EXPERIMENTAL BOLBOPHORUS DAMNIFICUS (DIGENEA: BOLBOPHORIDAE) INFECTIONS IN PISCIVOROUS BIRDS

Authors: Doffitt, Cynthia M., Pote, Linda M., and King, D. Tommy

Source: Journal of Wildlife Diseases, 45(3) : 684-691

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.3.684

Journal of Wildlife Diseases, 45(3), 2009, pp. 684–691

EXPERIMENTAL *BOLBOPHORUS DAMNIFICUS* (DIGENEA: BOLBOPHORIDAE) INFECTIONS IN PISCIVOROUS BIRDS

Cynthia M. Doffitt,[1] **Linda M. Pote,**[1,3] **and D. Tommy King**[2]

[1] Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, Mississippi 39762, USA

[2] US Department of Agriculture/Wildlife Services, National Wildlife Research Center, Mississippi State University, Starkville, Mississippi 39762, USA

[3] Corresponding author (email: pote@cvm.msstate.edu)

ABSTRACT: In order to determine potential definitive hosts of the digenetic trematode, *Bolbophorus damnificus*, two American White Pelicans (*Pelecanus erythrorhynchos*), two Double-crested Cormorants (*Phalacrocorax auritus*), two Great Blue Herons (*Ardea herodias*), and two Great Egrets (*Ardea alba*) were captured, treated with praziquantel, and fed channel catfish (*Ictalurus punctatus*) infected with *B. damnificus* metacercariae. Patent infections of *B. damnificus*, which developed in both American White Pelicans at 3 days postinfection, were confirmed by the presence of trematode ova in the feces. Mature *B. damnificus* trematodes were recovered from the intestines of both pelicans at 21 days postinfection, further confirming the establishment of infection. No evidence of *B. damnificus* infections was observed in the other bird species studied. This study provides further evidence that Double-crested Cormorants, Great Blue Herons, and Great Egrets do not serve as definitive hosts for *B. damnificus*.

Key words: Aquaculture, *Ardea alba*, *Ardea herodias*, *Bolbophorus damnificus*, Digenea *Ictalurus punctatus*, *Pelecanus erythrorhynchos*, *Phalacrocorax auritus*, piscivorous birds, trematode.

INTRODUCTION

Commercial aquaculture of channel catfish (*Ictalurus punctatus*) is a major industry in the United States, with the greatest concentration of production ponds in the northwestern region of Mississippi (Wellborn, 1988). This industry has experienced rapid growth since the first commercial catfish pond was established in 1965 (Wellborn, 1988). The increase in catfish ponds has been accompanied by a steady increase in numbers of piscivorous birds in the region (Mott and Brunson, 1997; Glahn and King, 2004; Overstreet and Curran, 2004). The birds most often observed feeding on channel catfish are American White Pelicans (*Pelecanus erythrorhynchos*), Double-crested Cormorants (*Phalacrocorax auritus*), Great Blue Herons (*Ardea herodias*), and Great Egrets (*Ardea alba*; Glahn et al., 1999, 2000; King and Werner, 2001; King, 2005).

In addition to predation of catfish, these birds may also serve as definitive hosts to digenetic trematodes that are infective to channel catfish (Sepúlveda et al., 1999; Overstreet et al., 2002; Flowers et al., 2004, Kinsella et al., 2004; Overstreet and Curran, 2004). In the late 1990s, channel catfish producers in Mississippi and Louisiana first began reporting catfish losses, which were attributed to a trematode tentatively identified as *Bolbophorus* spp. (Avery et al., 2001; Terhune et al., 2002). Since that time, the results of a series of infection studies, which were confirmed by molecular analysis, have established the trematode *Bolbophorus damnificus* as the causative agent of these losses (Levy et al., 2002; Overstreet et al., 2002; Yost et al., 2005). These studies also confirmed that one of the definitive hosts for this parasite is the American White Pelican, the first intermediate host is the rams-horn snail (*Planorbella trivolvis*), and the second intermediate host is the channel catfish (Overstreet et al., 2002; Overstreet and Curran, 2004).

