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ABSTRACT: A 22.4-ha impoundment experienced an outbreak of *Largemouth bass* (*Micropterus salmoides*) *virus* (LMBV) disease in the summer of 2006. All dead or dying largemouth bass observed throughout the entire event were recorded and removed. In this study, we estimated mortality and examined size distribution, condition, and biomass following the outbreak. Boat-mounted electrofishing was used to collect largemouth bass for a mark-recapture population estimate and other population metrics. Fish samples were examined for evidence of LMBV, other infectious diseases, and physical abnormalities. Cell cultures inoculated with samples from moribund fish developed cytopathic effects typical of LMBV, and polymerase chain reaction (PCR) confirmed the presence of LMBV. The total number ($N\pm95\%$ confidence interval) of stock-size largemouth bass remaining was estimated to be $2,301\pm528$ fish (103 bass/ha). The total observed mortality, including dead and dying individuals, during the LMBV outbreak was 176 largemouth bass (7% of the initial population). The total biomass remaining was estimated at 1,592 kg of stock-size bass and a relative biomass of 71.5 kg of stock-size largemouth bass per hectare. Largemouth bass size structure was dominated by quality and preferred (300–510 mm) size classes, with very few memorable-size or larger (>510 mm) fish, and the relative weight of largemouth bass was unusually variable. These results demonstrate that largemouth bass abundance and biomass in the reservoir remained very high despite mortalities attributed to a LMBV outbreak.

Key words: Epizootic, infectious diseases, largemouth bass virus, population effects.

INTRODUCTION

Largemouth bass virus (LMBV; family *Iridoviridae*; Plumb et al, 1996) is a pathogen that has become widely distributed throughout the southeastern and midwestern United States (Goldberg, 2002). This virus is known to contribute to high morbidity and mortality in wild largemouth bass (*Micropterus salmoides*) in North America, particularly adult fish (Grizzle and Brunner, 2003). Fish become infected either through the water (Plumb and Zilberg, 1999) or by consuming infected prey (Woodland et al., 2002). Infected fish lose equilibrium, tend to float near the surface, and may display characteristically enlarged or reddened swim bladders (Plumb et al., 1996). The virus has been associated with large-scale largemouth bass fish kills, particularly at warmer water temperatures. The fish that recover from the viral infection usually become chronic carriers of the virus (Hanson et al., 2001).

Outbreaks of LMBV appear to impact the size structure of largemouth bass populations by eliminating larger individuals. The virus was linked to threefold to 20-fold declines in memorable-size (≥2.27 kg or 51 cm) largemouth bass catch rates in Alabama, USA (Maceina and Grizzle, 2006). This corresponded to an increase of up to 43-fold in the fishing effort required to catch a fish >2.27 kg, suggesting that LMBV can have disastrous implications for trophy largemouth bass fisheries. However, there has not been documentation of the changes in the total number of fish following LMBV outbreaks and associated fish kills (Grizzle and Brunner, 2003).

A 22.4-ha reservoir in Arkansas, USA, experienced an outbreak of largemouth bass virus disease in the summer of 2006, which resulted in mass mortalities of largemouth bass that spanned 3 mo. This impoundment supported a valuable day-lease fishery, where anglers pay on a daily basis for the opportunity to catch and release largemouth bass. Anglers were likely

the source of the outbreak via contaminated boats, trailers, or tackle because no new fish releases were known to occur since 2002. Because of the economic importance and relatively small size of this system, the landowner removed and documented every dead or dying largemouth bass that could be found two to three times daily throughout the entire event. The objectives of this study were to estimate the percentage of largemouth bass lost to the mass mortality event and to examine the size distribution, condition, and biomass following a LMBV outbreak.

METHODS

Study site

This study occurred on a 22.4-ha well-fed impoundment near Stuttgart, Arkansas, USA (34°32′N, 91°41′W), which is leased on a daily basis for catch-and-release largemouth bass fishing. The lake was renovated in 2000 and restocked in the same year with green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), threadfin shad (*Dorosoma petenense*), gizzard shad (*Dorosoma cepedianum*), and grass carp (*Ctenophayngodon idella*). In April 2002, 5,500 15-cm Florida largemouth bass (*Micropterus salmoides floridanus*) were added into the established prey population and had reportedly grown up to 4 kg by the spring of 2006.

