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Largemouth Bass Virus: An Emerging Problem for Warmwater Fisheries?

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Abstract.—Largemouth bass virus (LMBV) is a recently discovered pathogen of warmwater fishes, most notably largemouth bass *Micropterus salmoides*. Its association with conspicuous fish kills has led to continuing concern about its origin, spread, and potential impact on wild fisheries. This, in turn, has helped foster ongoing research into the biology, pathogenesis, diagnosis and epidemiology of the virus. This article attempts briefly to summarize what is currently known about LMBV and to suggest how future research might contribute to answering the many questions that still remain.

Introduction

In 1995 investigation of a kill of approximately 1,000 largemouth bass in the Santee Cooper Reservoir of South Carolina led to the discovery of a new virus, largemouth bass virus (LMBV; Plumb et al. 1996). The appearance of this pathogen was surprising; no viruses had previously been associated with either systemic infection or epidemic mortality in wild largemouth bass. The finding generated considerable concern among fisheries biologists and anglers, leading to sustained public interest and scientific research.

LMBV was classified tentatively as an iridovirus (family *Iridoviridae*; Plumb et al. 1996), a finding later confirmed by molecular analyses (Mao et al. 1999). Iridoviruses are a diverse family of large DNA viruses infecting a variety of heterothermic vertebrate and invertebrate hosts (Williams 1996). LMBV is specifically a member of the genus *Ranavirus*. As such, it is closely related to certain iridoviruses of fish, amphibians and reptiles. It is distantly related to lymphocystis disease virus (genus *Lymphocystivirus*), a well-characterized iridovirus of fish with a broad host range (Leibovitz 1980; Anders 1989; Iidona and Darai 1997). Within the ranaviruses, LMBV is nearly identical (98% of nucleotide positions in the major capsid protein and viral methyltransferase genes) to Doctor fish virus (DFV) and Guppy virus 6 (GV6), two previously-characterized iridoviruses of *Labroides dimidatus* and *Poecilia reticulata*, respectively (Mao et al. 1999). This close relationship suggests that LMBV may have originated from the introduction of an exotic (Southeast Asian) pathogen of ornamental fish into North American waters.

LMBV has been documented only in the United States. Since its discovery in 1995, and through 2002, it has been found in 17 states (Figure 1). The occurrence of the first LMBV-associated fish kills in the Southeast seemed at first to imply a westward and northward expansion from a southeastern epicenter. Subsequent surveys have revealed, however, that LMBV is highly prevalent throughout its known range (Plumb et al. 1999; B.A.S.S. 2001, 2002). The apparent Southeast to Northwest expansion of LMBV may be real, or it may reflect the directed sampling efforts of states at the borders of the expanding known range. At present, not all states have surveyed for LMBV. The geographic distribution of the virus is, therefore, almost certainly more extensive than that shown in Figure 1.

LMBV has been found in populations of bass that have experienced documented fish kills, and also in apparently healthy populations. When LMBV has been found in association with fish kills, certain clinical features are characteristic (Plumb et al. 1999; B.A.S.S. 2001). Fish of both sexes, multiple age classes, and different genetic constitutions are affected. Affected fish float to the surface and exhibit loss of equilibrium prior to death, but show no external lesions. Hyperemia (increased blood flow, leading to darkened coloration) is occasionally seen, but is not consistently associated with infection. Internally, many

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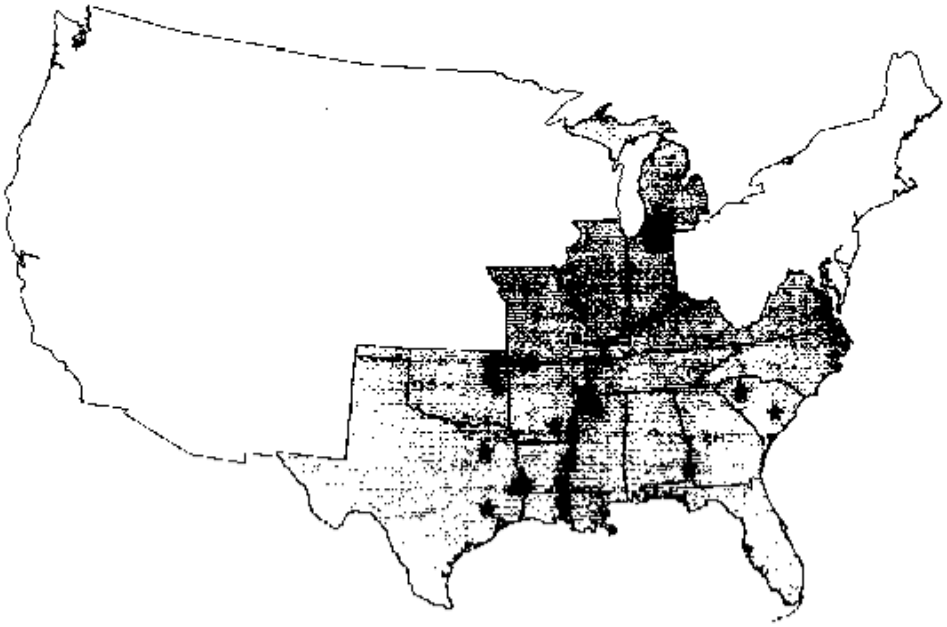