In channel catfish, *B. damnificus* cercariae penetrate the skin and form prodiplostomulum metacercariae in the superficial layers of the musculature (Overstreet et al., 2002). Hemorrhaging is often associated with cercarial penetration and metacercarial cyst development. In addition, kidney tubule necrosis and kidney inflammation may occur; however, the mechanism of this pathology is unknown (Overstreet et al., 2002; Terhune et al., 2002). High mortalities are observed in severely infected fingerling catfish. Larger catfish exhibit less mortality, but decreases in feeding can result in poor growth rates. Additionally, the meat of infected catfish is often unmarketable because of damage caused by encysted metacercariae (Terhune et al., 2002).

Although the American White Pelican has been confirmed as a definitive host for *B. damnificus*, other piscivorous birds, which commonly feed on commercially produced catfish, could potentially be hosts. In order to control the transmission of *B. damnificus* better, it is necessary to identify all of the hosts, especially the definitive hosts, which can introduce trematode eggs into the aquatic environment. This research investigated the potential for Double-crested Cormorants, Great Blue Herons, and Great Egrets to serve as definitive hosts for *B. damnificus*. These species were chosen because of their frequent association with commercial catfish production ponds and their potential for introducing digenetic trematodes into the fish population.

MATERIALS AND METHODS

Bird collection and care

Two individuals of each bird species (American White Pelican, Double-crested Cormorant, Great Blue Heron, and Great Egret) were live-captured from northwestern Mississippi with the use of modified padded leg-hold traps and methods previously described (King et al., 1998). Birds were weighed, marked with uniquely numbered bands, and housed outdoors in 3.0×3.0×1.8–m pens lined with outdoor carpet and outfitted with misting systems. Pelicans and cormorants were provided with 1,000-l recirculating filtered water tanks, and the herons and egrets were provided with 110-l tanks filled to 50% capacity with fresh water every 2–3 days. Pens were specially designed for long-term studies on piscivorous birds and were located at the Mississippi State University College of Veterinary Medicine. Birds were fed a diet of specific-pathogen–free (SPF) channel catfish daily at approximately the following rates: American White Pelicans, 1,500 g; Double-crested Cormorants, 600 g; Great Blue Herons, 400 g; Great Egrets, 400 g. Specific-pathogen–free channel catfish were obtained from enclosed hatcheries at Mississippi State University College of Veterinary Medicine in Starkville, Mississippi, USA and the Thad Cochran National Warmwater Aquaculture Center in Stoneville, Mississippi, USA. Each bird was observed daily for general health and body condition.

Following capture, birds were acclimated for 7 days prior to the initiation of the infection study (Day 0). On Day 0, each bird was given 26–30 mg/kg body weight of the anthelminthic drug praziquantel (Droncit® 34, Bayer Corporation, Shawnee Mission, Kansas 66201, USA). During the acclimation period and continuing for the duration of the trial, fresh fecal samples were collected and examined daily for the presence of trematode eggs with the use of a modification of the fecal sedimentation method (Foreyt, 2001). In order to remove excess fecal debris, a 0.5-g homogenized sample of fecal material was washed with a 1% soap solution and allowed to sit undisturbed for 5 min before removal of the supernatant. This process was repeated for 10 cycles. The fecal sample was then rinsed with distilled water and diluted to 10 ml. After the final water rinse, the sample was thoroughly mixed and a 1-ml aliquot of this preparation was quickly pipetted and viewed with a dissecting microscope (Olympus SZ60, Olympus America, Inc., Center Valley, Pennsylvania 18034-0610, USA) at 50× in order to enumerate the trematode eggs. The number of eggs per gram of feces (epg) was calculated according to the following formula: [(eggs in 1 ml)(10)]/weight (g) of feces.

Channel catfish were collected from a commercial catfish pond in northwestern Mississippi that had been experiencing *B. damnificus* infections. A subsample of the channel catfish (n=23) was examined prior to the challenge of the birds with these catfish to confirm the presence and number of *B. damnificus* metacercariae. Metacercariae from this subsample of catfish were excised and enumerated and a single metacercaria from each of the sampled fish was randomly selected for molecular analysis in order to

TABLE 1. Challenge of birds with live *Bolbophorus damnificus*–infected channel catfish.