Beginning in July 2006, dead and dying bass were observed in the lake. The lake owner made two to three trips around the lake daily to recover sick and dead fish, using a boat when necessary, and maintained accurate records of the number of fish collected. The event lasted until the end of September. Five moribund fish collected during the epizootic (late July) were transported alive to the University of Arkansas at Pine Bluff (Arkansas, USA), where they were examined microscopically for parasites and sampled for bacteriologic examination. Spleen and trunk kidneys from each fish were homogenized and inoculated onto fathead minnow

(FHM) cell cultures, followed by confirmatory polymerase chain reaction (PCR) techniques for detection of LMBV using standard Blue Book procedures (USFWS and AFS-FHS, 2007) with primers described by Grizzle et al. (2003).

Population sampling

Boat-mounted electrofishing standardized to an output of 3,000 W (Burkhardt and Gutreuter, 1995) was used to collect largemouth bass for a mark-recapture population estimate and other population metrics. The entire shoreline and available offshore habitat was electrofished on 6 October 2006, all largemouth bass collected were measured and weighed, and stock-size fish (≥200 mm) were marked by clipping the left pelvic fin before release.

Marked fish were given sufficient time to reintegrate into the population before recapture on 10 November 2006. During the recapture sampling, the entire shoreline and open water habitat was sampled using boat-mounted electrofishing, and all largemouth bass were collected, measured, and examined for marks. The number of stock-size largemouth bass was estimated using Chapman's modification of the Petersen index (Chapman, 1951), with a target 95% confidence interval of ±25% of n (Robson and Regier, 1964). Estimated population size was multiplied by the mean weight of stock-size largemouth bass to estimate biomass. Condition, size distribution, and relative stock density (RSD) were used to characterize the post-LMBV largemouth bass population. Condition was determined using the relative weight (W_r) index (Anderson, 1980), and RSD used the length categories proposed by Gabelhouse (1984). A sample of thin (poor condition, $W_r < 90$) and healthy (good condition, $W_r > 90$) largemouth bass were removed for a health check.

Health assessment

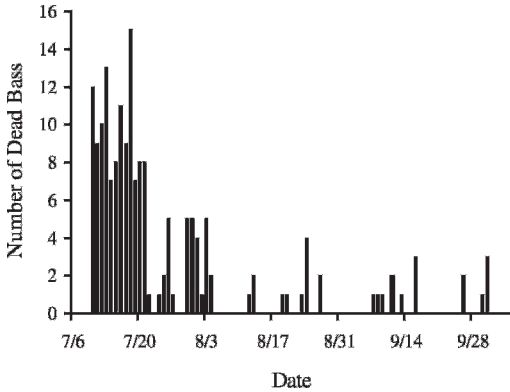During the marking event of the mark-recapture study, some largemouth bass

FIGURE 1. Distribution of largemouth bass mortality (dead and dying) during a largemouth bass virus epizootic event in a 22.4-ha impoundment in Arkansas, USA. Dead and dying largemouth bass were collected daily.
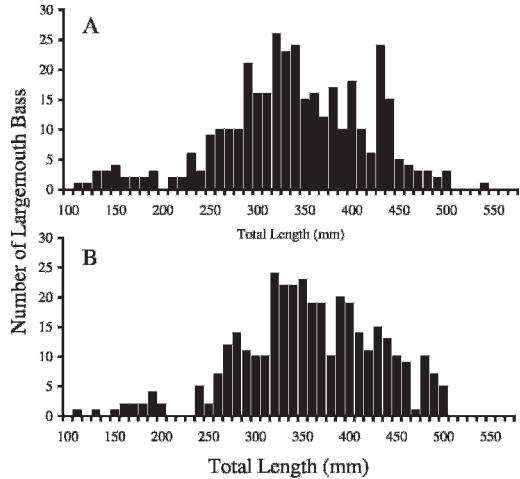

FIGURE 2. Length distributions of largemouth bass collected during (A) marking and (B) recapture efforts of a population estimate in a 22.4-ha Arkansas, USA, impoundment immediately following a largemouth bass virus epizootic event.

collected were underweight but were not retained. During the recapture sampling, a sample ($n=13$) of these fish were collected and were dissected and examined for evidence of parasites, infectious diseases, and physical abnormalities. DNA was extracted from spleen and trunk kidney samples and used as the template for LMBV PCR as described.

