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ARTICLE

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Abstract
A gram-negative bacterium, *Edwardsiella ictaluri*, is the cause of enteric septicemia of catfish (ESC), which is one of the most prevalent bacterial diseases in farm-raised catfish. The objective of this study was to identify risk factors associated with ESC mortalities and are reported by farm personnel. To identify risk factors a catfish management database was developed. The odds ratios (OR) of the final multivariable logistic regression model were: (1) volume of the pond (OR, 0.56), (2) interval from harvest until a mortality event (OR, 1.49), (3) interval from stocking until a mortality event (OR, 0.52), (4) nitrite measured within 14 d of a mortality (OR, 3.49), (5) total ammonia measured within 14 d of a mortality (OR, 20.48), and (6) sum of feed fed for 14 d prior to the disease outbreak (OR, 1.02), all of which were significantly ($P \leq 0.05$) associated with ESC occurrence. This study showed that some commonly recorded production variables were associated with ESC outbreaks and if monitored could help identify "at risk" ponds prior to disease outbreaks.

Enteric septicemia of catfish (ESC) is one of the most prevalent bacterial diseases in commercial catfish production (USDA 2003). It is caused by the gram-negative bacterium *Edwardsiella ictaluri* (Hawke et al. 1981). The epidemiology of ESC can be multifactorial. Outbreaks usually occur in the spring (April–June) and fall (September–November), months when water temperatures are 21–29°C (70–85°F) (Tucker et al. 2004). Enteric septicemia of catfish occurs in three forms: acute, subacute, and chronic. In the acute phase catfish show few clinical signs but go off their feed and swim listlessly or become motionless. Infected fish can have exophthalmia and distended abdomens. The subacute phase is characterized by a slower onset but cumulative mortalities may be high (Hawke and Khoo 2004). Catfish will have petechial and ecchymotic cutaneous hemorrhage on the abdomen and around the head along with small, shallow, white or red ulcers. Fish will go off feed more slowly than in acute ESC. Chronic-phase clinical signs may include hemorrhagic areas around the mouth and on the ventral sides of fish. Small

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white ulcers may be found on the fish's skin. Ulcers on the top of the head and between the eyes are considered pathognomonic for the disease and give it one of its common names, hole-in-the-head disease (Tucker et al. 2004). Fish suffer central nervous system involvement expressed as spinning, spiraling, and tail chasing (Hawke and Khoo 2004). Stress plays a key role in outbreaks. Stress factors such as handling, poor diet, poor water quality, overcrowding, and water temperature fluctuations can lead to an outbreak (Wise et al. 1993; Plumb and Shoemaker 1995). Culturing fish in mixed-age populations or stocking young naive fish in a pond with older fish also plays a key role in the spread of the disease to healthy fish. Surviving fish can carry the pathogen for up to 200 d in their kidney, liver, or brain. Stress may increase the susceptibility to infection and losses, but it is not a prerequisite for the disease. Immune status of the individual fish may also determine the outcome (Hawke and Khoo 2004).

Enteric septicemia of catfish is widespread throughout the U.S. catfish industry. The spread of the disease may be related to the shipment of infected but asymptomatic fingerlings. These fingerlings may be asymptomatic carriers outside of the temperature ranges in which the disease usually occurs (Klesius 1993). Bacteria may be maintained in a multibatch culture environment with the introduction of naïve fingerlings to a pond containing older exposed catfish.

Transmission of ESC between fish is from fecal shedding from sick fish or from the carcasses of dead fish (Earlix 1995). The bacterium can cross the intestinal epithelium, enter the blood stream, and migrate to the kidneys within 15 min of experimental intestinal infection (Baldwin and Newton 1993). Vertical transmission from infected broodstock to fry has not been demonstrated (Hawke and Khoo 2004). The presence of viable *E. ictaluri* in the intestinal contents of cormorants and herons suggest the fecal wastes from piscivorous birds are a potential source of infection (Taylor 1992). However, Waterstrat et al. (1999) found no viable *E. ictaluri* in feces, gastrointestinal tracts, or feathers in experimentally infected great blue herons *Ardea herodias* and concluded that great blue herons do not play a role in the transmission of ESC between catfish ponds.