Bird[a]	Challenge period (days)	Number of fish consumed	Estimated metacercariae dose[b]
AWPE 1	7	14	182
AWPE 2	7	12	156
DCCO 1	7	11	143
DCCO 2	7	7	91
GBHE 1	5	14	182
GBHE 2	4	14	182
GREG 1	7	12	156
GREG 2	7	5	65

[a] AWPE = American White Pelican, DCCO = Double-crested Cormorant, GBHE = Great Blue Heron, GREG = Great Egret.

[b] Estimated metacercariae dose = mean number of metacercariae/fish×number of fish eaten.

confirm that the catfish were infected with *B. damnificus*.

Seven days following praziquantel treatment (Day 7), the birds were fed live catfish that were naturally infected with *B. damnificus* to simulate natural infections in these captive birds. The number of fish and metacercariae consumed by each bird varied based on their individual feeding rates (Table 1). The infected catfish were fed to the birds over a period of up to 7 days, with each bird being fed to satiation each day. Attempts to infect the birds ceased once they had eaten the preferred dose of 14 fish (approximately 182 metacercariae) or once the 7-day challenge period expired (Day 14). After the challenge period expired, all birds were fed SPF fish for the duration of the trial.

Three weeks postchallenge (Day 28), all birds were euthanized with the use of carbon dioxide gas and necropsied. The gastrointestinal tract from the esophagus to the cloaca of each bird was removed, and opened longitudinally, and the intestinal contents were gently rinsed through a No. 200 stainless-steel screen (aperture=75 μm) with dechlorinated water (Pote et al., 1992). All intestinal contents were immediately examined with the use of a dissecting microscope, and all live parasites were collected and placed in 70% molecular-grade ethanol in preparation for staining and/or molecular analysis.

Identification of trematodes: The molecular identification of the eggs, metacercariae, and adult trematodes was based on polymerase chain reaction (PCR) with the use of oligonucleotide primers specific to *B. damnificus* (Levy et al., 2002). Genomic DNA was isolated from individual parasites according to the Gentra Purgene kit manufacturer's instructions (Gentra Systems, Inc., Minneapolis, Minnesota 55441, USA). PCR amplifications were performed in 25-μl reaction volumes composed of 2.0 μl template DNA, 0.625 units Takara Hot Start Taq Polymerase (Takara Bio Inc., Japan), 2.5 μl Takara 10× PCR buffer (Takara Bio Inc., Seta 3-4-1, Otsu, Shiga 520-2193, Japan), 200 μM dNTP mixture (Takara Bio), 200 nM forward primer, 200 nM reverse primer, and nuclease-free water added quantum satis to 25 μl. Reactions were performed in a MJ Research PTC-100 Peltier thermal cycler (Bio-Rad Laboratories, Inc., Waltham, Massachusetts 02451, USA) under the following conditions: 92 C for 5 min, followed by 34 cycles of 94 C for 1 min, 58 C for 1 min, 72 C for 1 min, and a final cycle of 72 C for 5 min. The primers used were specific to *B. damnificus* (forward 5′-TCA GTT TCG AAC GAT GAT GA-3′ and reverse 5′-CGG TCT ACG GTT CCA CC-3′; Levy et al., 2002). Both positive (known *B. damnificus* metacercariae) and negative (nuclease-free water) controls were used in each PCR reaction. The PCR products were visualized on a 1.2% agarose gel, which was poststained with Gelstar nucleic acid stain (Cambrex BioScience Rockland, Inc., Rockland, ME, USA) and observed under ultraviolet light.

Adult trematodes were stained in acetocarmine for 12 hr, destained in acid alcohol, dehydrated in a graded alcohol series (70, 95, and 100% ethanol), cleared in Citri-solve (Omega Laboratories, Inc., Houston, Texas 77080, USA), and mounted on slides with Permount (ProSciTech, Thuringowa Central Queensland 4817, Australia). Identifications of stained *B. damnificus* specimens were based on descriptions by Overstreet et al. (2002) and Levy et al. (2002). One stained specimen collected from each American White Pelican was deposited at the US National Parasite Collection in Beltsville, Maryland, USA (USNPC 101433.00).

