U.S. Fish and Wildlife Service
Division of Policy and Directives Management
Attn: Docket No. FWS-R9-FHC-2009-0093
4401 N. Fairfax Drive, Suite 222
Arlington, VA 22203

RE: Docket No. FWS-R9-FHC-2009-0093

My Response to the Defenders of Wildlife petition to the United States Fish & Wildlife Service to add all amphibians to the Lacy Act unless certified as Chytrid Free

1. About Me
Though I do not presently work with any amphibians, I have 15 years of previous hands-on experience in the captive husbandry and breeding of Dendrobatid frogs. My name is Webster Wheeler and I am a frog enthusiast and a researcher - not a scientist. Therefore, much of the information contained in my submission is cited from peer-reviewed studies by other scientists, rather than from my own original work with Dendrobatid frogs.

In my submission, I explore the Chytrid Fungus epidemic, analyze the scientific data and present my conclusions.

2. Origin of Chytrid Fungus
"In terms of a most likely candidate for spread from Africa, the number of frogs and geographic dissemination favor X. laevis. Soon after discovery of the pregnancy assay for humans in 1934, enormous quantities of the species were caught in the wild in southern Africa and exported around the world. The pregnancy assay is based on the principle that ovulation in X. laevis is induced by injection with urine from pregnant women because of high levels of gonadotropic hormones in the urine. X. laevis was selected as the most suitable amphibian for investigating the mechanism of the mating reflex because of the relative ease with which the animal can be maintained in captivity. For 34 years, the trade in X. laevis in South Africa was controlled by the then Cape of Good Hope Inland Fisheries Department (Western Cape Nature Conservation Board) at the Jonkershoek Fish Hatchery. As an indication of the numbers involved in this trade, 10,866 frogs were distributed in 1949, of which 3,803 (35%) were exported, and of the 20,942 frogs distributed in 1970, a total of 4,950 (24%) were shipped abroad. After the introduction of nonbiologic pregnancy tests, X. laevis became important as a model for the scientific study of immunity and later embryology and molecular biology. X. laevis could have carried the disease globally, particularly if the prevalence was similar to that seen in wild-caught X. laevis today. In the importing country, escaped frogs, the water they lived in, or both, could have come into contact with local amphibian species, and subsequent transmission of the disease could have followed. The establishment of feral populations of X. laevis in Ascension Island, the United Kingdom, the United States, and Chile in 1944, 1962, the 1960s, and 1985, respectively, show that transmission could have become ongoing if these feral populations were infected." (12.24)

The earliest evidence of Chytrid Fungus has been found in preserved museum specimens of Xenopus from Africa, from where the pathogen is thought to have spread (12.4). "The earliest case of Bd infection was in a specimen of Xenopus fraseri collected from Cameroon in 1933." (12.4)

"Xenopus laevis is among the few known asymptomatic carriers of Bd infection (Weldon et al., 2004). It has been hypothesized that the global trade in X. laevis, which began in the mid-third of the 20th century (primarily for use in the pregnancy assay for humans), led to Bd crossing geographical boundaries, which had previously confined the parasite to Africa (Weldon et al., 2004; Skerratt et al., 2007)." (12.4)

3. Magnitude of the Chytrid Fungus epidemic

The Chytrid Fungus, Batrachochytrium dendrobatidis (Bd), has been described as "the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted and its propensity to drive them to extinction" (12.1). The impact of chytridiomycosis, the disease caused by Bd, on frogs is the most spectacular loss of vertebrate biodiversity due to disease in recorded history. (12.2) Bd is already widespread throughout the world. Amphibians in Africa, Australia, Europe, South America, Central America, North America, and Oceania are reported to be infected with Bd. (12.3)

Bd is a waterborne pathogen mostly infecting frogs associated with permanent water, particularly streams, and it is these species of frogs that have experienced the most severe declines.Bd is pathogenic and virulent over a broad range of temperatures (12 degrees – 27 degrees Celcius) but has its greatest virulence at temperatures from 12 degrees – 23 degrees Celcius (Berger et al., 1998, 2004; Longcore et al., 1999; Woodhams et al., 2003; Carey et al., 2006). (12.2) Pathogenicity and virulence decreases significantly as temperatures are raised above 27 degrees Celcius. Frog species that occur at temperatures consistently below this upper threshold for Bd, are most affected (Berger et al., 1998, 2004; McDonald et al., 2005; Pounds et al., 2006). (12.2)

"Susceptibility to chytridiomycosis varies widely in amphibians, with some species being driven to extinction, whereas sympatric species remain apparently healthy carriers of infection (Daszak et al., 2003; Schloegel et al., 2006)." (12.4)

"Introduction of B. dendrobatidis to naive susceptible populations has caused epidemics of high mortality. If populations survive, the pathogen persists and becomes endemic, with reduced mortality rates and, in some cases, recovery (McDonald et al., 2005). This suggests that there is selection for host resistance against the disease." (12.25)

4. Chytrid Fungus in the United States

Chytrid Fungus was identified in the United States in at least two locations in 1974: Bufo canorus - Sierra Nevada, California (Green & Kagarise Sherman in Carey et al (1999)) and Rana pipiens - Colorado Rockies (Green, Carey & Corn in Carey et al (1999)). (12.3) Since 1974, Chytrid Fungus has been discovered in wild frogs in Arizona, Illinois, Maryland, North Carolina, South Carolina, (12.3) Indiana, Minnesota, Missouri, Virginia, Wisconsin, Wyoming, (12.8) Oregon, (12.13) Louisiana, Maine, Massachusetts, New Hampshire and Vermont (12.15).

