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FEATURE ARTICLE

Diagnosis of piscine francisellosis in Largemouth Bass from a public display exhibit in north-central Florida, USA

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Abstract

Objective: The Largemouth Bass *Micropterus salmoides* is an important freshwater fish that is native to the southeastern United States and is cultured for conservation, food, and for the sports fishing industry. *Francisella orientalis* is a globally distributed bacterial pathogen of warmwater fish species and is associated with granulomatous inflammation and high mortalities. Outbreaks of piscine francisellosis in the United States have been reported in only a few fish species. This study describes three case presentations of francisellosis in Largemouth Bass from a public display system in north-central Florida. Additionally, laboratory-controlled immersion challenges using an *F. orientalis* isolate from tilapia *Oreochromis* spp. evaluate susceptibility of Largemouth Bass fingerlings to *F. orientalis* infection and mortality through this exposure route.

Methods: Necropsy, histologic examination, immunohistochemistry, bacterial recovery and culture, and quantitative polymerase chain reaction were used as diagnostic tools to evaluate both the affected display fish and the immersion-challenged fingerlings.

Result: Although the display fish and immersion-challenged fingerlings presented with nonspecific clinical signs, gross and histological changes were indicative of granulomatous disease. Immunohistochemical and molecular testing methods confirmed *F. orientalis* infection in affected fish.

Conclusion: The three case presentations described here mark the first reporting of naturally occurring piscine francisellosis in Largemouth Bass that were held in a public display exhibit. Additionally, causality was proven in the Largemouth Bass fingerlings through the immersion challenges. These findings demonstrate susceptibility through immersion-based exposure and assert that francisellosis should be considered among the list of differential diagnoses for Largemouth Bass with granulomatous disease.

KEYWORDS

bacteria, disease and parasites, *Francisella orientalis*, Largemouth Bass, *Micropterus salmoides*, piscine francisellosis

INTRODUCTION

Freshwater sport fish species, including the Largemouth Bass *Micropterus salmoides*, are of great importance to Florida's economy. Data released by the American Sportfishing Association indicated that Florida is the premier sportfishing tourist destination, generating more fishing-related tourism and revenue than any other state (Southwick Associates 2012, 2020). The U.S. Fish and Wildlife Service estimated that freshwater sportfishing generates US1.7×10^9 annually for the Florida economy and supports over 14,000 jobs (U.S. Fish and Wildlife Service 2011). Furthermore, the same report indicated that the Largemouth Bass is the most fished freshwater species among both Florida residents and tourists. Thus, Largemouth Bass are the most economically valuable of Florida freshwater fishes (U.S. Fish and Wildlife Service 2011). For this reason, maintaining healthy Largemouth Bass populations is imperative both for the economy and for the conservation of the species.

Considering the importance of wild Largemouth Bass fisheries and the increasing importance of aquaculture production of this species, it is crucial to monitor the health status of these fish to detect important infectious and emerging diseases within wild and managed populations (Hussein et al. 2020). Largemouth Bass are also commonly displayed in public aquaria, and these facilities are often well equipped for disease detection and pathogen identification. Largemouth Bass are susceptible to bacteria from the genera *Mycobacterium* and *Edwardsiella*, both of which can be associated with nonspecific clinical signs and sudden mortalities, particularly within aged collections or densely stocked systems (Francis-Floyd et al. 1993; Francis-Floyd 2011; Fogelson et al. 2016). Most notably, these bacterial infections can result in a subacute to chronic disease process that is usually characterized by granulomatous inflammation affecting a variety of organs, including the head kidney, liver, and spleen; lesions can also form on the eyes, gills, skeletal muscle, and other organs (Francis-Floyd et al. 1993; Noga 2010; Phillips et al. 2017; Rajme-Manzur et al. 2021). These two genera are often differentiated using histochemical staining and molecular techniques. Despite the bacillary shape of both pathogens, the characteristic mycolic acid cell wall of *Mycobacterium* spp. allows these bacteria to be distinguished from *Edwardsiella* spp. through acid-fast staining. However, due to the prevalence and ubiquitous nature of mycobacteria in closed recirculating systems, mortalities associated with characteristic granulomatous visceral lesions are often attributed to mycobacteriosis without confirmatory diagnostic testing. Although *Mycobacterium* spp. and *Edwardsiella* spp. are commonly diagnosed in fish with granulomatous disease, other organisms, such as *Francisella* spp., should be considered in fish with this type of inflammation.

Impact statement

Largemouth Bass are the most popular Florida freshwater sport fish and are also commonly raised in hatcheries and displayed in public aquaria. Disease can threaten the health and longevity of both wild and managed fish populations, making it important to identify infectious and emerging diseases, including those caused by bacteria. The discovery of new diseases in Largemouth Bass can illuminate opportunities for improved management and husbandry practices pertaining to this species.