RESULTS

The initial sample of moribund largemouth bass was confirmed to be LMBV positive in July 2006. Cell cultures (on FHM) inoculated with homogenized organ samples from moribund bass developed cytopathic effect (CPE) typical of LMBV, and PCR confirmed the presence of LMBV in all five fish examined. No significant bacterial or parasitic infections were found. Fish began dying on 10 July 2006, and the epizootic continued until 1 October 2006, although 85% of the mortality occurred during the first month. Interestingly, the occurrence of dead and dying fish appeared to be somewhat episodic (Fig. 1), with a peak interval of about 2 wk. The total observed mortality during the LMBV outbreak was 176 largemouth bass, including both dead and dying fish removed from the lake.

The landowner did not measure collected largemouth bass but noted that, although most were large, all sizes of adult fish were collected. Although it is likely that some fish were not recovered (e.g., sank to the pond bottom), the landowner was confident that most of the dead and dying fish were counted from lakewide assessment two to three times daily.

Following the LMBV epizootic, 344 stock-size largemouth bass were fin-clipped during the marking period, and 346 were collected during the recapture period; 51 of which displayed fin-clips. The stock-size largemouth bass population size was estimated to be 2,301 with a 95% confidence interval of ±528 (±23% of n) or 103±24 stock-size largemouth bass/ha. The total biomass was estimated as 1,592 kg of stock-size bass or a relative biomass of 71.5 kg of stock-size largemouth bass/ha.

The majority of largemouth bass collected were stock size or larger (Fig. 2). Largemouth bass RSD-Q, or the proportion of quality-size fish (≥300 mm) to stock-size fish (≥200 mm), was 79, and the proportion of preferred-size (≥380 mm) to stock-size largemouth bass (RSD-P) was 36. Only two largemouth bass exceeding memorable
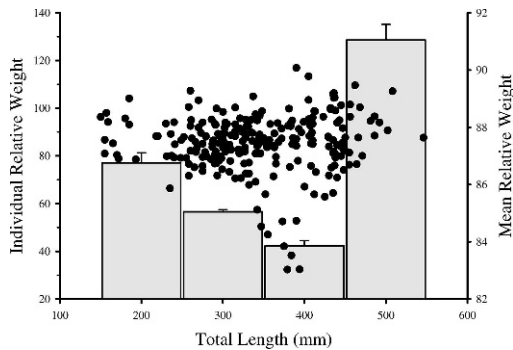
FIGURE 3. Individual and mean relative weight by 100-mm length groups for largemouth bass during marking efforts of a mark-recapture population estimate following a largemouth bass virus epizootic event. Error bars represent standard error (SE) of the mean. Note the difference of scale between individual and mean values.

size ($\geq$510 mm; RSD-M<1) were collected, and no trophy-size fish were seen.

Relative weight of largemouth bass was unusually variable, ranging from 32 to 117, with a number of fish demonstrating W_r values <60 (Fig. 3). The unusually low W_r values for several fish were not erroneous. Five of the fish (2.0%) from the sample (n=252) had W_r values ranging from 32 to 50 and were clearly thin and emaciated. Overall, mean W_r was 85 (SE<1), and only the largest fish had a mean relative weight >90. All fish in extremely poor condition were 340–400 mm in length. Examination of largemouth bass in a sample retained during the recapture event revealed that some fish in poor condition (W_r=68–89) had damage to the jaw, gills, or stomach, most likely attributable to hooking injuries. One fish (W_r=68) had a disarticulated jaw hinge, two exhibited broken jaws (W_r=72 and 76), and one (W_r=68) had a tear in the wall of the stomach. Two fish in good condition (W_r=90 and 104) exhibited moderate to severe gill erosion. All five of the poor condition fish examined were LMBV positive by PCR done on tissue extracts, while only three of the eight fish in good condition (W_r=90–116) were positive for LMBV. No significant bacterial or parasitic infections were found.