Figure 1. Map of the United States, showing the known geographic distribution of largemouth bass virus (as of 2002). States in which the virus has been identified are shaded. Stars represent the locations of documented LMBV-associated fish kills.

(but not all) fish display exudative inflammation and enlargement of the swim bladder (Plumb et al. 1999; Zilberg et al. 2000; B.A.S.S. 2002). Finally, kills tend to be overwhelmingly dominated by largemouth bass. Other centrarchid species and, preliminarily, the chain pickerel *Esox niger* have been found to carry the virus in the wild, but so far none appear to have shown clinical signs (B.A.S.S. 2001, 2002). The virus is experimentally pathogenic in striped bass *Morone saxtalis* (Plumb and Zilberg 1999a), but no LMBV-associated kills have been documented in this species. The exact mechanism by which LMBV kills fish is not yet known.

The modes of transmission and pathogenesis of LMBV are not well understood. Experimentally, bass can be infected by injection with cultured virus, immersion in water containing virus, cohabitation with infected fish, or ingestion of experimentally-infected prey (Plumb and Zilberg 1999a; B.A.S.S. 2001). Both direct transmission and transmission from reservoir species (e.g., prey) could maintain the virus in bass populations. Furthermore, LMBV can remain viable for extended periods in water and in frozen fish (7 and ≥ 155 days, respectively; Plumb and Zilberg 1999b; B.A.S.S. 2002), suggesting that persistence in the physical environment could also be important.

The epidemiology of LMBV infection and clinical disease has been difficult to investigate. Surveys for the virus have not generally been systematic. Extensive surveys have been hampered by the lack of a nonlethal diagnostic test. The current diagnostic test for LMBV involves isolation of the virus from homogenized visceral tissues, and, therefore, requires sacrifice of the fish. As a result, practical testing of populations for LMBV has become difficult and politically charged. This is especially so because the generally-accepted ideal sample for LMBV testing is 60 fish per population. This number was derived from a binomial sampling distribution to reflect the sample size that would yield a 95 percent probability of detection of at least one positive fish in a population with a prevalence of disease of five percent. This number, however, assumes perfect sensitivity and specificity of the test. In fact, the sensitivity and specificity of the current test for LMBV have not been reported, making both negative and positive test results difficult to interpret. Fortunately, nonlethal test development is currently underway. Future tests may involve PCR amplification of viral DNA from biopsied tissues and/or immunological (ELISA) tests for antibodies to LMBV in the sera of fish. The latter test strategy would have the added advantage of being able to

indicate prior exposure of a fish to LMBV, not just active infection.

Despite the current lack of a nonlethal diagnostic test, some states are surveying for LMBV. The most extensive survey to date has been conducted in Texas, and has spanned multiple years (B.A.S.S. 2002). In Texas, LMBV was found in 19/54 (35%) of reservoirs tested across the state, and in 9/14 (64%) of the major river basins in which these reservoirs are located. Prevalences of LMBV-positive fish in individual reservoirs in 2000 ranged from 1.7 to 13.3 percent, with a mean prevalence overall of 5 percent.

A central database specific for LMBV is being maintained by the U. S. Fish and Wildlife Service Warm Springs Regional Fisheries Center, in addition to the recently released National Wild Fish Health Survey Database. Preliminary results of these surveys indicate that the virus is highly prevalent both as a proportion of total samples tested within states (between 7% and 49%) and as a proportion of sites tested (between 22% and 75% of sites in states in which at least 10 sites were tested; B.A.S.S. 2001). Within lakes the prevalence of LMBV appears to vary considerably from year to year and may decline after a fish kill. Lakes positive in a given year appear to remain so, but "conversions" from negative to positive status have been documented.

