and for notifiable diseases (8). Special care should be taken to understand both the fixation and preservation methods based on the organism type and type of fixative.



Supplemental Figure 1. Field guide to collection of marine disease samples

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