Many agricultural industries use production databases to help improve production. Feed costs are the largest expense in ictalurid catfish production. Catfish are fed daily as much as they will eat during warm months. Catfish are fed to maximize growth and minimize waste because overfeeding can have a negative effect on water quality. Monitoring feed intake is an important management tool. Some catfish producers use a database, FISHY that was developed by the Mississippi State University Agricultural Economics Department to help catfish producers improve their production-management decision making. The FISHY database concentrates on feeding and projecting fish growth. The catfish management database developed for this research includes data from 2004 to 2007. Not only does this database allow the farmer to manage feed, stocking, harvesting, and mortality, it can also be used to generate user-defined reports designed to (1) incorporate production data already being recorded for generating reports for use at managerial meetings focused on feeding rates, feed conversion ratios, mortalities, and harvesting events; (2) be easily used by a catfish farmer to collect management data in order to analyze production efficiency; (3) provide the farm with easy access to management reports; and (4) improve a pond's efficiency and cost of production. Additional customized reports were generated as requested by the farm management.

The objective of this study was to identify risk factors associated with ESC mortalities. Of particular interest was determining whether the production variables collected from the farms could be used to predict the occurrence of ESC mortality events.

METHODS

Sampling and data collection.—A large commercial catfish enterprise agreed to share their production records. Over 500 ponds from five farms from multiple counties in the Mississippi Delta and dedicated to food fish production were included in this analysis. These ponds had an average size of 5.0 ± 1.66 ha (mean $\pm$ SD).

The catfish management database was programmed in Microsoft Access (Microsoft, Redmond Virginia). A permanent unique pond identification number (ID) greater than 1 was assigned to each pond. Data recorded by the producer from 2004 to 2007was imported into the newly constructed database. Briefly, when a mortality event occurred, the date, pond ID, and reason or cause of the mortality event as well as biomass, average size, and number of fish lost were recorded. Occasionally, affected fish were submitted to the Mississippi State University College of Veterinary Medicine Diagnostic Laboratory located in Stoneville, Mississippi, for laboratory confirmation of ESC.

Pond and production information, later used as explanatory variables in statistical models, were recorded or classified into four main groups: (1) physical characteristics of the ponds, (2) time interval between fish handling and a mortality event, (3) daily feed consumption, and (4) water quality. Physical characteristics of each pond included the surface area (hectares) and average depth (meters), which were used to calculate the volume in hectare-meters (ha-m). Surface area was determined from GIS files (ESRI, San Antonio, Texas) while pond depth was recorded as a single point measurement supplied by farm management. Disease-pond age was defined as the age of the pond at the time of a mortality event and was calculated from the time of original construction or from the time when the pond was rebuilt. A second group of variables included two calculated variables. The disease-stocking interval was defined as the interval from the time fish were stocked into the pond until a mortality event occurred. Stocking event information was recorded and included the source of the fish stocked, date the pond was stocked, and the number, size, and weight of fish stocked. The disease-to-harvest interval was defined as the time from a harvesting event until a mortality event occurred.

Harvesting event information was recorded and included the date of the harvest, the weight, and the number of fish harvested.

The third group of variables dealt with feed consumption. The catfish management database contained the feeding records in terms of total kilograms of feed fed for each pond on a daily basis. The total feed was then aggregated for periods of 7, 14, 21, and 30 d prior to the ESC-related mortality event. These values did not take into account the varying sizes of the ponds. To compare feed usage the aggregate totals were divided by pond area to calculate feed per hectare, divided by pond depth to calculate feed per meter of depth, and divided by pond volume to calculate feed per hectare-meter. These calculations allowed comparisons between ponds of differing sizes, depth, and volumes.

The fourth group of variables involved water quality measurements. A separate water quality database was developed and located in a water quality testing laboratory on the farm. All testing for pond water quality variables was performed in the central laboratory by one technician. Pond water was tested for total ammonia nitrogen (TAN), nitrite, and chloride. Chloride was measured only if the TAN level was considered high (>6 mg/L). The database was designed to automatically generate a report of ponds that exceeded the management-defined total chloride-to-nitrite ratio. The water quality database was constructed in 2005 so data from 2005 to 2007 were included in the analysis. Water quality data were collected on a weekly or biweekly basis during the growing season (March–November) and monthly during the nongrowing season.

An observation was defined as a pond with a positive mortality event due to ESC as defined by the farm management. Ponds without a history of mortality events associated with ESC were selected as negative ponds and served as controls. A negative pond was defined as a pond that did not have a mortality event 60 d before and 60 d after the mortality event date being analyzed.