5. Chytrid Fungus around the world

"Some sites in which B. dendrobatidis is associated with die offs are in remote and seemingly undisturbed locations such as high-elevation sites in the Sierra Nevada (California), Central America, and northern Australia." (12.18)

"In Queensland, Australia, there have been extinctions or major declines of at least 14 frog species in undisturbed, high elevation rainforest streams, commencing in 1979–1981 in the Conondale and Blackall Ranges (268509 S, 1528419 E), followed in 1985–1986 in the Eungella region of the Clarke Range (218079 S, 1488299 E), and, in 1990–1995, in the Wet Tropics bioregion (178229 S, 1458499 E) (Laurance et al. 1996; McDonald and Alford 1999). Laurance et al. (1996, 1997) suggested that an epidemic disease was responsible for all these declines, without proposing an agent. Despite the presence of B. dendrobatidis in ill and dead frogs collected from the Big Tableland in the Wet Tropics in 1993, it was not recognised as a pathogenic organism until 1998 (Berger et al. 1998) and was described as a new species of fungus in 1999 (Longcore et al., 1999). B. dendrobatidis has been suggested as the causative agent of many of the east coast Australian declines (Berger et al. 1999a), although only the decline at Big Tableland (McDonald and Alford 1999) had direct evidence of an association with the presence of B. dendrobatidis." (12.19)

4. Chytrid Fungus detection and Chytrid Fungus persistance

"We have shown that the use of a singlicate assay in qPCR detection of chytridiomycosis can provide significant economic benefits over the use of a triplicate assay, while sacrificing no significant loss of accuracy. ...A maximum saving of $7.66 sample–1 can be attained by using the singlicate assay when there is no chytridiomycosis detected in the population. This is a reduction in costs of up to 59% compared with the triplicate assay, and makes qPCR comparable in cost to other diagnostic techniques in current use. At any (Bd) prevalence less than 80.2%, it is more economical to use the singlicate assay. The reduced costs involved in the singlicate qPCR assay will also assist those involved in the exportation of amphibians for the zoo, pet or food trades, as it will enable them to increase the number of amphibians surveyed for chytridiomycosis, thereby reducing the chances that they unwittingly transport the disease to naïve amphibian populations. Alternatively, a researcher whose sole goal is to determine whether or not chytridiomycosis is present or absent in a population may have no interest in the disease status of individuals, the severity of their infections, or even the prevalence in the population. In such a case, using a singlicate assay and pooling 5 to 10 samples per well might be a highly cost-effective option. A singlicate re-analysis of any positive pools could be used to confirm that the positive result was not due to contamination (triplicate re-analysis with a full set of standards would be unnecessary since quantitation is not required). The efficacy and details of pooling samples should be more thoroughly explored." (12.14)

"Many researchers worldwide are currently utilizing conventional PCR techniques (Annis et al. 2004) for the detection of chytridiomycosis. This is likely due to 2 factors: (1) monetary restrictions, i.e. the conventional PCR method does not use a triplicate assay, and is therefore less expensive than the previously described (Boyle et al. 2004) qPCR technique. The singlicate qPCR technique described herein, however, is approximately equal in cost to conventional PCR techniques. (2) There is a false perception that the difference in sensitivity between conventional PCR and qPCR is negligible. However, qPCR is widely regarded as being more sensitive than conventional PCR (Luthra et al. 1998, Helps et al. 2001, Borg et al. 2003, Emery et al. 2004). With regard to the detection of Batrachochytrium dendrobatidis, the qPCR method described by Boyle et al. (2004) can detect a single chytrid zoospore, whereas the conventional PCR method described by Annis et al. (2004) sets a lower detection limit of about 10 zoospores, under which reproducibility is poor." (12.14)

"In summary, the methods developed here (SYBR/Boyle vs. Taqman/Wood quantitative PCR assays) allow for the assessment of Bd distribution and abundance in water and sediment. ...Our field sampling demonstrates that water can now be sampled, with or without concurrent amphibian sampling. This technique will allow researchers to study the implications of Bd's presence in water bodies, to monitor water bodies before reintroduction efforts and to investigate the spread of Bd across the landscape." (12.13)

"We show that B. dendrobatidis will survive in tap water and in deionized water for 3 and 4 weeks, respectively. In lake water, infectivity was observed for 7 weeks after introduction. The knowledge that water can remain infective for up to 7 weeks is important for the formulation of disease control and quarantine strategies for the management of water that has been in contact with amphibians." (12.12) "For quarantine purposes, all water, moist soil, and wet fomites imported into a country with amphibians should be regarded as infectious for B. dendrobatidis unless the amphibians are shown to be uninfected. A similar strategy should be adopted when introducing new amphibians into a captive colony or collection. Similarly, water and any items coming into contact with amphibians moved within countries should be regarded as infectious for B. dendrobatidis. In practical terms, storage alone for a period of time should not be used as a means of ensuring water that has been in contact with an amphibian is not contagious. All water and wet soil in contact with an amphibian should be disinfected before discharge into the wastewater system or the natural environment." (12.12) "To comply with the intentions of Office Internationale des Epizootes listing, amphibians, when moved between countries, should be placed in a different container on arrival; all water, soil, plants, and litter in contact with the amphibian during transport should be adequately disinfected by using techniques capable of killing B. dendrobatidis." (12.12)