All procedures used in this study were approved by the US Department of Agriculture/Wildlife Services (USDA/WS) National Wildlife Research Center's (NWRC) Institutional Animal Care and Use Committee under NWRC QA-1138.

RESULTS

The subsample (n=23) of channel catfish used in the challenge was found to be infected with an average of 13 (range

0–73) metacercariae per fish. Eighteen of the 23 (78%) sampled catfish were confirmed to be infected with *B. damnificus*. No metacercariae were found in three of the catfish (13%). The two remaining sampled catfish (9%) were infected with metacercariae that were not *B. damnificus*, but are likely to be *Hysteromorpha triloba*, another larval digenetic trematode infecting channel catfish musculature (Hoffman, 1999). Given that the metacercarial doses were based on a subsample of the channel catfish population used and that a few of the sampled channel catfish were not infected with metacercariae or with non–*B. damnificus* metacercariae, all data pertaining to metacercarial doses are estimations. Although we identified three catfish as negative for metacercariae in our subsample, we may have underestimated the number of positive catfish in the population, because the detection method relies on the microscopic gross examination of fish muscle tissue.

Three of the study birds received the full dose of 182 metacercariae (American White Pelican 1 [AWPE1], Great Blue Heron 1 [GBHE 1], and Great Blue Heron 2 [GBHE 2]). Three received nearly the full dose (American White Pelican 2 [AWPE 2], Double-crested Cormorant 1 [DCCO 1], and Great Egret 1 [GREG 1] at 156, 143, and 156 metacercariae, respectively). Because of the individual feeding rates of the study birds, two of the study birds received lower doses (Double-crested Cormorant 2 [DCCO 2] and Great Egret 2 [GREG 2] at 91 and 65 metacercariae, respectively; Table 1).

Both AWPE 1 and AWPE 2 shed *B. damnificus* eggs beginning on Day 10 (3 days postchallenge); AWPE 1 ceased shedding by Day 15 (8 days postchallenge, Fig. 1), whereas AWPE 2 shed eggs intermittently until Day 24 (17 days postchallenge; Fig. 1). The Double-crested Cormorants (DCCO 1 and DCCO 2), GREG 1 and GREG 2, and one Great Blue Heron (GBHE 2) did not shed trematode eggs in the feces at any point during the study period. The other Great Blue Heron (GBHE 1) shed low numbers of trematode eggs intermittently (Days 2, 4, and 6) following treatment with praziquantel; however, based on both morphology and molecular analysis, the eggs were not those of *B. damnificus*.

One adult *B. damnificus* was recovered from AWPE 1. It was stained and identified based on its morphology. Five adult *B. damnificus* were recovered from the intestine of AWPE 2. All adult trematodes were morphologically identical. Of these five trematodes, one was identified based on PCR, two were stained and identified morphologically, and the remaining three were archived in 70% molecular-grade ethanol. No adult *B. damnificus* were found in the intestinal contents of either of the Double-crested Cormorants, Great Blue Herons, or Great Egrets. However, a single gravid adult trematode was recovered from DCCO 1. This trematode was stained for morphologic comparison and identified as *Drepanocephalus spathans* (Yamaguti, 1958; Rietschel and Werding, 1978; Kostadinova et al., 2002; Jones et al., 2005).

DISCUSSION

The two American White Pelicans were successfully infected with *B. damnificus*. Both exhibited patent trematode infections beginning on Day 10 (3 days postchallenge) and shed eggs intermittently until the termination of the trial (Day 28). This timing of parasite maturation and egg production in the American White Pelicans is similar to that of *B. damnificus* in previous studies (Overstreet et al., 2002). Following necropsy (Day 28), the infections were verified by the presence of adult *B. damnificus* in the gastrointestinal tracts of both pelicans. At necropsy, a single adult *B. damnificus* was recovered from AWPE 1, which had a lower egg shedding rate (peak=1,680 eggs/g feces [epg], Fig. 1) for a shorter duration (Days 10–15). Conversely, five adult *B. damnifi-*