Statistical procedures: risk-factor modeling, variable selection.—Logistic regression was used to assess the strength of association between the dichotomous outcome of interest, ESC occurrence in ponds, and various independent variables that represented potential risk factors for the disease. The data in the study was hierarchically structured, which called for multilevel modeling (Guo and Hongxin 2000) with ponds (level 1) nested in farms (level 2) that are nested in the catfish enterprise (level 3). Biases in variable estimates could result from ignoring observations that are more highly correlated and within clusters or levels. Linear and binary models underestimate standard errors when clustering is not taken into account and the assumption of independence is violated. Multilevel modeling provides more accurate standard errors, confidence intervals, and significance tests by correcting these biases (Guo and Hongxin 2000). Generalized linear mixed models designating a binomial distribution with a logit link function were fitted using the GLIMMIX procedure in SAS 9.2 software for Windows (SAS Institute, Cary, North Carolina) to conduct the logistic regression analysis. Random effects were incorporated to account for the repeated measures of ponds and variability among the participating farms and possible intrafarm correlation. In the screening process, each risk factor was evaluated in the basic model as a single fixed-effects factor, and if associated with the outcome ($P \leq 0.20$) was retained for further analysis. In the second step, all continuous variables considered as risk factors were retained from the screening step and investigated for pairwise collinearity using the CORR procedure in SAS for Windows version 9.2. Each case of collinearity, defined at $R \geq 0.6$, detected was evaluated separately on the significance of the association with the occurrence of ESC and relationship with other explanatory variables.

To build the final multivariable model, the fixed-effects risk factors retained from the screening and collinearity investigations were offered to the basic model all at once as fixed-effects factors. After each model run, the fixed-effects factor with the highest P-value was removed until a final model with all the fixed-effects variables significant at $P \leq 0.050$ was developed. Further refinement of the developed full final model was pursued to obtain the most parsimonious model while preserving its explanatory ability. A limited number of tools are available to evaluate the performance of generalized linear mixed models with a different set of predictors for a given outcome. There is no best way to estimate goodness of fit for multilevel models. In nonmultilevel logistic regressions, the chi-square goodness-of-fit test is appropriate when an assumption of independence of observations and data are not very sparse (Schukken et al. 2003). These assumptions are not met in multilevel modeling with clustering so the chi-square test of goodness of fit is not appropriate.

The models were compared using the Akaike information criterion (AIC) score. Models that had AIC score differences of greater than 2 from the model with the minimum AIC score were eliminated from the analysis (Burnham and Anderson 2001). Models with the lowest AIC scores were selected as the final model. In the descriptive statistics, means were reported with their SD values. A strength of association between variables was reported as the odds ratio (OR).

RESULTS

In ESC-related mortality events, mean losses were $4{,}053 \pm 224$ individuals, at 0.6 ± 0.01 kg per fish for a total weight of $2{,}303 \pm 120$ kg per mortality event. Enteric septicemia of catfish accounted for 18.91% of the observed mortalities from 2004 to 2007. On a monthly basis, farm-recorded ESC mortalities peaked in September and October (Figure 1). Average pond surface area was 4.65 ± 1.52 ha (range, 4.4–4.8 ha) and pond depth was 1.76 ± 0.33 m (range, 1.71–1.81 m). This farm has undergone a very aggressive pond rebuilding program over the last 3 years with newer rebuilt ponds being deeper. Average pond volume was 8.48 ± 3.84 ha-m (range, 7.9–9.1 ha-m).

The screening process for the data set identified 27 variables that had an association ($P \leq 0.2$) with the occurrence of