The emerging pattern is that LMBV tends to strike in summer months, possibly precipitated by stressors such as elevated temperature, low oxygen, and angling. Because sampling and observation have been neither systematic nor random with respect to season, strong inferences should not yet be made from this pattern. LMBV-associated fish kills have not been reported to occur in lakes repeatedly in consecutive years, but to date longitudinal observations have been limited. Lakes that do experience fish kills also suffer observable declines in catch-per-unit-effort and possibly in the proportion of large fish caught, but these effects seem to be transient. LMBV, therefore, appears to be as variable over time as it is across space.

Future directions

Whenever new pathogens are discovered, debate inevitably ensues as to whether the pathogen is truly new, or surveillance and detection methods have simply improved. LMBV is no exception. LMBV could be a recently introduced exotic pathogen, or it might have existed in the United States for years unnoticed. An iridovirus genetically identical (in a portion of the major capsid protein gene sequence) to LMBV was recovered in 1991 from largemouth bass in Lake Weir, Florida (B.A.S.S. 2002). LMBV may, therefore, have been present in the U. S. for years before its characterization in 1995.

The high degree of genetic similarity between LMBV and DFV/GV6 (Mao et al. 1999) and among LMBV isolates (Plumb et al. 1999) has implied to some that LMBV has only recently entered North America. Such conclusions are unjustified, however, without accurate knowledge of the rate at which the iridoviral major capsid protein gene evolves. Indeed, it is known that this gene is conserved enough to be useful for the systematic classification of iridoviruses (Tidona et al. 1998), but that within iridoviral species variation is minimal or nonexistent (Hyatt et al. 2000). As a result, the utility of the iridoviral major capsid protein gene for molecular epidemiological inference is questionable.

Nevertheless, LMBV is an emerging pathogen in the sense that its visibility in the scientific and political arenas is increasing. As such, LMBV is an excellent model system for understanding both the politics and the biology of emerging infections wildlife/fisheries diseases, and the ways in which these interact. For example, public concern as represented by angling interest groups has helped drive the focus of LMBV research. The questions, hypotheses, and preliminary data outlined in this article originated largely from LMBV Workshops sponsored by the Bass Anglers Sportsman Society.

One of the most vexing questions about LMBV, recognized by scientists and nonscientists alike, is why some populations of infected bass experience fish kills while others, also infected, remain clinically normal. In the broadest terms, the variability in clinical response to LMBV infection could result from factors operating at one, two, or all of three interacting levels: the host, the pathogen and the environment. Some potential factors are given in Table 1.

On the "host side," certain populations of largemouth bass may possess innate resistance or susceptibility to LMBV. Previous exposure to LMBV or to similar pathogens with which LMBV cross-reacts immunologically could render bass populations resistant to fish kills as a result of "herd immunity." Host genetics may also play a role. Bass populations may be either excessively inbred or outbred, for example, either of which could reduce host immunity (Allendorf and Leary 1986; Templeton 1986).

On the "pathogen side," the degree of genetic variability of LMBV is largely unknown. The existence of multiple strains is entirely possible, for

Table 1. Factors that may account for the variability in clinical response of fish populations to infection with LMBV.

Factor[a]	Proposed mechanism
Host factors	
Host immune status	Increased susceptibility in populations immunologically naïve to LMBV or similar pathogens
Host genetics	Immune system variability
Innate susceptibility/resistance	Increased rates of infection/viral replication in genetically-predisposed populations
Inbreeding depression[b]	Increased vulnerability of populations with low immunological heterogeneity
Outbreeding depression[b]	Lowered resistance due to disruption of coadapted immune system gene complexes
Pathogen factors	
Strain variation	Strain-related variation in virulence and/or strain-specific virulence factors
Other pathogens[c]	Pathogenic interactions with unidentified coinfecting agents
Environmental factors	
Environmental factors affecting hosts	Physiological stress and immunosuppression
High temperature	Lowered physiological resistance to viral infection and replication
Low oxygen	Lowered physiological resistance to viral infection and replication
Dissolved toxins	Lowered physiological resistance to viral infection and replication
Angling/tournament stress	Behavioral stress, lowered physiological resistance and increased contact rates
Crowding[d]	Behavioral stress, lowered physiological resistance and increased contact rates
Environmental factors affecting the pathogen	Altered viral survival and replication
Temperature[e]	Rapid viral replication and maximum infectivity near optimal temperature
Water quality[f]	Altered efficiency of viral transmission and persistence in the environment
Reservoir hosts[g]	Enhanced efficiency of viral transmission and persistence in the environment