Francisella orientalis has been recognized as an important emerging bacterial pathogen that has caused mortalities and economic losses in numerous farmed species worldwide, particularly in tilapia *Oreochromis* spp. (Colquhoun and Duodu 2011; Soto et al. 2014). *Francisella orientalis* is a gram-negative, pleomorphic, non-acid-fast, nonmotile, facultative intracellular coccobacillus; infection causes a chronic disease known as piscine francisellosis in a variety of economically important fish species within freshwater and saltwater aquaculture settings (Soto et al. 2014, 2019). Francisellosis was first reported in the continental United States between 2005 and 2006, at which time it was attributed to a novel *Piscirickettsia*-like or *Rickettsia*-like organism (Mauel et al. 2005; Ostland et al. 2006). Similar to other bacterial pathogens, clinical signs are nonspecific and can include wasting, anorexia, ascites, lethargy, buoyancy and swimming abnormalities, scale loss and larger areas of ulceration, exophthalmia, and pale gills with white nodules (Soto et al. 2009, 2019). Notable gross and microscopic features resemble lesions caused by mycobacteriosis and edwardsiellosis. Changes are characterized by organomegaly caused by widespread, multifocal, white to tan inflammatory foci, which can occur in any organ but are primarily seen in the head kidney, liver, and spleen (Soto et al. 2009, 2019; Birkbeck et al. 2011; Francis-Floyd 2011; Fogelson et al. 2016). Despite documented mortalities in several other economically valuable fish species, *F. orientalis* has not been previously identified from Largemouth Bass.

CASE PRESENTATIONS

In 2017 and 2019, three separate cases of naturally occurring *F. orientalis* infection were documented in

Largemouth Bass that had been housed since 2016 at a public display exhibit in north-central Florida. All three cases occurred in mature female wild-caught Largemouth Bass originating from the southeastern United States, with a mean weight ± SD of 4.46 ± 1.52 kg. Throughout all three cases, water quality characteristics were within normal limits for the system: dissolved oxygen ranged from 8.6 to 9.0 mg/L; water temperature was 21°C; water pH ranged from 7.6 to 7.9; salinity was 3‰; total ammonia nitrogen was 0 mg/L; nitrite was less than 0.010 mg/L; and alkalinity ranged from 100 to 120 mg/L. The 45,425-L (12,000-gal) closed recirculating system was stocked primarily with Largemouth Bass but also displayed other wild-caught or aquaculture-raised indigenous Florida freshwater and estuarine fishes, including hybrid striped bass (White Bass *Morone chrysops* × Striped Bass *Morone saxatilis*), Suwannee Bass *Micropterus notius*, Channel Catfish *Ictalurus punctatus*, Florida Gar *Lepisosteus platyrhincus*, Bowfin *Amia calva*, and Red Drum *Sciaenops ocellatus*. Between 2017 and 2019, this collection consisted of approximately 50 total fish, including 30 Largemouth Bass. The exhibit was supported with sand and carbon filtration as well as UV sterilization. The diet consisted of frozen Bluegill *Lepomis macrochirus*, minnows, and shrimp as well as Zeigler aqua-stable pellets and a Mazuri low-fat aquatic gel diet.

All three fish cases (hereafter referred to as bass 1, 2, and 3) experienced an acute onset of perimortem clinical signs followed by rapid decline and death. Abnormal buoyancy and position within the water column were observed in all three cases, including loss of buoyancy control, negative buoyancy, and lateral recumbency. Bass 1 and bass 3 also presented with rapid opercular movement and gasping behavior. Bass 1 died 3 days after the first onset of perimortem clinical signs, bass 2 died 24 h after the onset of clinical signs, and bass 3 was humanely euthanized after the onset of clinical signs. Euthanasia was performed in accordance with current euthanasia guidelines using a buffered tricaine methanesulfonate (MS-222; Syndel) overdose comprised of a 250-mg/L immersion sedation dose and a 2000-mg/L lethal dose applied directly to the gills.

On gross examination, copious amounts of viscous to gelatinous, amber to serosanguinous fluid was present in the coelom of all three Largemouth Bass. In bass 1 (Figure 1A), this fluid retention contributed to severe coelomic distention and the development of a ventral skin ulcer that exposed the underlying musculature (Figure 1B). Slightly raised, white and tan, pinpoint to 2-mm, multifocal to coalescing soft-tissue nodules were noted in the head kidney, liver, and spleen of all three fish (Figure 1C,D). These nodules were randomly disseminated throughout capsular and cut parenchymal surfaces of each affected organ. Similar nodular lesions were present throughout the viscera of bass 2. Furthermore, bass 3 had several cardiac nodules as well as a hard, approximately 3-cm mass on the medial aspect of the spleen.