"B. dendrobatidis has no known resting or saprophytic stage; such stages, however, occur in other Chytridiomycetes (Powell 1993). B. dendrobatidis grows well in culture, grows on dead frogs and snake skin (both keratin substrates), and thus may grow saprophytically in the wild (Longcore et al. 1999). Zoospores can survive for an extended period in sterilized water (Johnson & Speare 2003), raising the possibility of such survival in the wild." (12.18)

5. Global spread of Chytrid Fungus today

"There is a high prevalence of chytridiomycosis in the international amphibian trade including the pet trade (Berger et al., 1999; Cunningham et al., 2005), the scientific trade (e.g. Weldon et al., 2004), the food trade (e.g. Mazzoni et al., 2003; Hanselmann et al., 2004), the ornamental trade (Daszak et al., 1999) and the introduction of frogs into zoological collections (Nichols et al., 1998, 2001; Pessier et al., 1999; Banks & McCracken, 2002; Schloegel et al., 2006). Infected frogs imported into zoos have caused epidemics of chytridiomycosis in established amphibian collections, but few of these cases have been published (Nichols et al., 1998; Pessier et al., 1999). This may reflect potential problems with quarantine and hygiene procedures within zoological collections." (12.25)

5.1 Scientific and medical research

"If X. laevis did carry B. dendrobatidis out of Africa as we propose, other amphibian species subsequently could have distributed it between and within countries. The American bullfrog, Rana catesbeiana, has been proposed as an important vector, mainly through international trade as a food item, but also within countries as populations established for the food trade escape and spread. The earliest current record for the occurrence of chytridiomycosis in R. catesbeiana is 1978 in South Carolina, 40 years after the first record in southern Africa, but details on the intensity of the search for chytridiomycosis in archived bullfrogs are not available. The transmission of chytridiomycosis globally may involve a series of key steps: 1) occurrence of B. dendrobatidis in an amphibian vector in southern Africa that is relatively resistant to disease (X. laevis), 2) sudden rise in 1935 of export trade in this vector because of technologic advances (Xenopus pregnancy test), 3) escape of the pathogen from the exported Xenopus to establish new foci in other countries (possibly expedited in some countries by establishment of feral populations of X. laevis), 4) transmission into other vector amphibians (food and pet trade), and 5) further transmission to other countries along different trade routes in key amphibian vectors that move in high numbers and become established in commercial populations and closely interact with wild frogs, which likely leads to feral populations (food frogs R. catesbeiana). Spread through native amphibian populations with epidemic disease in some species could have occurred at any point after B. dendrobatidis entered a naïve native species." (12.24)

"The three most widely introduced species, the African clawed frog Xenopus laevis, the North American Bullfrog Rana catesbeiana and the Cane toad Bufo marinus, have established feral populations in the Americas, Europe, Australia, Asia and many oceanic and coastal islands. Weldon et al. (2004) reported the first case of Bd infections in Xenopus laevis collected and preserved in the late 1930s. It was at this time Xenopus spp. were first being exported to the USA, Australasia and Europe as pregnancy assays. Subsequently, Xenopus has become the model amphibian genus for primary research and continues to be widely traded (Weldon et al. 2004). Captive colonies of Xenopus spp. are known to harbour Bd-infected animals (Parker et al. 2002; Fisher unpubl. data) and Xenopus escapes and establishments are still being reported (Cunningham et al. 2005)." (12.6)

"(T)he American bullfrog (Rana catesbeiana) appears resistant to mortality caused by B. dendrobatidis and may be a carrier (Daszak et al. 2003; Hanselmann et al. 2004). Bullfrogs have been introduced in many regions of the world in attempts to culture them for human food (Kupferberg 1997). These introductions began in the 1800s (Kupferberg 1997) and have continued at least into the 1990s (Hanselmann et al. 2004). Results of retrospective studies show that the earliest cases of B. dendrobatidis primarily date back only to the 1960s–1990s (Fig. 1b); searches of museum specimens, however, may not be comprehensive in many species and locations. Weldon (2002) andWeldon et al. (2004) propose Xenopus laevis as a potential intercontinental carrier based on their discovery that B. dendrobatidis has occurred in African populations of X. laevis since the 1930s (Fig. 1b) and that this species can asymptomatically carry B. dendrobatidis. X. laevis were frequently transported intercontinentally during the 1940s–1950s for human pregnancy testing (Hansen 1960). In Australia, where many declines have occurred (Berger et al. 1998), X. laevis are restricted to laboratories and are neither widespread nor free ranging. In California introduced X. laevis populations exist only in southern California (Tinsley & McCoid 1996), yet major declines in native frogs have been documented mostly farther north (Jennings & Hayes 1994; Davidson et al. 2001). Thus, additional vectors (amphibian or nonamphibian) must be involved in order for the pathogen to have spread from these carriers." (12.18)