[a]Factors may interact positively, such that multiple factors may be necessary to precipitate a fish kill.
[b]Both inbreeding depression and outbreeding depression could occur naturally (e.g. as a result of natural cycles of population expansion and decline) or artificially (e.g. as a result of stocking).
[c]To date, no coinfecting agents have been identified in consistent association with LMBV-related fish kills.
[d]Crowding could occur naturally (e.g. during the breeding season or because of congregation around localized resources) or artificially (e.g. due to intensive management practices such as in hatchery rearing).
[e]The optimal temperature for LMBV replication in cell lines under laboratory conditions is 30° C (Piaskoski et al. 1999).
[f]Water quality factors directly affecting the pathogen could include such variables as pH, organic content and concentrations of dissolved solids.
[g]Reservoir hosts that might be especially important are those that interact directly with largemouth bass (e.g. predator or prey species).

several reasons. First, iridoviruses are notoriously variable as a family, as are ranaviruses as a genus (Williams 1996). Second, the current diagnostic test for LMBV uses cultured cells permissive for a wide variety of iridoviruses, and that test may not discriminate between genetically different strains (Piaskoski et al. 1999). Third, the only gene thus far sequenced in geographically-distinct LMBV isolates (the major capsid protein gene, also targeted for PCR-based diagnostic test confirmation) is known to be conserved within iridoviral species (Hyatt et al. 2000). Lack of genetic differences between LMBV isolates in the DNA sequence of this gene (Plumb et al. 1999) does not, therefore, preclude the existence of strain-level variability at other loci. Indeed, preliminary evidence indicates that, in a controlled laboratory setting, the original South Carolina LMBV isolate may kill bass more quickly than isolates from Illinois, where no LMBV-related fish kills have been confirmed to date (B.A.S.S. 2002).

If genetically-distinct viral strains do exist, then molecularly-based diagnostic tests could be developed to distinguish between strains of low and high

virulence. Phylogenetic analysis of a suitably variable viral locus or loci could help reconstruct the origin and spread of LMBV within the United States. Such data would ideally be coupled with information about the actual geographic distribution of the virus, and about its prevalence in different watersheds. The epicenter of the epidemic should correspond to an area of high prevalence and high viral genetic diversity. Viruses from this area should appear basal on a phylogenetic tree of isolates collected from throughout the full geographic range of the virus.

On the "environment side," environmental factors could affect either the host or the pathogen (or both) directly. For example, there is a general consensus that LMBV-associated fish kills are precipitated by physiological stress. Most kills occur in late summer, at peaks of water temperature and nadirs of dissolved oxygen, and when angling pressure is heaviest. High temperature, however, is a condition that would also favor optimal viral replication, which has been shown to occur at 30°C in the laboratory (Hlaskoski et al. 1999). LMBV-associated fish kills may, therefore, result from the synergistic interactions of viral infection, reduced host immune capacity, maximum potential for transmission, and optimal conditions for viral replication. Water quality declines, anthropogenic toxins, and habitat degradation could very well potentiate such effects as well.

Finally, studies are needed to investigate the modes of transmission of LMBV between water bodies. Natural LMBV reservoir species may exist that could play a role in the geographic spread of the virus. LMBV has already been isolated from wild fishes other than largemouth bass (B.A.S.S. 2001, 2002); it could have a host range broad enough to infect even nonfish species, such as amphibians, that are capable of transporting the virus across land barriers. The roles of warm-blooded animals (e.g., waterfowl) may prove important also, especially if LMBV can be shown experimentally to survive passage through the gastrointestinal tract of such species. The potential for anglers and boats to transmit LMBV between water bodies should not be ignored, especially considering preliminary results indicating that LMBV may be resistant to disinfection with standard concentrations of bleach and phenol-based disinfectants (B.A.S.S. 2002).

Understanding the modes of LMBV transmission would be necessary for limiting the entry of the pathogen into uninfected waters. Similarly, understanding what factors increase or decrease the susceptibility of bass populations to LMBV would be critical to ameliorating the effects of the virus where it has arrived. Elucidating the biology of LMBV in both of these respects should be a primary goal of future LMBV research.

Is LMBV a Threat?

Is LMBV a threat to the sustainability of warmwater fisheries? Given that kills have not yet been reported for LMBV-positive lakes in consecutive years, and given that the population-level effects of LMBV appear to be transient, it is tempting to conclude that the answer is "no". This conclusion, however, would be premature. Disease epidemics are not unknown in largemouth bass (e.g., Noga et al. 1990; Francis Floyd et al. 1993), but none have been as extensive across space or as protracted over time as that attributed to LMBV.

Too little is yet known about the pathogenesis, epidemiology, and natural history of the virus to predict its future impact. LMBV is probably not, in and of itself, sufficient to cause fish kills. Rather, it appears to interact in complex ways with other environmental factors in precipitating such events. Sub-lethal effects of LMBV infection may also exist. Even if the virus does not kill fish outright, it could alter their behavior or physiology in ways that decrease a population's sustainability or its utility to recreational anglers. Given this, and given the fact that LMBV does not appear to be present everywhere throughout its range, it is best seen as a pathogen in the process of emerging. Its consequences have yet to be played out in full.