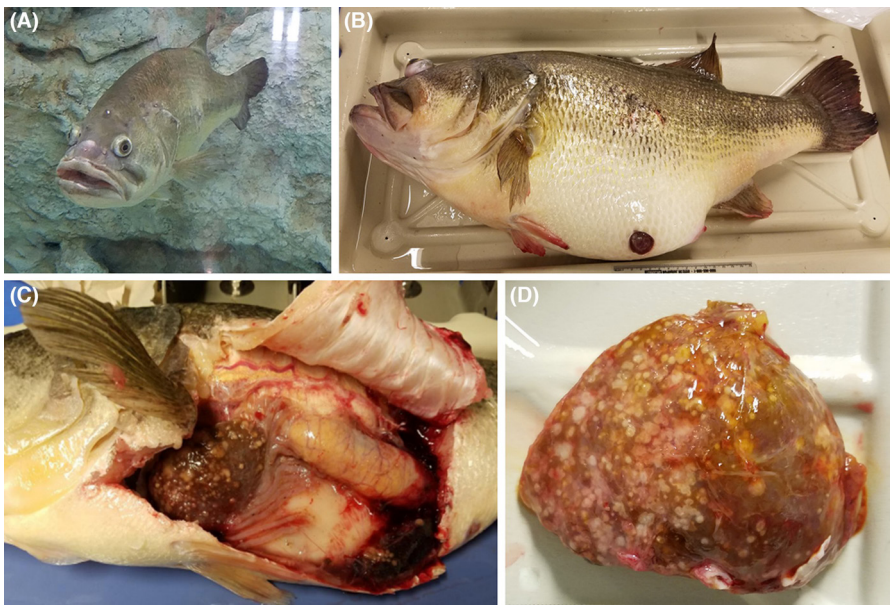

FIGURE 1 Gross changes in Largemouth Bass infected with *Francisella orientalis*: (A) bilateral exophthalmia of bass 1, with concurrent rostral swelling secondary to a previously resolved nasal cavity infection; (B) severe coelomic distention of bass 1, with multifocal ulcerative skin lesions and a large ventral skin ulceration exposing the underlying musculature; (C) severe organomegaly caused by multifocal white nodules in the liver and serosanguinous ascites of bass 3; and (D) lateral aspect of the liver from bass 1, showing multifocal, white to tan nodular soft tissue effacing over 80% of the liver capsule.