DISCUSSION

This study indicates that the mortality event attributed to LMBV had a minimal impact on biomass of largemouth bass. At least 176 largemouth bass, or 7% of the population, died during the LMBV epizootic. Even if mortality was underestimated because of overlooked mortalities, the abundance and biomass estimates after the epizootic remained high. The post-epizootic estimates from this lake were as high or higher than many published estimates from other systems, which were not impacted by LMBV. For example, Durocher et al. (1984) reported that the highest density of largemouth bass, 250 mm or larger, collected from 30 Texas, USA, reservoirs was <50 fish/ha. This compared with 99 largemouth bass/ha of 250 mm or larger in this study.

Likewise, biomass estimates in this study were considerably higher than largemouth biomass estimates reported in the literature. Jenkins (1975) reported a mean standing crop of largemouth bass of 10 kg/ha for 170 US reservoirs; most of which were similar in latitude (30°–39°N) to Stuttgart, Arkansas, USA. Carlander (1955) reported a mean standing crop in 34 North American lakes and reservoirs of 19.2 kg/ha with the maximum of <70 kg/ha, whereas Durocher et al. (1984) found that the greatest biomass reported in 30 Texas, USA, reservoirs was about 36 kg/ha. The average largemouth bass biomass in 44 Oklahoma, USA, ponds was somewhat higher at 49 kg/ha (Jenkins, 1958), which is not surprising given that farm ponds are generally more productive than reservoirs. However, after the LMBV epizootic in the study lake, biomass was estimated to be 71.5 kg/ha, much higher than most reports from reservoirs, lakes, or ponds.

Condition of largemouth bass during the marking period displayed unusual

patterns, particularly the presence of large (340–400 mm) fish in very poor condition. These fish were thin and malformed and approximately the same length. Unfortunately, these fish were not retained during the marking period. Health inspection of fish in somewhat poor condition sampled during the recapture sampling revealed probable fishing injuries that likely interfered with predation, consumption, or digestion. However, injuries were not detected in all of the low-condition fish. Surprisingly, although there were fish in poor condition found during the recapture effort, the severely emaciated fish were not observed and had presumably died from their condition. The precipitous loss of these poor condition fish may be due to a greater susceptibility to LMBV disease or to the stresses associated with high summer temperatures, or the condition could have been a symptom of the virus itself. Alternatively, the severely emaciated fish may have succumbed to fishing injuries without any involvement of LMBV. Pay-fishing activities were suspended during the epizootic, so observed injuries likely occurred several months before they were reported.

Size-structure indices indicated that, despite the high density of largemouth bass, the population was composed of quality-size or larger fish. Gablehouse (1984) suggested that balanced largemouth bass populations should have RSD-Q values of 40–70 and RSD-P values of 10–40. The population size structure appears skewed toward larger fish, with limited recruitment to smaller-size classes. This is not surprising considering the population is managed to produce quality largemouth bass fishing, and the high density of large fish likely leads to cannibalism of some potential recruits. It is notable that the population contains very few memorable-size fish (RSD-M<1), despite anecdotal reports of large fish (>4 kg) being caught by anglers before the LMBV outbreak.

The lack of pre-epizootic data limits conclusions regarding the impact of LMBV on the larger-size classes. Likewise, it could be argued that the sample size of only five largemouth bass used to confirm the epizootic was insufficient to attribute the entire mortality event to LMBV. However, the characteristics of the event were consistent with LMBV, the virus was confirmed, and there were no other mortality vectors apparent. Thus, it can be concluded LMBV was responsible for the mass mortality but that the overall impact of LMBV on population abundance was limited.

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