"One frog escaped after 16 d and was not recovered." (12.20)

5.2 Agriculture and aquaculture

"Intentional introductions of bullfrogs across western North America began in the 1930s and continued for decades afterwards(Green 1978; Nussbaum et al. 1983) and there is a history of bullfrog farming in the native range (Stoutamire 1932; Baker 1942). Nace et al. (1971) reported 22 private outlets marketing bullfrogs for research in the 1960s, undoubtedly representing a small component of the trade in bullfrogs dominated by the food industry (Teixeira et al. 2001). Introduced populations of bullfrogs are consistent in harbouring infected frogs and tadpoles (Garner et al. 2006). First reports of chytridiomycosis in Australia are from 1978 (Rachowicz et al. 2005). Cane toads were introduced in Australia as biocontrols in 1935 (Lever 2001), which precedes the hypothetical emergence out of Africa. However, like Xenopus, cane toads were used as pregnancy assays (Bivens 1950) in South America, the Caribbean and Australia (Lavergne & Trapido 1951; McDonald & Taft 1953; Floch & Fauran 1955). The species was commercially traded in Australia by the thousands during the 1960s

(Tyler 1999). Thus, for the three most widely introduced species that asymptomatically harbour Bd infection, the establishment of trade and mass transport of animals and the potential for horizontal transmission among them predates the emergence of the disease." (12.6)

"By demonstrating that R. catesbeiana is likely to be an efficient carrier of this pathogen, our experimental data add weight to the hypothesis that this host species is important in the spread of chytridiomycosis, particularly by commercial activities." (12.5)

"Batrachochytrium dendrobatidis is the chytridiomycete fungus which has been implicated in global amphibian declines and numerous species extinctions. Here, we show that introduced North American bullfrogs (Rana catesbeiana) consistently carry this emerging pathogenic fungus. We detected infections by this fungus on introduced bullfrogs from seven of eight countries using both PCR and microscopic techniques. Only native bullfrogs from eastern Canada and introduced bullfrogs from Japan showed no sign of infection. The bullfrog is the most commonly farmed amphibian, and escapes and subsequent establishment of feral populations regularly occur. These factors taken together with our study suggest that the global threat of B. dendrobatidis disease transmission posed by bullfrogs is significant." (12.7)

5.3 Pet industry

"A previous report (6–10 October 2003) addresses the results of an OIE questionnaire on amphibian diseases and states.. 'the indication so far is that the trade in pet amphibians is of limited scale and may not be sufficient to present significant risk of disease transfer.'" (12.6)

6. Novel vs. Endemic Pathogen Hypothesis

"Changes to the environment have been implicated in the declines of amphibians worldwide (reviewed in Alford & Richards 1999). Most likely multiple factors are involved and no one factor can explain all amphibian declines (Blaustein & Wake 1995; Kiesecker et al. 2001a; Collins & Storfer 2003). Although the available genetic evidence suggests B. dendrobatidis is a novel pathogen (Morehouse et al. 2003), one should not rule out the possibility that B. dendrobatidis is an old amphibian associate that has emerged recently as a pathogen in some species. We caution that one hypothesis on the origin of the disease may not apply to all species or all areas. Because strategies for studying and managing the effects of emerging pathogens differ substantially depending on whether the pathogens are novel or endemic, we suggest that discriminating between the novel pathogen and endemic pathogen hypotheses should be an early step in investigations of all emerging pathogens. This is particularly important for pathogens of wildlife, where the fact that a pathogen escaped previous detection is weak evidence for its novelty." (12.18)

"It is intriguing that we found similar seasonal fluctuations in infection levels within each year, but no evidence of variation among the 4 y of the study. This suggests strongly that B. dendrobatidis has become endemic and relatively stable in prevalence in these populations. Given that frog numbers and diversity remained broadly similar over this period, it suggests that some form of host–pathogen equilibrium has become established, in contrast to the epizootic that may have occurred 10 y previously." (12.19)

"In this study, infection levels of B. dendrobatidis were significantly higher during winter and spring than during summer and autumn. A survey of ill and dead frogs from eastern Australia from 1995 to 1999 showed a similar seasonal prevalence of chytridiomycosis (Berger et al. 2004). In the laboratory, the growth of B. dendrobatidis has been shown to peak at about 23 8C, with death or arrested growth occurring in vitro at temperatures above 30 8C (Longcore et al. 1999; Johnson et al. 2003; Piotrowski et al. 2004). Further, infection in some frog species can be cleared in the laboratory by exposing them to temperatures in excess of 37 8C (Woodhams et al. 2003). At Eungella, 23 8C is a typical daytime maximum temperature for winter, with water and air temperatures along streams generally remaining at or below 23 8C for the entire 24-h daylength period. In summer, however, daytime air temperatures along Eungella’s streams regularly exceed 23 8C, and at sites such as Rawson Creek can reach as high as 37 8C (R. Retallick, unpublished data)." (12.19)

