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How Relevant is Opportunistic *Bd* Sampling: Are We Ready for the Big Picture?

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Understanding the distribution of chytridiomycosis, both at global and local scales, is important to controlling its impacts on host species (e.g., biocontrol or eradication) and to managing host amphibian populations (e.g., reintroduction and habitat management). In response to this, efforts to map observations of *Batrachochytrium dendrobatidis* (*Bd*) are underway to better understand its distribution and impact on amphibian populations (e.g., www.spatialepidemiology.net\Bd).

While there are many legitimate reasons to sample opportunistically for this pathogen, we question the validity of continuing this sort of exercise without a more cohesive and directed effort to apply the information to management of *Bd*. We use a recent example (Scalera et al. 2008) and some additional data from Denmark to discuss this question.

Scalera et al. (2008) published data from frogs (*Rana esculenta* [now *Pelophylax esculentus*], and *R. temporaria*) at sites in Denmark on the islands of Zealand (1 of 7 positive for *Bd*) and Fyn (1 of 4 positive for *Bd*). Two sites on the Jutland mainland of Denmark were negative according to Scalera et al. (2008) and a single positive record is known from the island of Bornholm, the most eastern portion of Denmark (www.spatialepidemiology.net). To these published data we add information from the island of Lolland where we tested 10 adult *R. esculenta* (three female and seven male) for *Bd* (sensu Berger et al. 2005). These animals were from a single pond in the backyard of a private residence on the outskirts of Nakskov (54.85°N, 11.01°E). Swabs were analyzed using PCR (Annis et al. 2004) at Pisces Molecular (Boulder, Colorado, USA) and *Bd* was detected on 0 of 10 animals.

Our data increase the range of sampling in Denmark to include four of six of the major islands (Zealand, Lolland, Fyn, and Bornholm). To date, there are no samples from Mon, Falster and the smaller islands of Als, Aero, and Langeland. The information reported here and by Scalera et al. (2008) is interesting because it is from a region lacking in data and provides the most northerly published records of this fungus in Europe (www.spatialepidemiology.net\Bd). However, there are serious constraints to these data.

Sampling in both reports (Scalera et al. [2008] and the present one) was ad hoc, sample sizes were low, and most sites yielded negative results. Scalera et al. (2008) tested a maximum of four adults or three juveniles per site. We tested 10 adults at one site. Skerratt et al. (2008) provides a good example of necessary sample sizes that contrast with those reported here and in many other reports (e.g., Zellmer et al. 2008, N = six per site; Adams et al. 2007, N = 2–17 per site). For example, if we assume perfect specificity of the PCR, and use an apparent prevalence ≥ 5%, 59 individuals must be sampled to be 95% certain of detecting *Bd* on at least one animal (Skerratt et al. 2008). This suggests that the sample sizes from locations in Denmark need to be at least six times greater to be confident in the negative reports. Alternatively, small sample sizes can be informative at high prevalence rates, but given reported sample sizes, prevalence rates would have to be 30% in our work, and at least 75% in the work by Scalera et al. (2008), to be 95% certain of detecting *Bd* on at least one animal. In populations that are not declining, we would expect the prevalence of *Bd*, if it is present, to be low in contrast to high values reported in amphibian communities where *Bd* is causing extinctions (e.g., 98% prevalence, Lips et al. 2006).

Clearly, neither the data presented by Scalera et al. (2008) nor our data are adequate to determine if the amphibian chytrid is absent from portions of Denmark. Additionally, these data cannot speak to the prevalence of the disease where it was detected (Skerratt et al. 2008). These reports beg the question, "What do we really know about the presence of the amphibian chytrid fungus in Denmark?" We submit that these reports tell us very little. Specifically, the information does not provide a clear direction or purpose for further testing because we are uncertain about the applicability (area of inference) and the degree of confidence we should attribute to the results. This hampers our ability to discuss the relationship of these data to other *Bd* occurrences in northern Europe, an exercise that has the potential to be relevant to at-risk amphibians in northern Europe and amphibian declines in general.