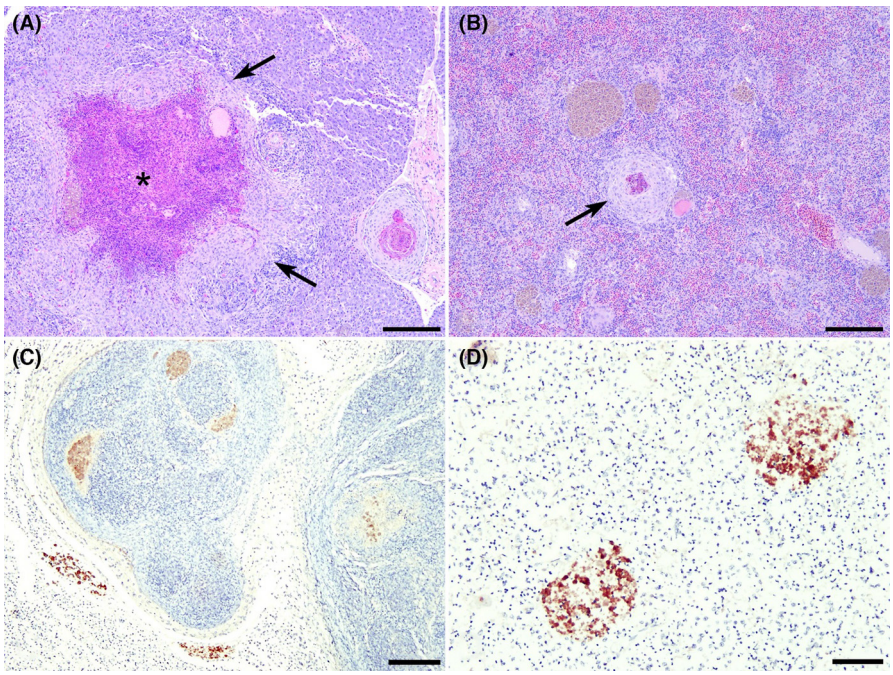

FIGURE 2 Histologic and immunohistochemical findings for two Largemouth Bass (bass 3 and bass 2, respectively) infected with *Francisella orientalis*: (A) photomicrograph of the liver of bass 3, showing large areas of necrotic cellular debris (asterisk) surrounded by broad zones of epithelioid macrophages (arrows; hematoxylin and eosin [H&E] stain; scale bar = 100 μm); (B) photomicrograph of the spleen of bass 3, showing a discrete granuloma (arrow; H&E stain; scale bar = 100 μm); (C) immunohistochemistry (IHC) of the spleen of bass 2, showing granulomatous foci with aggregates of organisms immunolabeled for *F. orientalis* antigen (scale bar = 100 μm); and (D) higher magnification of the IHC-stained splenic granulomas from bass 2, demonstrating the immunolabeling of organisms (scale bar = 50 μm).

The major organs from bass 2 and bass 3 were processed for histologic examination. Granulomatous inflammation was identified in the spleen, kidney, liver, heart, gastrointestinal tract, and episcleral layer of the eye of bass 2. These foci were few to numerous, were discrete to occasionally coalescing, and contained foci of mineralization. Special histochemical staining of affected organs in bass 2 consisted of Ziehl–Neelsen and Fite's acid-fast stain, Twort's tissue Gram stain, and Gomori's methenamine silver stain. No evidence of acid-fast organisms, bacteria, or fungi was noted using these staining techniques. Histologic examination of organs from bass 3 revealed multifocal to coalescing granulomatous inflammation that often formed discrete granulomas in the head kidney, liver, and spleen (Figure 2A,B). Bacteria were not observed in tissues using acid-fast stain or Gram histochemical stains. Additionally, the heart of bass 3 showed evidence of lymphohistiocytic epicarditis consistent with chronic antigenic stimulation as opposed to the monomorphic histiocytic infiltrates or granulomas seen in other organs. The presence of nematode, trematode, pentastomid, and microsporidian parasites and the presence of parasite-related granulomatous inflammation were noted as incidental findings in both cases, as both fish were wild caught.

The three cases were screened for *F. orientalis* using species-specific quantitative polymerase chain reaction (qPCR) and immunohistochemistry (IHC). The DNA was extracted from homogenized spleen and liver tissue samples by using the Qiagen DNeasy Blood and Tissue Kit and was evaluated for the presence of the *F. orientalis* intracellular growth loci gene (*iglC*) following a previously published protocol (Soto et al. 2012). The qPCR results from all three cases verified the presence of *F. orientalis*. Formalin-fixed, paraffin-embedded spleen samples from bass 2 were processed for IHC analysis using the NovaRED Horseradish Peroxidase Substrate Kit (Vector Laboratories) and previously published methods (Soto et al. 2012). Numerous organisms immunolabeled for *F. orientalis* antigen were visible within the splenic granulomas (Figure 2C,D).

EXPERIMENTAL CHALLENGE

Previous experimentally induced *F. orientalis* (strain AOD 104086) infections via intracoelomic injection have established a precedent that Largemouth Bass are susceptible to francisellosis (Poudyal et al. 2020). To further study francisellosis in this species and to best replicate the route of infection in the three naturally occurring mortalities, 30 fingerlings donated from the Florida Bass Conservation Center (Webster, Florida) were utilized in an *F. orientalis*

immersion challenge experiment. Fish had a mean total length ± SD of 5.76 ± 0.52 cm and a mean weight ± SD of 2.19 ± 0.54 g. The fingerlings were fed a Largemouth Bass diet provided by the Florida Bass Conservation Center; the diet was administered at an estimated 1% of body weight. *Francisella orientalis* (LADL 07-285A) isolated from cultured tilapia was used for this study (Soto et al. 2009). *Francisella orientalis* was grown in modified Thayer–Martin agar (Thermo Fisher) and cation-adjusted Mueller–Hinton broth (CAMHB; Sigma-Aldrich) with 1% IsoVitaleX (Fisher Scientific) as in Soto et al. (2009). Fifteen fingerlings were randomly assigned to both the exposure and negative control groups and were kept in 15-L aquaria receiving flow-through freshwater at approximately 18°C at the Center for Aquatic Biology and Aquaculture, Davis, California. Exposed fish were immersion challenged with *F. orientalis* at 1×10^7 colony-forming units/mL for 3 h, while the negative control fingerlings were treated similarly but were only exposed to sterile CAMHB media for 3 h. The fingerlings were visually monitored for clinical signs for 30 days postchallenge (dpc). Survival curves were compared with log-rank (Mantel–Cox) and Gehan–Breslow–Wilcoxon tests using GraphPad Prism version 9.1.0 (GraphPad Software). A *p*-value of 0.05 or less was considered to indicate statistical significance for all tests. At the end of the trial, the survivors from both treatments were humanely euthanized with buffered MS-222 (Syndel) at a concentration of 500 mg/L. All fingerlings used in the study were subjected to postmortem examination following death or euthanasia. Necropsy findings are summarized in Table 1. Challenged fish showed 66.66% survival at 30 dpc ($n = 10$), while controls scored 100% survival (Figure 3). A total of five exposed fingerlings died prior to termination of the 30-day challenge period; time-of-death intervals were 10, 17, 21, 24, and 26 dpc. No overt clinical signs were observed in the fingerling that died at 10 dpc. The fingerlings that died at 17, 21, 24, and 26 dpc as well as two of the 30-day survivors exhibited gross changes that included pale erosive to ulcerative skin lesions, white nodules on and within the spleen, and organomegaly (Figure 4). No clinical signs or gross changes were observed in the remaining 30-day survivors or in the negative control fish.