7. How Chytrid Fungus kills frogs

"Although our results do not detail the exact mechanism by which Batrachochytrium dendrobatidis disrupts epidermal functioning, the severe reduction in plasma electrolytes is a plausible cause of mortality. Reduced plasma osmolality and reduced plasma electrolyte concentrations, particularly hyponatremia (low sodium) and hypokalemia (low potassium), are potentially life-threatening conditions because these electrolytes are crucial in cell membrane function. In addition, sodium and potassium facilitate the conduction of action potentials in smooth and cardiac muscle and are important in multiple physiological processes. The 3 Litoria caerulea that appeared healthy despite infection had no significant changes in plasma osmolality or electrolyte concentrations. These results suggest that electrolyte reductions occur only in terminal stages of infection and may account for the neurological signs such as muscle tetany that precede death (Berger et al. 2007). While most amphibians can tolerate changes in plasma electrolyte levels (Deyrup 1964), the observed decrease of approximately 30% plasma sodium and 50% plasma potassium concentrations in diseased frogs may be too extreme." (12.21)

8. How Chytrid Fungus causes frog extinctions

"Both epidemiological theory and observations suggest that where a pathogen drives a host species to extinction, there is likely to be a reservoir host within which the pathogen has a reduced effect and is therefore maintained at a higher prevalence (McCallum and Dobson 1995, 2002). L. wilcoxii/ jungguy appears not to have declined at the same time as T. eungellensis and R. vitellinus and, in our data, it has a high prevalence of infection. It therefore is a candidate reservoir host. Whether the prevalence of B. dendrobatidis differs between the sibling species L. wilcoxii and L. jungguy at sites where they are sympatric would be an interesting question, but cannot be answered from our study. The L. lesueuri complex has a widespread distribution throughout streams on the east coast of Australia (Barker et al. 1995; Donellan and Mahony 2004) and could therefore play a substantial role in the maintenance and spread of chytrid infection throughout that region. If a species is acting as a reservoir, the prevalence of infection in that species should be higher than in species that are declining as a result of infection (McCallum and Dobson 1995). The prevalence of infection we observed in L. wilcoxii/jungguy exceeded that observed in T. eungellensis, but we do not have clear evidence that this represents a higher prevalence in the populations as a whole. It is also possible that B. dendrobatidis exists as a saprophyte in the environment independent of amphibians (Longcore et al. 1999; Johnson and Speare 2003), or may use tadpoles (which seem relatively little affected by the pathogen) as a reservoir (Daszak et al. 1999)." (12.19)

9. Controling the Chytrid Fungus epidemic

"Using heat, we cleared infection from half of the surviving frogs, leaving the other half infected, then continued to monitor mortality and weight. Mortality ceased among disinfected frogs but continued among infected frogs. Disinfected frogs gained weight significantly more than infected frogs, to the point of becoming indistinguishable from controls, demonstrating that at least some of the effects of sub-lethal chytridiomycosis on hosts can be non-permanent and reversible." (12.20)

"Exposure to 70% ethanol, 1 mg Virkon ml–1 or 1 mg benzalkonium chloride ml–1 resulted in death of all zoosporangia after 20 s. The most effective products for field use were Path-XTM and the quaternary ammonium compound 128, which can be used at dilutions containing low levels (e.g. 0.012 or 0.008%, respectively) of the active compound didecyl dimethyl ammonium chloride. Bleach, containing the active ingredient sodium hypochlorite, was effective at concentrations of 1% sodium hypochlorite and above. Cultures did not survive complete drying, which occurred after <3 h at room temperature. B. dendrobatidis was sensitive to heating, and within 4 h at 37°C, 30 min at 47°C and 5 min at 60°C, 100% mortality occurred." (12.11)

"Itraconazole has been used successfully both orally (2–13mg kg-1 daily for 9–28 days) and via shallow immersion (0.01% suspension for 5 minutes daily for 11 days) to treat some adult amphibians (Taylor et al., 1999; Nichols & Lamirande, 2000; Taylor, 2001). A commercial solution of malachite green (0.1mg litre-1) and formaldehyde (25 p.p.m.) (Formalite IIIs) effectively treated African clawed frog Xenopus tropicalis (Parker et al., 2002), but malachite green can cause developmental deformities and is therefore not recommended for use in threatened species. Although temperature elevation to 37C was effective in treating L. chloris (Woodhams et al., 2003), this treatment has not worked in other species (G. Marantelli, unpubl. data). Furthermore, some species may not tolerate these high temperatures. Itraconazole, fluconazole and F10SC Veterinary Disinfectants baths for tadpoles were either ineffective or toxic (Marantelli et al., 2000; B. McMeekin, unpubl. data). Treatment of water with terbinafine hydrochloride (2–4mg litre-1 for 7 days) appears to be non-toxic to tadpoles and its efficacy in eliminating infection is currently being investigated (B. McMeekin, unpubl. data)." (12.25)

"In conclusion, Tri-Gene and F10 are preferable for use in the field over the previously recommended DDAC products, as the former are active at much lower concentrations and appear to have no record of environmental toxicity. Any of these 3 disinfectants is recommended instead of bleach due to their efficacy at lower concentrations and less hazardous qualities." (12.22)