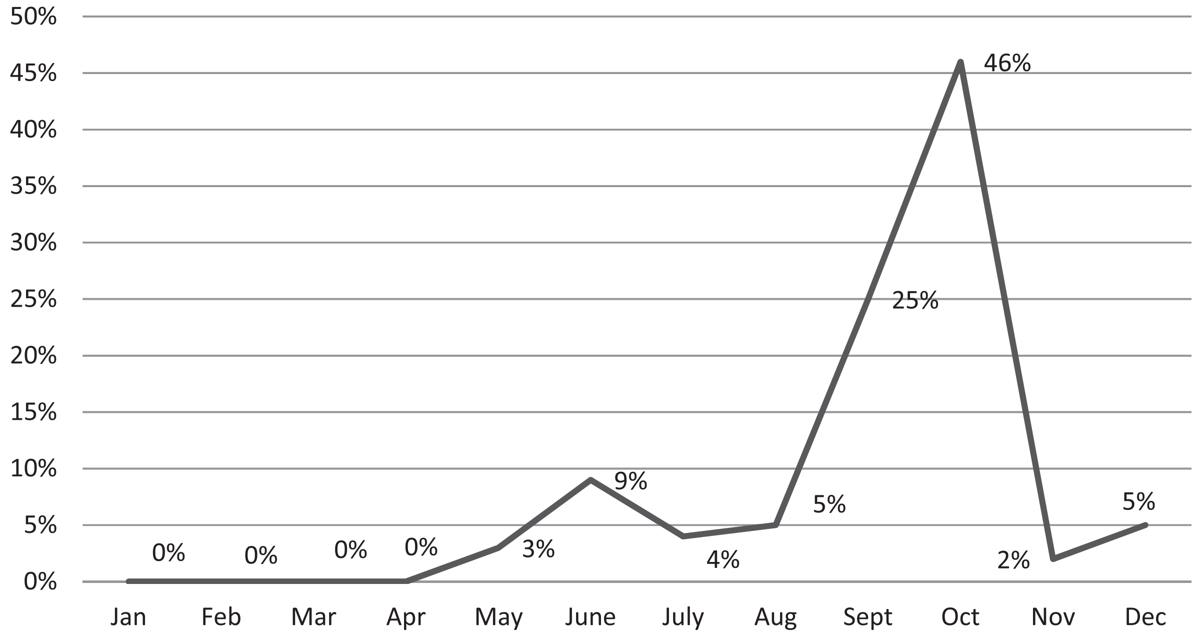

FIGURE 1. Farm-reported percent of ESC mortalities in catfish (number of cases per month, 2004–2007).

ESC and were considered as candidates in a multivariable model (Table 1). During the variable screening process 1,215 observations were identified.

The most parsimonious multivariable model included six effects in the final model: (1) pond volume, (2) interval from stocking until a mortality event, (3) interval from harvest until a mortality event, (4) nitrite measured within 14 d of a mortality event, (5) TAN measured within 14 d of a mortality event, and (6) sum of feed fed for 14 d prior to the disease outbreak. As pond volume and the interval from stocking to a mortality event increased, the odds of a mortality event associated with ESC occurring decreased. As the interval from harvest to a mortality event, nitrite and ammonia levels within 14 d, and sum of feed fed for 14 d prior to a mortality event increased, the odds of a mortality event associated with ESC occurring in a pond increased (Table 2). For the ponds included in this analysis, the mean $\pm$ SD pond surface area was 4.8 ± 1.60 ha and pond depth was 1.84 ± 0.364 m.

DISCUSSION

The objective of this study was to identify potential risk factors associated with mortality events that the farm managers attributed to ESC. Wagner et al. (2002) using the 1997 National Animal Health Monitoring System (NAHMS) survey of catfish farmers found that the most frequently reported (35.7%) average loss per outbreak of ESC and columnaris combined was 90–900 kg (200–2,000 lb) per outbreak. Only 18.3% of the operations reported losses classified as severe (>900 kg). In contrast, the ESC-related mortality events in this study resulted in losses (mean $\pm$ SD) of $3{,}156 \pm 3{,}317$ fish, at 0.5 ± 0.02 kg per fish for a total weight of $1{,}615 \pm 1{,}835$ kg per mortality event. Enteric septicemia of catfish accounted for 18.91% of the observed mortalities from 2004 to 2007. Wagner et al. (2002) found that 78.1% of the farms surveyed and 42.1% of all ponds experienced ESC or columnaris problems.

In the model pond volume was significantly associated with ESC occurrence. Ponds with more volume had reduced odds of a mortality event associated with ESC. Since depth is a key component of volume (area $\times$ depth) this result in not unexpected. Hanson et al. (2008) found that as pond depth increased, catfish losses from weather-related causes decreased, because the deeper ponds were not as sensitive to windstorms, droughts, and freezing. In contrast, Cunningham et al. (2012) found that the incidence of columnaris increased as pond depth increased. Greater pond depth offers more habitat for the catfish; shallower ponds or older ponds that have filled in (Steeby et al. 2004) provide less space and may lead to crowding and increased stress on the catfish. Pond depth data were a single measurement reported by the farm. However, catfish ponds are sloped and are shallower at the margins and/or at one end and deeper at the opposite end. Pond depth can be influenced by the age of the pond and sediment accumulation. Increased stress can lead to greater chance of disease occurring; deeper ponds may reduce this stress leading to reduced odds of a disease occurring.