Selected fingerlings from both the exposed and control groups were chosen for additional testing via bacterial recovery and culture, qPCR, and histology as summarized in Table 1. Bacterial culture of splenic tissue was attempted for 10 of the immersion-challenged fish and six control fish. Splenic isolates were cultured on modified Thayer–Martin agar (Thermo Fisher) and were assessed for bacterial growth after incubation at 25°C for up to 7 days. Eighty percent of the cultures from immersion-challenged fish were positive for *F. orientalis* growth, whereas none of

TABLE 1 Macroscopic lesions, gross changes, and diagnostic results observed in immersion-challenged Largemouth Bass fingerlings and negative control fingerlings throughout a 30-day postchallenge (dpc) period. Diagnostic results are designated as follows: did not test (DNT), significant findings (SF), no significant findings (NSF), positive result (+), and negative result (−). Abbreviations are as follows: *igIC*, intracellular growth loci gene; qPCR, quantitative polymerase chain reaction.

	Immersion-challenged fish ($n = 15$)						
Variable	**10 dpc ($n = 1$)**	**17 dpc ($n = 1$)**	**21 dpc ($n = 1$)**	**24 dpc ($n = 1$)**	**26 dpc ($n = 1$)**	**30 dpc ($n = 10$)**	**Control fish at 30 dpc ($n = 15$)**
Number of fingerlings with gross pathology							
Skin lesion	0	1	1	1	0	0	0
Splenic nodules	0	1	1	1	1	2	0
Splenomegaly	0	1	0	0	0	2	0
Renomegaly	0	0	0	0	0	1	0
Mottled liver	0	1	0	0	0	0	0
Diagnostic results for fingerlings							
Bacterial recovery	DNT	DNT	+	+	+	+ ($n = 5$); − ($n = 2$)	− ($n = 6$)
F. orientalis igIC qPCR	+	DNT	DNT	+	+	+ ($n = 7$)	− ($n = 4$)
Histology	DNT	SF	DNT	DNT	DNT	SF ($n = 3$)	NSF ($n = 3$)

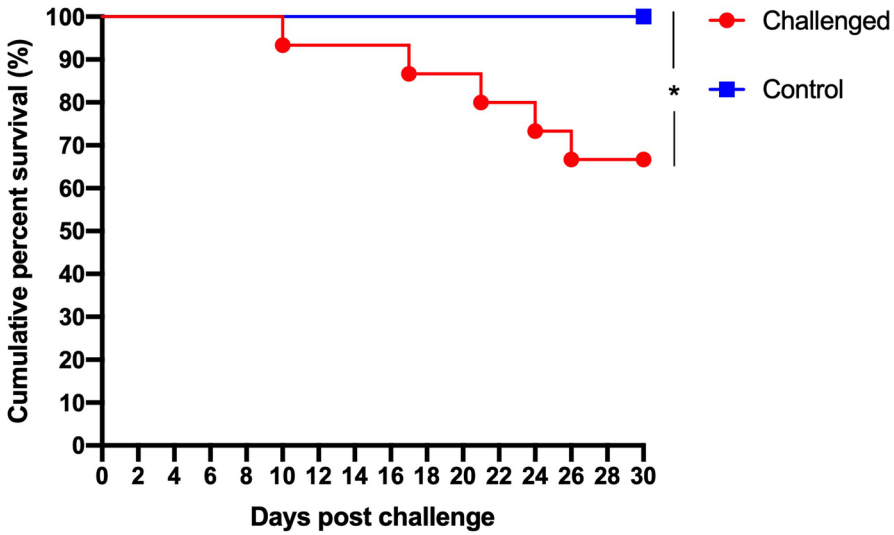

FIGURE 3 Cumulative percent survival observed in *Francisella orientalis*-exposed Largemouth Bass fingerlings during laboratory-controlled immersion challenges ($n = 15$ fish/group). Fish were exposed to *F. orientalis* and were monitored throughout a 30-day postchallenge period. Survival curves were significantly different between the challenged group and the control group (df = 1, $p = 0.0159$).