Perhaps the greatest concern is warranted not for the specific effects of LMBV, but rather for what the presence of this new pathogen may signify. Wildlife diseases in general appear to be emerging at an accelerated rate (Dobson and Foufopoulos 2001). The growing consensus is that anthropogenic environmental change underlies this phenomenon (Daszak et al. 2001). The expanding impact of human populations on the natural environment and the concomitant pressures levied on wildlife may account for the recent emergence of so many novel pathogens.

If so, then LMBV might be a harbinger of things to come. LMBV itself may turn out to be only a minor player in the population dynamics of warmwater fish populations, but it should give us cause for concern anyway. It may be an early indicator of a coming wave of health-related problems in which the sustainability of our wild fisheries declines by our own hand.

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References

Allendorf, F. W., and R. F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. Pages 57–76 *in* M. E. Soulé, editor. Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Inc., Sunderland, United Kingdom.

Anders, K. 1989. Lymphocystis disease of fishes. Pages 141–160 *in* W. Ahne and E. Krustak, editors. Viruses of lower vertebrates. Springer-Verlag, New York.

B.A.S.S. 2001. Report from the 2001 Largemouth Bass Virus Workshop. Bass Anglers Sportsman Society, Jacksonville, Florida.

B.A.S.S. 2002. Report from the 2002 Largemouth Bass Virus Workshop. Bass Anglers Sportsman Society, Little Rock, Arkansas.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change, and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Dobson, A., and J. Foufopoulos. 2001. Emerging infectious pathogens of wildlife. Philosophical Transactions of the Royal Society of London, Series B 356:1001–1012.

Francis-Floyd, R., P. Reed, B. Bolon, J. Estes, and S. McKinney. 1993. An epizootic of *Edwardsiella tarda* in largemouth bass (*Micropterus salmoides*). Journal of Wildlife Diseases 29:334–336.

Hyatt, A. D.and seven coauthors. 2000. Comparative studies of piscine, and amphibian iridoviruses. Archives of Virology 145:301–331.

Leibovitz, L. 1980. Lymphocystis disease. Journal of the American Veterinary Medical Association 176:202.

Mao, J., J. Wang, G. D. Chinchar, and V. G. Chinchar. 1999. Molecular characterization of a ranavirus isolated from largemouth bass *Micropterus salmoides*. Diseases of Aquatic Organisms 37:107–114.

Noga, E. J., R. A. Bullis, and G. C. Miller. 1990. Epidemic oral ulceration in largemouth bass (*Micropterus salmoides*) associated with the leech *Myzobdella lugubris*. Journal of Wildlife Diseases 26:132–134.

Piaskoski, T. O., J. A. Plumb, and S. R. Roberts. 1999. Characterization of the largemouth bass virus in cell culture. Journal of Aquatic Animal Health 11:45–51.

Plumb, J. A., J. M. Grizzle, H. E. Young, A. D. Noyes, and S. Lamprecht. 1996. An iridovirus isolated from wild largemouth bass. Journal of Aquatic Animal Health 8:265–270.

Plumb, J. A.and five coauthors. 1999. Isolation and identification of viruses from adult largemouth bass during a 1997–1998 survey in the southeastern United States. Journal of Aquatic Animal Health 11:391–399.

Plumb, J. A., and D. Zilberg. 1999a. The lethal dose of largemouth bass virus in juvenile largemouth bass and the comparative susceptibility of striped bass. Journal of Aquatic Animal Health 11:246–252.

Plumb, J. A., and D. Zilberg. 1999b. Survival of largemouth bass iridovirus in frozen fish. Journal of Aquatic Animal Health 11 94–96.

Templeton, A. R. 1986. Coadaptation and outbreeding depression. Pages 105–116 *in* M. E. Soulé, editor. Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Inc., Sunderland, United Kingdom.

Tidona, C. A., and G. Darai. 1997. Molecular anatomy of lymphocystis disease virus. Archives of Virology, Supplemental 13:49–56.

Tidona, C. A., P. Schnitzler, R. Kehm, and G. Darai. 1998. Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution? Virus Genes 16:59–66.

Williams, T. 1996. The iridoviruses. Advances in Virus Research 46:345–412.

Zilberg, D., J. M. Grizzle, and J. A. Plumb. 2000. Preliminary description of lesions in juvenile largemouth bass injected with largemouth bass virus. Diseases of Aquatic Organisms 39:143–146.