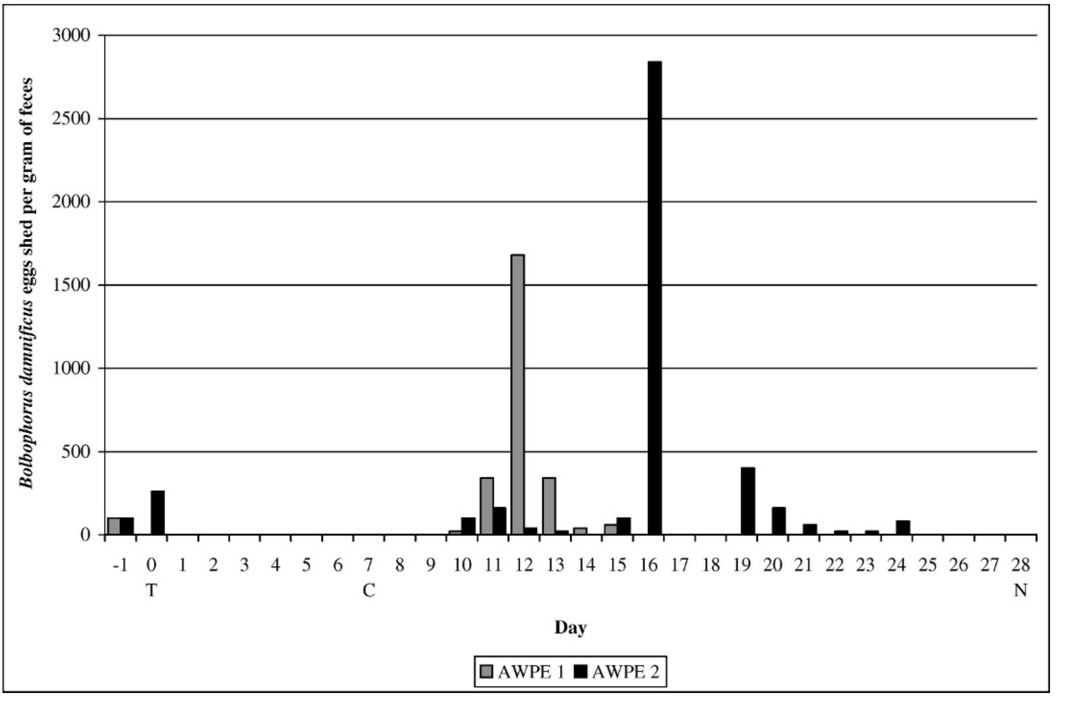

FIGURE 1. Daily *Bolbophorus damnificus* egg shedding rates for American White Pelican (*Pelecanus erythrorhynchos*) 1 and 2. Reported as eggs shed per gram of feces (epg). T=praziquantel treatment, C=challenge with live *B. damnificus*–infected channel catfish, N=euthanasia and necropsy.

cus were recovered from AWPE 2, which had a higher egg shedding rate (peak= 2,840 epg) for a longer duration (Days 10–24; Fig. 1).

The recovery of a single *B. damnificus* adult from AWPE 1 at necropsy may provide valuable insight into the life cycle of this parasite. Trematode egg counts obtained from fecal sedimentations of host fecal material, while demonstrating the presence of gravid adults within the avian host, are often confounded by the presence of several adult trematodes. However, the fecal trematode egg data generated from AWPE 1 may provide important information about the egg output of a single adult trematode. In this and previous studies (Yost et al., 2005), the fecal egg data from American White Pelicans infected with multiple adult *B. damnificus* was cyclic with periods of intermittent shedding that continued for up to several weeks. However, the fecal egg data from AWPE 1 did not exhibit this cyclic pattern. The cyclic nature of egg production observed in multiple infections is likely the effect of individual trematodes maturing and beginning egg production at varying rates.