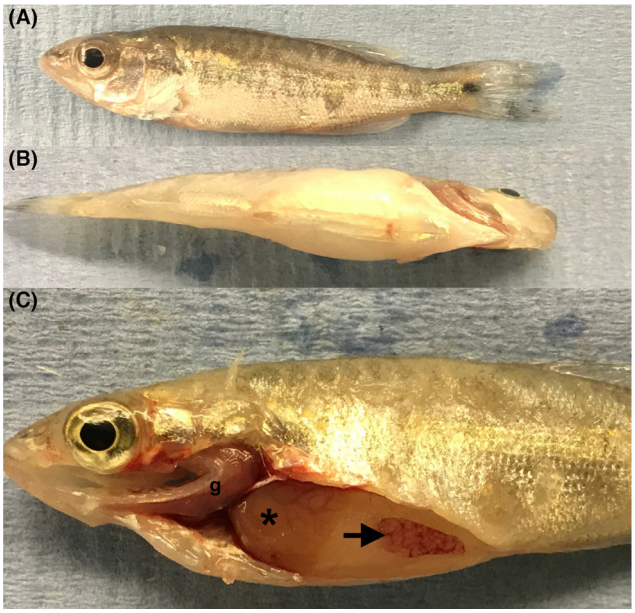

FIGURE 4 Largemouth Bass that were experimentally challenged with *Francisella orientalis* showed nonspecific gross changes, such as (A) loss of scales; (B) coelomic distention; and (C) pale gills, hyperemic liver, and splenomegaly with widespread white nodules (g = gills; asterisk = liver; arrow = spleen).

the cultures from negative control fish produced growth. We extracted DNA from homogenized head kidney and liver tissue samples to establish the presence of *F. orientalis iglC* using previously published qPCR methods (Soto et al. 2012). All 10 samples from the immersion-challenged fish amplified the *F. orientalis iglC* gene, and no amplification occurred in the negative control samples. Four of the immersion-challenged fish and three negative control fish were evaluated for microscopic pathology. Following coelomic incision, the fingerlings were fixed in 10% neutral-buffered formalin (Sigma) and then decalcified in Kristensen's solution (Clin-Tech). Each fish was trimmed into serial transverse sections from snout to caudal fin. After routine processing and sectioning at 5 µm, each tissue section was stained with hematoxylin and eosin. Histologic lesions consistent with chronic *F. orientalis* infection were observed in the fingerling that died at 17 dpc. Discrete and coalescing granulomas, which were interspersed with ill-defined foci of granulomatous inflammation, replaced and expanded much of the head kidney and splenic tissue (Figure 5A). Central regions of hypereosinophilic cellular debris surrounded by broad mantles of epithelioid macrophages with peripherally scattered lymphocytes and rare eosinophilic granulocytes characterized the granulomas. The cytoplasm of scattered macrophages contained a single large, clear vacuole that occasionally contained small numbers of coccobacilli (Figure 5B). Additionally, select sections with prominent lesions and suspect organisms were also stained with Giemsa stain for better visualization of bacteria (Figure 5C). Loosely organized mixtures of similar inflammatory cells, including macrophages with intracellular bacteria, formed multiple, irregularly delineated foci within the liver, dermis, and skeletal muscle at the base of the caudal fin. In the three 30-day survivors of the immersion challenge, one fish each contained rare, small foci of lymphocytes, eosinophilic granular cells, and vacuolated macrophages in various proportions located transmurally in the walls of pyloric ceca and adjacent adipose, in peduncular skeletal muscle, and in the head kidney (Figure 5D). No bacteria were observed. The three negative control fish were microscopically normal.