"Current ideas on ameliorating the effects of B. dendrobatidis on amphibian populations include practices that limit movement of the fungus among areas, such as quarantine (Australian Government, 2004). While such practices are important, it appears that this fungal species is already geographically widespread in many areas of the world and that containment will be difficult to achieve (Speare and Berger, 2000). An exciting aspect of a cutaneous antichytrid flora is that it could be harvested from resistant individuals and inoculated onto susceptible individuals. In addition, amphibians would likely transmit the beneficial skin flora among themselves during activities such as mating, aggressive interactions, and congregating in hibernacula. A flora that inhibits B. dendrobatidis may help amphibian individuals already affected by stressors such as pesticides, drier conditions, and high population density, to avoid the lethal effects of chytridiomycosis." (12.9)

"One factor that may contribute to disease resistance is innate immune defense. Innate skin defenses including antimicrobial peptides and symbiotic microbial barriers may be crucial for defending amphibians against the skin-invasive fungus, Bd (Harris et al., 2006;Woodhams et al., 2006a,b; Lauer et al., in press). Granular glands in the skin of R. muscosa secrete a mixture of peptides including antimicrobial peptides (ranatuerin-2Ma, 2Mb, and temporin-1M). These peptides inhibit the growth of Bd in vitro (Rollins-Smith et al., 2006). Symbiotic bacteria of amphibians are not well-known and have not been previously described in R. muscosa. However, the salamanders Plethodon cinereus and Hemidactylium scutatum host beneficial bacteria that inhibit growth of Bd in vitro and may be important for resisting Bd colonization of skin (Harris et al., 2006; Lauer et al., in press). Here we describe the microbiota of R. muscosa that contribute to resistance against Bd, and suggest that populations which host beneficial bacteria may be more likely to persist with Bd." (12.23)

9. Going forward

"Effective management of chytridiomycosis requires national surveillance to determine accurately disease distribution, protection of disease-free populations, rapid detection of and response to new outbreaks, restriction of amphibianmovements, implementation of national hygiene and quarantine protocols, and routine monitoring of stock for chytridiomycosis. Captive breeding can play a critical role in limiting the impact of chytridiomycosis on wild amphibian populations by providing supplemental numbers of threatened species for restocking and research (Australian Government Department of the Environment and Heritage, 2006). It may be possible to select for innate disease resistance by collecting tadpoles from declining populations, breeding from individuals that survive metamorphosis and restocking field sites with their progeny. Individuals must not be returned to their point of origin unless they can be shown to be disease-free, and diagnostic screening procedures must be implemented to ensure this. Augmenting remnant populations through restocking has been successfully used in Australia with Corroboree frog Pseudophryne corroboree and this strategy appears to have played an important role in slowing the decline of this Critically Endangered species (IUCN, 2006; G. Marantelli, unpubl. data). An intensive captive-breeding programme for the last remaining Wyoming toad Bufo baxteri population in Wyoming prevented extinction and efforts to reintroduce this species to the wild are under way (AmphibiaWeb, 2006). The success of this programme was largely a result of early recognition of the precarious status of wild populations, extensive collaborative captive-breeding efforts involving government and zoological institutions, and habitat protection through land purchase. The urgent need for captive-breeding programmes for a number of other threatened species has been recognized but many of these programmes are in their infancy owing to limited captive-breeding success. Restocking of threatened species may increase their chance of survival when high mortality rates threaten to cause extinction. This strategy can increase the time available for a species to develop disease resistance and maintain population numbers during adverse conditions that favour the pathogen. Captive reared amphibians used for restocking must be kept disease-free, and restocking must be implemented before the final population of a species is under threat, or extinction may not be preventable (Australian Government Department of the Environment and Heritage, 2006). This is demonstrated by the case of Sharp-snouted day frog Taudactylus acutirostris in Australia, where frogs and tadpoles were transferred from the last remaining population into captivity. As the wild population crashed from chytridiomycosis, the captive specimens also died from the disease, resulting in extinction of the species (Banks & McCracken, 2002; Schloegel et al., 2006; Australian Government Department of the Environment and Heritage, 2007)." (12.25)

"It is unlikely that a means for treating amphibian populations for chytridiomycosis in the field will be developed in the near future, so manipulative methods for controlling the pathogen should concentrate on captive populations and the development of appropriate strategies for reintroductions. In some cases, it has proved possible to treat captive frogs for chytridiomycosis, either with elevated temperatures (Woodhams et al. 2003) or by chemotherapy (Parker et al. 2002). Further research is necessary to identify appropriate strategies for reintroduction of captive-reared animals to areas from which they have disappeared." (12.17)

"Reducing or banning the trade and transport of research amphibians would directly and negatively impact the research community and may actually increase the risk of disease release as independent research facilities attempt to accommodate more Xenopus lines." (12.26)

"In the United States, the U.S. Department of Agriculture (USDA) may be the most suitable regulatory body for the bullfrog trade since these animals are a commercial agricultural product. No current USDA regulations exist for the identification of infectious agents in amphibians." (12.16)

"With the use of validated assays and sampling protocols for the detection of B. dendrobatidis, it is now possible to undertake validated testing and surveys within both individual animals and populations. This can include studies involving the pathogenesis of chytridiomycosis, assessing the absence of B. dendrobatidis in captive breeding programs and in endangered species, in addition to the screening of the environment for the fungus by swabbing endemic amphibians, other organisms and inanimate objects." (12.10)