As the stocking-to-disease interval increased, the odds of a disease outbreak associated with ESC decreased. Therefore, a decreased stocking-to-disease interval would be associated with increased odds of ESC, suggesting that contaminated equipment used in stocking or stress due to the stocking event could have contributed to disease occurrence. The odds of a mortality event due to ESC increased as the harvest-to-disease interval

TABLE 1. Logistic regression analysis results for variables associated with the occurrence of ESC in catfish (*P*-values < 0.20). ha-m = hectare-meter.

Variable	Measured unit or comparison	*N*	Odds ratio	Confidence interval	*P*-value
Depth	m	1,215	0.11	0.03–0.47	0.0027
Volume	ha-	1,215	0.78	0.66–0.91	0.0019
Size	ha	1,216	0.68	0.51–0.92	0.0124
Year	year	1,216			<0.0001
	2005 versus 2007		0.02	0.01–0.05	
	2006 versus 2007		0.37	0.28–0.49	
Disease-pond age	d	1,215	1.23	1.15–1.32	<0.0001
Prediseasefeed sum	kg	1,215	3.14	2.54–3.86	<0.0001
Disease-harvest interval	d	1,215	1.47	1.40–1.53	<0.0001
Disease-stock interval	d	1,215	0.44	0.30–0.67	<0.0001
Nitrites, 8–14 d	mg/L	1,215	3.44	2.06–5.74	<0.0001
Ammonia, 8–14 d	mg/L	1,215	7.41	4.13–13.28	<0.0001
Nitrites, 14–21 d	mg/L	192	7.48	0.95–58.82	0.056
Feed, 0–7 d	kg	1,215	1.00	1.00–1.00	0.1058
Feed, 0–14 d	kg	1,215	1.00	1.00–1.00	0.0127
Feed, 0–21 d	kg	1,215	1.00	1.00–1.00	<0.0001
Feed, 0–30 d	kg	1,215	1.00	1.00–1.001	<0.0001
Feed, 0–7 d/ha	kg/ha	1,215	1.00	0.99–1.00	0.1230
Feed, 0–14 d/ ha	kg/ha	1,215	1.00	1.00–1.001	0.0167
Feed, 0–21 d/ha	kg/ha	1,215	1.00	1.00–1.00	<0.0001
Feed, 0–30 d/ha	kg/ha	1,215	1.001	1.001–1.001	<0.0001
Feed, 0–7 d/ha-m	kg/ha-m	1,215	0.999	0.998–1.000	0.1297
Feed, 0–14 d/ha-m	kg/ha-m	1,215	1.001	1.000–1.001	0.0058
Feed, 0–21 d/ha-m	kg/ha-m	1,215	1.001	1.000–1.001	<0.0001
Feed, 0–30 d/ha-m	kg/ha-m	1,215	1.002	1.002–1.002	<0.0001
Feed, 0–7 d/ha-m	kg/m	1,215	1.000	1.000–1.000	0.1606
Feed, 0–14 d/ha-m	kg/m	1,215	1.000	1.000–1.000	0.0022
Feed, 0–21 d/ha-m	kg/m	1,215	1.000	1.000–1.000	<0.0001
Feed, 0–30 d/ha-m	kg/m	1,215	1.000	1.000–1.000	<0.0001

increased. This could be caused by fewer fish in the pond after harvest leading to decreased fish density, which would lower the odds of an ESC break. As the pond is restocked and the fish density is increased, stress will also increase, increasing the risk of an ESC break. These intervals could be used as indirect indicators of fish handling stress or the use of contaminated equipment.

Increased total feed fed increased the odds of a disease outbreak associated with ESC. Catfish ponds have a finite capacity to process waste without affecting water quality. Water quality problems including low dissolved oxygen (DO) will increase in severity and frequency if feed exceeds the waste-processing capacity of a pond. Catfish ponds fed at a high rate, defined as a maximum of 78 kg/ha, had lower DO levels at dawn, reduced

TABLE 2. Odds ratio (OR) of the final logistic regression model.