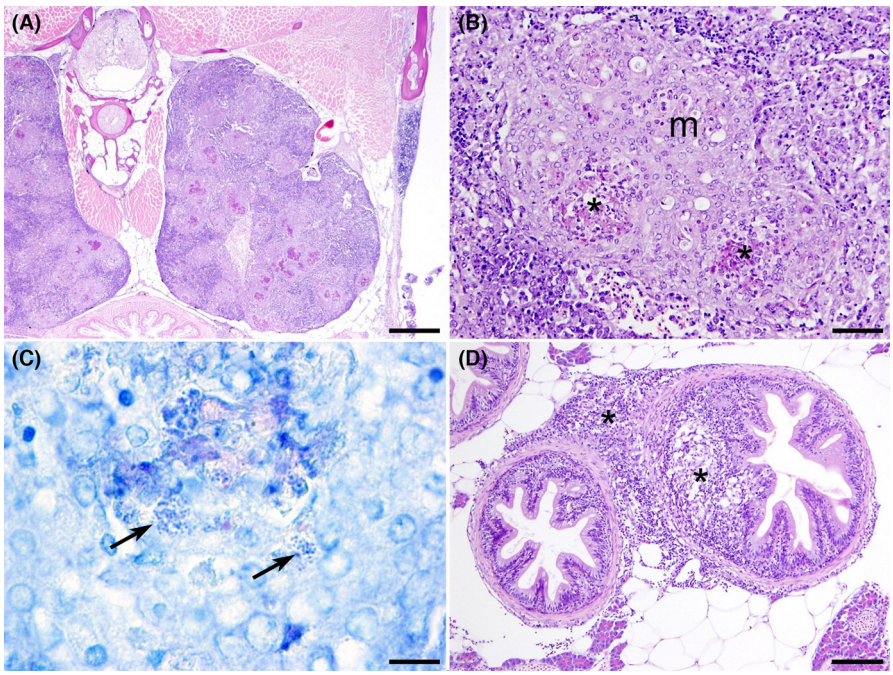

FIGURE 5 Histologic sections from Largemouth Bass fingerlings that were immersion challenged with *Francisella orientalis*: (A) low-magnification image of head kidney from the fingerling that died at 17 days postchallenge (dpc), with lesions indicative of chronic infection and coalescing granulomas replacing extensive areas of the parenchyma (hematoxylin and eosin [H&E] stain; scale bar = 500 μm); (B) higher magnification image of a head kidney granuloma with central areas of necrotic cellular debris (asterisk) surrounded by broad zones of epithelioid macrophages (m; H&E stain; scale bar = 50 μm); (C) head kidney granuloma with small coccoid bacteria visible within cytoplasmic vacuoles of macrophages (arrows; Giemsa stain; scale bar = 10 μm); and (D) transmural granulomatous inflammation affecting the pyloric cecal wall and adjacent coelomic adipose (asterisks), suggestive of early infection, in a fingerling that survived for 30 dpc (H&E stain; scale bar = 100 μm).

DISCUSSION

The current case presentations document a history of naturally occurring *F. orientalis* infection and mortality within a public display collection. Furthermore, they mark the first reporting of a natural *F. orientalis* infection in Largemouth Bass. The immersion challenges demonstrated that *F. orientalis* infection and mortality can occur in Largemouth Bass through immersion-based exposure; this expands upon previous knowledge that francisellosis can develop in Largemouth Bass after experimental intracoelomic injection (Poudyal et al. 2020). The described immersion challenges better replicated the natural route of infection and allowed for more valid comparisons to be made between the naturally and experimentally infected fish. Fish from both the public display system and the experimental challenges presented with white to tan nodular lesions consistent with francisellosis. Disparity in the severity of granulomatous inflammation throughout organ systems can be explained by differences in exposure duration and subsequent infection between these two groups. The three fish within the collection were likely continuously exposed to *F. orientalis* in the system and displayed gross and histologic signs that were consistent with chronic infection. The necropsy findings for the display fish suggested a chronic disease syndrome, with the sudden onset of signs and death likely related to insufficient functional parenchyma in vital organ systems. It is also conceivable that environmental stressors, the noted concurrent incidental parasitism, or increased energy demands associated with chronic infection could have accelerated the decline of these fish. Conversely, the immersion-challenged fingerlings were exposed to *F. orientalis* for a singular 3-h exposure interval and developed all observed signs of disease in 30 days or less. Future environmental studies that aim to quantify *F. orientalis* concentration within the water and biofilms of the public display system could illustrate the level to which these bacteria persist within this environment and could determine the risk of future infections. Furthermore, quantifying the concentration of *F. orientalis* in the system could better inform the design of future immersion challenge experiments.

Attempts to culture *F. orientalis* from the frozen tissues of the three fish from the exhibit proved unsuccessful; therefore, the strain that was specific to the managed population mortalities could not be utilized in the immersion challenges to fulfill Koch's postulates. *Francisella*

orientalis is a fastidious organism, and tissue samples from these case study fish were not processed or stored in a manner that would be optimal for bacterial survival and subsequent culture (Soto et al. 2009). However, the genomes of *F. orientalis* strains are known to be highly homogeneous, making the use of a tilapia aquaculture-derived *F. orientalis* strain in the immersion challenges an appropriate substitution (Soto et al. 2009).