"We believe that the state of knowledge, and science, of the epidemiology of chytridiomycosis has reached the stage where a dialog can successfully be opened between major stakeholders, namely the Government, animal importers, traders and breeders, zoological gardens, scientists and research organisations. Initiating such a dialog is increasingly necessary in order to take measures to control the emergence of this increasingly destructive pandemic infectious disease." (12.6)

"If it could be shown that susceptible frog populations were able to develop resistance to the amphibian chytrid, then captive breeding and artificial selection for resistance would provide an avenue for management of this threat to critically endangered species." (12.19)

"One exciting area of hope is the possibility that there may be evolution of either resistance in frogs or decreased virulence in the pathogen following initial declines (Retallick et al. 2004). If this is the case, then selection for resistance in captive populations before reintroduction may be the best longtermprospect for the recovery of populations affected by B. dendrobatidis." (12.17)

"There is no quick fix when confronting such a volume of trade, and indeed sharp regulatory responses to questionable wildlife trade practices have historically yielded other than the desired results. Imposing restrictions and outright bans on trade has consistently driven trade underground, and some estimate the black market trade in wildlife to be second only to drugs in terms of economic value." (12.26)

"We agree that the financial benefits of the amphibian pet trade may currently be less than the food or research trades and that this component of the amphibian trade maybe more difficult to justify. Nevertheless, it is still a reality and we suggest that regulation should first be made for the benefit of the trade rather than in direct opposition to it. Early experiences with the pet trade in Europe have been quite encouraging. Most exhibitors at a regional dendrobatid trade show were willing to participate in swab sampling for qPCR testing, pet shop owners in the United Kingdom were far more likely to participate in a survey than were zoo and research staff, and the general outcome of survey work is that most involved in the pet trade would not be adverse to improving amphibian health surveillance Trenton W. J. Garner et al. and certification (E. Wombwell, unpublished data, 2008). Costs of surveillance and certification would be passed on directly to the consumer and could serve to reduce the volume of trade without impacting the economic benefits to wholesalers and retailers. Ideally, the move should be toward captive bred species (a move that is already to some degree underway), which would facilitate both screening and treatment if and when infectious disease is detected. However, there are conservation benefits to be accrued from the pet trade sector as it stands." (12.26)

"Many species in the pet trade are closely related both phylogenetically and ecologically to important target conservation species. These species can be used to train staff at regional centers so that when target species are brought into captivity the likelihood of successful ex situ programs will improve. In cases where target species are in the pet trade, they may prove to be the best or only source of breeding stock. This is no small beer; most zoos have far better developed reptile husbandry and very few institutions boast of breeders and keepers who are specialized in amphibian care, health, and reproduction. Some zoos are already using amphibians purchased from the private sector to develop the skills necessary to implement the Amphibian Ark plans. From a conservation perspective, this is not the time to alienate the pet trade sector, which may be the most useful repository of captive breeding and husbandry know how and arguably has the greatest success rate at breeding rare, difficult to keep, and difficult to breed species." (12.26)

10. My response to the proposal

I have researched the Chytrid Fungus epidemic and I have the following observations:

1. Chytrid Fungus is present on every continent except Asia
2. Chytrid Fungus is spread globally via scientific and medical research as well as agricultural/aquacultural food production
3. Chytrid Fungus is spread locally via infected amphibians, water and moist soil containing the fungus
4. The pet trade has not played a very significant role in the spread of Chytrid Fungus
5. The pet trade maintains and breeds a large number of captive exotic frog species and has general husbandry and captive breeding know-how that zoos and scientific institutions are just beginning to understand
6. The pet trade is willing to participate in preventing the spread of Chytrid Fungus
7. Government, scientific and medical institutions, agriculture/aquaculture, zoos and the pet trade are all valuable participants in the fight against the spread of Chytrid Fungus and the control of Chytrid Fungus

11. My conclusion

I feel that the role of the U.S. Fish & Wildlife Service should be to facilitate further research, cooperation and coordination among all interested parties. At this time, I am opposed to adding all amphibians to the Lacy Act, unless certified as Chytrid free. I feel that the Lacy Act was never intended to be used in such a way and is the wrong way to address this very serious problem.

12. LITERATURE CITED:

12.1 Gascon, C., Collins, J.P., Moore, R.D., Church, D.R., McKay, J.E. & Mendelson, J.R., III (eds) (2007) Amphibian Conservation Action Plan. IUCN/SSC Amphibian Specialist Group, Gland, Switzerland/Cambridge, UK, 59 pp.

12.2 Lee Francis Skerratt, Lee Berger, Richard Speare, Scott Cashins, Keith Raymond McDonald, Andrea Dawn Phillott, Harry Bryan Hines, Nicole Kenyon (2007) Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs, EcoHealth 2007 DOI: 10.1007/s10393-007-0093-5

12.3 Speare R, Berger L. Global distribution of chytridiomycosis in amphibians. World Wide Web -

http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm. 11 November 2000.