Although neither of the Double-crested Cormorants was positive for trematode eggs in the feces, a single gravid adult trematode was recovered from the intestine of DCCO 1 at necropsy. This trematode was identified as *D. spathans*, which has been previously reported from this species (Threlfall, 1982; Fedynich et al., 1997; Flowers et al., 2004). Infections by trematodes in the genus *Drepanocephalus* have also been reported in other *Phalacrocorax* species (Nasir and Marval, 1968; Lamothe-Argumedo and Perez-Ponce de Leon, 1989; Kostadinova et al., 2002). *Drepanocephalus spathans* has not been reported to infect channel catfish. The larval stage of this helminth has been reported in the cichlid fish, *Cichlasoma fenestratum* (Garcia, 1993; Salgado-Maldonado et al., 2005) and *Cichlasoma urophthalmus* (Salgado-Maldonado and Kennedy, 1997). However, the presence of this trematode was unexpected because all birds were treated with a dose of praziquantel previously shown to be efficacious against digenetic trematodes (Overstreet et al., 2002; Yost et al., 2005). The presence of *D. spathans* may indicate that praziquantel is less effective against this species. Another explanation is that *D. spathans* could be infective to channel catfish, despite the fact that it has not been previously reported in channel catfish. No trematodes were recovered from the gastrointestinal tract of DCCO 2.

No adult trematodes were recovered from either of the Great Blue Herons at necropsy. However, GBHE 1 shed trematode eggs intermittently following treatment with praziquantel on Days 2, 4, and 6. These eggs were confirmed to be non–*B. damnificus* eggs with the use of molecular analysis. The absence of eggs in the feces of GBHE 1 pre- and postchallenge indicated a failure of *B. damnificus* to establish an infection in this host. This was confirmed by the absence of adult trematodes in either of the Great Blue Herons at necropsy. No trematode eggs were detected in the feces of either of the Great Egrets during the study period and no adult trematodes were recovered from the intestines at necropsy.

Because it has been previously documented that American White Pelicans serve as definitive hosts (Overstreet et al., 2002), their *B. damnificus* infections indicate that the metacercariae used for the challenge were infective and that the doses given were sufficient to induce an infection. American White Pelicans (AWPE 1 and AWPE 2) were challenged with an estimated dose of 182 and 156 metacercariae, respectively (Table 1). These doses resulted in the maturation of one mature adult in AWPE 1 and five mature adults in AWPE 2. Although two of the study birds (DCCO 2 and GREG 2) received lower estimated doses of metacercariae, three birds (AWPE 1, GBHE 1, and GBHE 2) received the full estimated dose of 182 metacercariae. The remaining birds (AWPE 2, DCCO 1, and GREG 1) received comparable estimated doses ranging from 143 to 156 metacercariae (Table 1). Consequently, at least one individual of each species studied likely ingested a metacercariae dose sufficient to induce an infection, as evidenced by the successful infection of AWPE 2 with approximately 156 metacercariae. This demonstrates that the lack of *B. damnificus* patent infections in the Double-crested Cormorants, Great Blue Herons, and Great Egrets was not due to an insufficient metacercariae dose.

This research provides further confirmation that American White Pelicans serve as definitive hosts for *B. damnificus*. Additionally, we have demonstrated that Double-crested Cormorants, Great Blue Herons, and Great Egrets, when subjected to the same experimental infection parameters as the American White Pelicans, were refractory to *B. damnificus*, indicating that they are unlikely to serve as natural definitive hosts for this parasite. It is necessary to identify all potential definitive hosts of *B. damnificus* in order to better understand the life cycle of this parasite. This information will be used by researchers and commercial channel catfish producers to focus control measures in an effort to reduce the impact of this parasite on the industry.

It is possible that the failure to infect Double-crested Cormorants, Great Blue Herons, or Great Egrets with *B. damnificus* was related to experimental design. However, we attempted to mimic the most efficacious natural conditions for this infection to occur in commercial channel catfish production ponds. To that end, live channel catfish naturally infected with *B. damnificus* were used to challenge birds; this indicates that our challenge model was valid. Previous studies using this method were successful in the establishment of patent infections in the American White Pelican (Overstreet et al 2002). Another issue that may have confounded our results is the relatively small avian sample size. In order to maximize our results but minimize the number of protected birds used in the study, we chose to use two birds of each species.

Because experimental infection studies cannot completely mimic natural conditions, a helminthologic survey of gastrointestinal trematode infections in each of these four bird species is currently under way. The data collected in that study will complement the present study by documenting all naturally occurring trematode infections, including *B. damnificus*, in each of these bird species. These two studies, in conjunction, will provide further evidence about the ability of Double-crested Cormorants, Great Blue Herons, and Great Egrets to serve as definitive hosts for *B. damnificus*.