Because granulomatous disease is common in Largemouth Bass and is often attributed to *Mycobacterium* spp. in densely stocked or aged systems, histopathology was not pursued for bass 1 because this fish marked the first mortality with the described clinical signs in the public display system. It was serendipitous that frozen tissues were archived from bass 1, allowing for future qPCR analysis once additional cases presented and warranted differential diagnostics. Due to shared characteristic gross pathology among *Francisella* spp., *Mycobacterium* spp., and *Edwardsiella* spp. infections, definitive diagnosis is challenging without the use of specialized microbiological, molecular, or histologic techniques. Diagnosis is further complicated when nonbacterial causes of granulomatous disease, such as microsporidians, digenetic trematode larvae, cestode larvae, fungi, or foreign body reactions, are also considered. Furthermore, as observed in bass 2 and bass 3, a paucity of bacteria in chronically infected moribund fish may render histochemical staining alone an unreliable diagnostic tool for francisellosis. Specialized diagnostics and histochemical stains may not be readily accessible to aquarists, hatchery personnel, or fisheries managers, thereby limiting the identification of emerging pathogens such as *F. orientalis*. However, francisellosis should be considered among the list of differential diagnoses for fish with granulomatous disease.

The Largemouth Bass is a freshwater sport fish that is important for the Florida economy; therefore, differential diagnostics of potential emerging pathogens is prudent. Considering that the Largemouth Bass in these cases are native to freshwater ecosystems within the southeastern United States, the present findings could be more broadly applied to study the potential for *F. orientalis* to emerge as a cause of disease within wild populations of this economically important game fish species. *Francisella* spp. are known to be emergent pathogens in cultured tilapia and have also been identified in cultured hybrid striped bass (White Bass ×Striped Bass) and in French Grunt *Haemulon flavolineatum* and Casear Grunt *H. carbonarium* from Florida (Ostland et al. 2006; Soto et al. 2011, 2014). Through aquaculture escape and intentional release, tilapia species have become highly invasive within the southeastern United States, including Florida (Bradford et al. 2011). Although *Francisella* spp. have not yet been documented within wild tilapia, given the susceptibility of these species to francisellosis, the possibility warrants consideration. It is possible that naturalized tilapia within freshwater ecosystems could serve as a reservoir and vector for *F. orientalis* horizontal transmission to other species, including Largemouth Bass.

Although management options are limited, with no treatment or vaccines yet available for francisellosis, it is important to identify emerging diseases within public display exhibits and hatcheries so that husbandry and stocking practices can be amended as needed. The diagnosis of francisellosis within only female Largemouth Bass from the public display system is likely explained by a stocking bias toward female fish, as females of this species more readily reach the large sizes preferred for this collection. The route of *F. orientalis* introduction to the public display system remains uncertain. Previous challenge experiment results indicated that Pumpkinseed *Lepomis gibbosus* are susceptible to *F. orientalis* infection, so the possibility of transmission through frozen sunfish diets should be considered (Lewisch et al. 2016). Alternatively, *F. orientalis* could have been introduced into the system through an infected wild-caught or aquaculture-raised fish. The collection included three hybrid striped bass, and this species has previously been demonstrated to be susceptible to francisellosis (Colquhoun and Duodu 2011). The identification of an emerging disease within a collection could warrant selective culling and cessation of the transfer or release of fish from affected systems or facilities. Because *F. orientalis* can cause chronic disease and can persist in both freshwater and marine environments, this emerging disease is of concern. To limit horizontal transmission between hatchery-reared fish and wild conspecifics, it may be imperative to test hatchery-reared fish prior to release (Colquhoun and Duodu 2011). Although reports of *Francisella* spp. within wild fish populations are currently limited, *F. orientalis* has been documented within aquaria after the introduction of wild-caught fish (Camus et al. 2013; Soto et al. 2014). Reports such as these highlight the possibility that *F. orientalis* is an emerging pathogen within wild fish populations (Soto et al. 2014).

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CONFLICTS OF INTERESTS STATEMENT

The authors declare that they have no conflicts of interest related to the findings discussed within this manuscript.

ETHICS STATEMENT

All fish used in the experimental challenges at the Center for Aquatic Biology and Aquaculture, University of California–Davis, were handled as per approved by the Institutional Animal Care and Use Committee protocols for animal welfare in research.

DATA AVAILABILITY STATEMENT

All data presented in this manuscript are original, and data related to the experimental challenges are stored at the Center for Aquatic Biology and Aquaculture, University of California–Davis. Case records and reports pertaining to the public display fish are archived by Amanda Sheehy. Data can be accessed upon request.

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