As observations of *Bd* are published and added to existing databases, it is tempting to try to interpret the "patterns" that begin to emerge which can be at best heuristic and at worst seriously misleading. Because of the well-known impact of chytridiomycosis on amphibians, the relative ease of sampling for the disease and the awareness level in the herpetological community, sampling and testing for *Bd* is often undertaken. Such opportunistic sampling is generally motivated by goals other than determining the distribution of *Bd* in a particular region or country. It can be quite useful to know whether *Bd* is present at a particular study site. For example, testing surrogate local amphibians or testing the environment for *Bd* (Kirshstein et al. 2007, Walker et al. 2007) is likely to be a critical element in translocation programs involving susceptible species (Walker et al. 2008). There is some utility to reporting the ad hoc testing of amphibians and locations for *Bd*, even when results are negative, especially when reports: 1) come from areas with no information at all; or 2) can be combined to present a picture of an area where a more concerted effort is unfunded or logistically difficult. Perhaps the real utility of these data lies in the archiving and mapping realm effectively implemented at www.spatialepidemiology.net\Bd.

On the other hand, we can argue that by continuing to report on isolated instances of positive and negative results, we are losing focus on the bigger issue of a single pathogen with global impacts. Two main issues surface, first, sample sizes are often low and ad hoc reports generally lack details on assay specificity, and laboratory and collection conditions. Second, much of the opportunistic sampling occurs in portions of the world where amphibians are already declining, the *Bd* "hot spots" of South and Central America and Australia. Are other locales being neglected because there is no globally coordinated sampling plan? Northern Europe is far removed from typical *Bd* "hot spots" where species are highly impacted by this fungus and it is possible that declines in amphibian populations, caused or exacerbated by *Bd*, could go unnoticed in countries such as Denmark. While data exist on *Bd* for almost every biome in the world, including the temperate zone (e.g., Bosch et al. 2001 [Spain], Muths et al. 2003 [U.S.], Ouellet et al. 2005 [Canada]), there are information gaps where no scientists or funded projects exist, especially in regions where the perceived threat from *Bd* is low (i.e. northern regions).

There is no global amphibian authority to provide direction for coordinated sampling or to enforce collaboration, however, the Amphibian Conservation Action Plan (Gascon et al. 2007) calls specifically for more coordinated research into this disease. Perhaps an effort by the amphibian research community to follow a standard sampling plan is a first step. For example, Skerratt et al. (2008) have laid out a *Bd* sampling protocol using systematic sampling and taking into consideration time of year, accessibility, likelihood of infection, life stage, potential for pooling samples, and the specificity of the assay. This protocol was designed for the large land mass of Australia, but is applicable to any regional or country-wide effort in the rest of the world and offers the potential for a great deal of consistency in efforts to map and understand the distribution of *Bd*.

There is a significant challenge to moving ahead toward an understanding of the spatial epidemiology of chytridiomycosis and continued ad hoc additions to the existing databases are unlikely to yield answers. A systematic approach to building and testing hypotheses and bridging data gaps is necessary. Fortunately the substantial body of knowledge about the geographical distribution of this pathogen gained from coordinated regional efforts, as well as opportunistic sampling, provides the data necessary to develop such an approach.

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Seasonal Variation in the Detection of *Batrachochytrium dendrobatidis* in a Texas Population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*)

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Blanchard's Cricket Frog (*Acris crepitans blanchardi*) is a small anuran with a broad distribution in North America (Conant and Collins 1998), and recently has been suggested for elevation to full species (Gamble et al. 2008). Cricket Frog populations are in decline throughout much of the northern portion of their range, although causes for these declines are unclear (Lannoo 2005). However, *A. c. blanchardi* is often the most abundant and conspicuous member of anuran breeding assemblages throughout much of the year in eastern and central Texas. Central Texas supports a particularly robust population, with Cricket Frogs found in virtually any natural or artificial aquatic environment, including ephemeral streams and pools, spring outflows, stock tanks, roadside ditches, and along riverbanks. At the eastern edge of the Edward's Plateau, *A. c. blanchardi* is sympatric with a number of endangered, threatened, or endemic amphibians including the Houston Toad (*Bufo* [*Anaxyrus*] *houstonensis*) (Hillis et al. 1984) and several species of plethodontid salamanders (*Eurycea nana*, *E. neotenes*, *E. pterophila*, *E. rathbuni*, *E. sosorum*) (Chippindale et al. 2000) each with increasing conservation concern in this rapidly developing region.

Amphibian populations are declining at an alarming rate world-