ACKNOWLEDGMENTS

This research was supported in part by grants from the USDA Southern Regional Aquaculture Center (Grant 2002-38500-11805), USDA-NRI (Grant 2002-35204-11678), USDA/APHIS (Cooperative Agreement 06-7428-0499), the Mississippi Agricultural and Forestry Experiment Station (MAFES), and Mississippi State University College of Veterinary Medicine. We thank P. Fioranelli, K. Hanson, S. Lemmons, R. Singleton, and S. Woodruff for assistance with bird capture. We also thank J. Grady, P. Fioranelli, K. Hanson, S. Lemmons, K. Obringer, P. Siefker, R. Singleton, E. Thornton, and S. Woodruff for assistance with animal care. M. Yost and K. Obringer provided help with trematode egg counts. T. Lenarduzzi assisted with bird euthanasia. C. Panuska, D. Minchew, M. Mauel, and S. Barras assisted with editorial reviews.

LITERATURE CITED

AVERY, J., J. TERHUNE, D. WISE, AND L. KHOO. 2001. New trematode in channel catfish. Thad Cochran National Warmwater Aquaculture Center Fact Sheet No. 004 (revised). Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS.

FEDYNICH, A. M., D. B. PENCE, AND J. F. BERGAN. 1997. Helminth community structure and pattern in sympatric populations of double-crested and neotropic cormorants. Journal of the Helminthological Society of Washington 64: 176–182.

FLOWERS, J. R., M. F. POORE, J. E. MULLEN, AND M. G. LEVY. 2004. Digeneans collected from piscivorous birds in North Carolina, U.S.A. Comparative Parasitology 71: 243–244.

FOREYT, W. J. 2001. Veterinary parasitology reference manual. Iowa State University Press, Ames, Iowa, 235 pp.

GARCIA, M. I. J. 1993. Fauna helminthologica de *Cichlasoma fenestratum* (Pisces: Cichlidae) del Lago de Catemaco, Veracruz, Mexico. Anales del Instituto de Biologia Universidad Nacional Autonoma de Mexico Serie Zoologica 64: 75–78.

GLAHN, J. F., AND D. T. KING. 2004. Bird depredation. *In* Biology and culture of channel catfish, C. S. Tucker and J. A. Hargreaves (eds.), Developments in aquaculture and fisheries science 34. Elsevier, Amsterdam, The Netherlands, pp. 503–529.

———, E. S. RASMUSSEN, T. TOMSA, AND K. J. PREUSSER. 1999. Distribution and relative impact of avian predators at aquaculture facilities in the northeastern United States. North American Journal of Aquaculture 61: 340–348.

———, D. S. REINHOLD, AND C. A. SLOAN. 2000. Recent population trends of double-crested cormorants wintering in the delta region of Mississippi: Responses to roost dispersal and removal under a recent depredation order. Waterbirds 23: 38–44.

HOFFMAN, G. L. 1999. Parasites of North American freshwater fishes. 2nd Edition. Comstock Publishing Associates, Ithaca, New York, 539 pp.

JONES, A., R. A. BRAY, AND D. I. GIBSON. 2005. Keys to the trematoda, Vol. 2. CAB International and The Natural History Museum, London, 745 pp.

KING, D. T. 2005. Interactions between the American white pelican and aquaculture in the southeastern United States: An overview. Waterbirds 28 (Special Publication 1): 83–86.

———, AND S. J. WERNER. 2001. Daily activity budgets and population size of American white pelicans wintering in south Louisiana and the delta region of Mississippi. Waterbirds 24: 250–254.

———, J. D. PAULSON, D. J. LEBLANC, AND K. BRUCE. 1998. Two capture techniques for American white pelicans and great blue herons. Colonial Waterbirds 21: 258–260.

KINSELLA, J. M., M. G. SPALDING, AND D. J. FORRESTER. 2004. Parasitic helminths of the American white pelican, *Pelecanus erythrorhynchos*, from Florida, U.S.A. Comparative Parasitology 71: 29–36.

KOSTADINOVA, A., C. VAUCHER, AND D. I. GIBSON.