12.4 Claudio Soto-Azat1, Barry T. Clarke, John C. Poynton, Andrew A. Cunningham (2010) Diversity and Distributions Volume 16, Issue 1, pages 126–131, January 2010 DOI: 10.1111/j.1472-4642.2009.00618.x

12.5 P. Daszak, A. Strieby, A. A. Cunningham, J. E. Longcore, C. C. Brown, D. Porter. (2004) Experimental Evidence That The Bullfrog (R. Catesbeiana) Is A Potential Carrier Of Chytridiomycosis, An Emerging Fungal Disease Of Amphibians, Herpetological Journal, Vol. 14, pp. 201-207 (2004)

12.6 Matthew C. FISHER, Trenton W. J. GARNER (2007) The relationship between the emergence of Batrachochytrium dendrobatidis, the international trade in amphibians and introduced amphibian species,

12.7 Trenton W. J. Garner, Matthew W. Perkins, Purnima Govindarajulu, Daniele Seglie, Susan Walker, Andrew A. Cunningham and Matthew C. Fisher, (2006) The emerging amphibian pathogen Batrachochytrium dendrobatidis globally infects introduced populations of the North American bullfrog, Rana catesbeiana, Biol. Lett. (2006) 2, 455–459 doi:10.1098/rsbl.2006.0494 Published online 24 May 2006

12.8 Oullet M, Mikaelian I, Pauli BD, Rodrigue J, Green DM. (2005) Historical evidence of widespread chytrid infection in North American amphibian populations, Conservation Biology 2005;19:1431-1440

12.9 Harris RN, James TY, Lauer A, Simon MA, Patel A. (2006) Amphibian pathogen Batrachochytrium dendrobatidis is inhibited by the cutaneous bacteria of amphibian species. EcoHealth 2006;3:53-56. DOI: 10.1007/s10393-005-0009-1

12.10 Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, Campbell R, Marantelli G, Gleason F, Colling A. (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms 2007;73:175-192

12.11 Johnson M, Berger L, Phillips L, Speare R. (2003) In vitro evaluation of chemical disinfectants and physical techniques against the amphibian chytrid, Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms 2003;57:255-260

12.12 Johnson M, Speare R. (2003) Survival of Batrachochytrium dendrobatidis in water: quarantine and control implications. Emerging Infectious Diseases 2003;9(8):922-925

12.13 Kirshtein JD, Anderson CW, Wood JS, Longcore JE, Voytek MA. (2007) Quantitative PCR detection of Batrachochytrium dendrobatidis DNA from sediments and water. Diseases of Aquatic Organisms 2007;77:11-15

12.14 Kriger KM, Hero JM, Ashton KJ. (2006) Cost efficiency in the detection of chytridiomycosis using PCR assay. Diseases of Aquatic Organisms 2006;71:149-154

12.15 Longcore JR, Longcore JE, Pessier AP, Halteman WA. (2007) Chytridiomycosis widespread in anurans of northeastern United States. Journal of Wildlife Management 2007;71(2):435-444

12.16 Mazzoni R, Cunningham AC, Daszak P, Apolo A, Perdomo E, Speranza G. (2003) Emerging pathogen of wild amphibians in frogs (Rana catesbiana) farmed for international trade. Emerging Infectious Diseases 2003;9(8):995-998

12.17 McCallum H. (2005) Inconclusiveness of chytridiomycosis as the agent in widespread frog declines. Conservation Biology 2005;19(5):1421-1430

12.18 Rachowicz LJ, Hero JM, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, Collins JP, Briggs CJ. (2005) The novel and endemic pathogen hypothesis: competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology 2005;19(5):1441-1448

12.19 Retallick RWR, McCallum H, Speare R. (2004) Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLOS Biology 2004;2(11):e351

12.20 Retallick RWR, Miera V. (2007) Strain differences in the amphibian chytrid Batrachochytrium dendrobatidis and non-permanent, sub-lethal effects of infection. Diseases of Aquatic Organisms 2007;75:201-207

12.21 Voyles J, Berger L, Young S, Speare R, Webb R, Warner J, Rudd D, Campbell R, Skerratt LF. (2007) Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Diseases of Aquatic Organisms 2007;77:113-118

12.22 Webb R, Mendez D, Berger L, Speare R. (2007) Additional disinfectants effective against the amphibian chytrid fungus Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms 2007;74:13-16

12.23 Woodhams DC, Vredenburg VT, Simon MA, Billheimer D, Shakhtour B, Yu Shyr, Briggs CJ, Rollins-Smith LA, Harris RN. (2007) Symbotic bacteria contribute to innate immune defences of the threatened mountain yellow-legged frog, Rana muscosa. Biological Conservation 2007:138(3-4):390-398

12.24 Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R. (2007) Origin of the amphibian chytrid fungus. Emerging Infectious Diseases 2004;10(12):2100-2105

12.25 Young S, Berger L, Speare R. (2007) Amphibian chytridiomycosis: strategies for captive management and conservation. International Zoo Yearbook 2007;41:1-11

12.26 Trenton W. J. Garner, Ian Stephen, Emma Wombwell, Matthew C. Fisher. (2009) The Amphibian Trade: Bans or Best Practice? EcoHealth DOI: 10.1007/s10393-009-0233-1