2002. Redescriptions of two echinostomes from birds in Paraguay, with comments on *Drepanocephalus* Dietz, 1909 and *Paryphostomum* Dietz, 1909 (Digenea: Echinostomatidae). Systematic Parasitology 53: 147–158.

LAMOTHE-ARGUMEDO, R., AND G. PÉREZ-PONCE DE LEON. 1989. Tremátodos de aves II. Descripción de una especie nueva del género *Drepanocephalus* Dietz, 1909 (Trematoda: Echinostomatidae) de *Phalacrocorax olivaceus* en Teapa, Tabasco. Anales del Instituto de Biologia Universidad Nacional Autónoma de México Serie Zoologica 59: 15–20.

LEVY, M. G., J. R. FLOWERS, M. F. POORE, J. E. MULLEN, L. H. KHOO, L. M. POTE, I. PAPERNA, R. DZIKOWSKI, AND R. W. LITAKER. 2002. Morphologic, pathologic, and genetic investigations of *Bolbophorus* species affecting cultured channel catfish in the Mississippi Delta. Journal of Aquatic Animal Health 14: 235–246.

MOTT, D. F., AND M. W. BRUNSON. 1997. A historical perspective of catfish production in the southeast in relation to avian predation. *In* Proceedings of the Eastern Wildlife Damage Management Conference 7: 23–30.

NASIR, P., AND F. H. MARVAL. 1968. Two avian trematodes, *Drepanocephalus olivaceus* n. species and *Calactosomum puffini* Yamaguti, 1941, from Venezuela. Acta Biologica Venezuelica 6: 71–75.

OVERSTREET, R. M., AND S. S. CURRAN. 2004. Defeating diplostomoid dangers in USA catfish aquaculture. Folia Parasitologica 51: 153–165.

———, ———, L. M. POTE, D. T. KING, C. K. BLEND, AND W. D. GRATER. 2002. *Bolbophorus damnificus* n. sp. (Digenea: Bolbophoridae) from the channel catfish *Ictalurus punctatus* and American white pelican *Pelecanus erythrorhynchos* in the USA based on life-cycle and molecular data. Systematic Parasitology 52: 81–96.

RIETSCHEL, G., AND B. WERDING. 1978. Trematodes of birds from northern Colombia. Zeitschrift fur Parasitenkunde 57: 57–82.

SALGADO-MALDONADO, G., AND C. R. KENNEDY. 1997. Richness and similarity of helminth communities in the tropical cichlid fish *Cichlasoma urophthalmus* from the Yucatan Peninsula, Mexico. Parasitology 114: 581–590.

———, R. AGUILAR-AGUILAR, G. CABAÑAS-CARRANZA, E. SOTO-GALERA, AND C. MENDOZA-PALMERO. 2005. Helminth parasites in freshwater fish from the Papaloapan river basin, Mexico. Parasitology Research 96: 69–89.

SEPÚLVEDA, M. S., M. G. SPALDING, J. M. KINSELLA, AND D. J. FORRESTER. 1999. Parasites of the great egret (*Ardea albus*) in Florida and a review of the helminths reported for the species. Journal of the Helminthological Society of Washington 66: 7–13.

TERHUNE, J. S., D. J. WISE, AND L. H. KHOO. 2002. *Bolbophorus confusus* infections in channel catfish in northwestern Mississippi and effects of water temperature on emergence of cercariae from infected snails. North American Journal of Aquaculture 64: 70–74.

THRELFALL, W. 1982. Endoparasites of the double-crested cormorant (*Phalacrocorax auritus*) in Florida. Proceedings of the Helminthological Society of Washington 49: 103–108.

WELLBORN, T. L. 1988. Channel catfish: Life history and biology. Southern Regional Aquaculture Center Publication No. 180. Southern Regional Aquaculture Center, Stoneville, Mississippi.

YAMAGUTI, S. 1958. Systema helminthum, Vol. 1: Digenetic trematodes of vertebrates: Part 1. Interscience Publishers, Inc., New York, 979 pp.

YOST, M., B. S. DORR, AND L. M. POTE. 2005. Confirmation of *Bolbophorus damnificus* life cycle and characterization of all life stages. Southeastern Biology 52: 163.

Received for publication 17 January 2008.