Variables	Measured unit	OR	Confidence interval	*P*-value
Volume	ha-m	0.56	0.42–0.74	<0.0001
Disease-stocking interval	d	0.52	0.34–0.81	0.0035
Disease-to-harvest interval	d	1.49	1.41–1.57	<0.0001
Nitrites, 14 d	mg/L	3.49	1.66–7.33	0.0010
Total ammonia, 14 d	mg/L	20.48	9.96–42.11	<0.0001
Feed, 0–14 d	100 kg	1.02	1.01–1.03	<0.0001

growth rate, poorer feed conversion, and increased mortality compared with medium (56 kg/ha) and low (34 kg/ha) feeding rates (Tucker and Boyd 1979). In 50% of the ponds fed at the higher rate the mortality rate ranged from 7% to 32%. Cole and Boyd (1986) found that net fish production increased in proportion to feed fed up to 112 kg/ha per day but then decreased at higher feeding rates. Feed conversion (increased feed fed per unit of weight gain) was constant when feed fed was between 28 and 112 kg/ha but quickly increased at higher feeding levels (>112 kg/ha per day) at which point fish did not consume all of their feed, resulting in increased waste accumulation and decreased water quality

The odds of a pond having an ESC outbreak were 3.49 times greater for each one unit increase in nitrite measured 14 d prior to a disease event and 20.48 times greater for each one unit increase in TAN levels measured for this same time period. Water quality measures that potentially affect fish health include nitrite, ammonia, and oxygen levels. High nitrite can result from overfeeding and decomposition of organic materials. Therefore, routine monitoring of these levels in ponds is considered to be an essential best management practice (BMP) towards the prevention of mortalities due to toxic levels. Water quality data were collected on a weekly or biweekly basis during the growing season (March–November) and monthly during the nongrowing season. Weekly measurements give the farm time to identify ponds at risk. The addition of salt (NaCl) to ponds is a common management practice aimed towards the treatment and prevention of disease. Elevated ammonia levels can cause physiological, biochemical, histological, and behavioral effects in fish. Un-ionized ammonia (NH_3) is excreted by passive diffusion from the gills of Channel Catfish *Ictalurus punctatus*. The pH and temperature of the blood determine the proportion of TAN ($NH_3 + NH_4^+$) that is partitioned between ionized and un-ionized forms. Gill epithelium diffusion of NH_3 is a function of water pH, plasma pH, and total ammonia concentration (Hargreaves and Tomasso 2004). High levels of NH_3 in water will cause decreased diffusion resulting in increased levels in plasma. Low DO levels can increase the effect of high ammonia levels. Although ammonia concentrations that cause death are seldom observed in catfish ponds, sublethal effects such as compromised immune status and reduced growth rate are observed. As oxygen levels decrease even low levels of total ammonia (0.43 mg/L) can reduce voluntary feed consumption by 68% (Hargreaves and Tomasso 2004). The chloride-to-nitrite ratio is important to determine methemoglobin levels in catfish. Nitrite from the pond water is actively transported to the catfish circulatory system producing a life threatening condition known as brown blood disease or methemoglobinemia (Durborrow et al. 1997). Nitrite : chloride ratios of 20:1 or greater are recommended. Lower ratios can result in brown blood disease (Hargreaves and Tomasso 2004). The database was designed to automatically generate a report of ponds that do not meet the management-defined chloride : nitrite ratio. This ratio is important because the potential toxicity of nitrite is reduced by increasing the chloride concentration of the pond water by adding salt. Chloride : nitrite–nitrogen ratios of 30:1 allow little nitrite to enter the catfish's blood stream, but producers routinely maintain pond water chloride concentrations at 100–150 mg/L to maintain a safety margin (Tucker and Hargreaves 2004).

The data were used in the management of the catfish farm and assumed accurate. Compared with a prospective study a disadvantage of this retrospective study was that key variables such as pond DO levels, temperature, and pH were not recorded. The farm recorded DO content on each pond up to eight times per night. They did not record these observations (>3,500 per night) due to their high number. They responded to any low DO events by aeration. Pond water temperature was available from a nearby weather station, but we did not use it because the water temperature would be the same in ponds with and without ESC outbreaks. The variables described in this study are associated with ESC mortality events but do not necessarily cause ESC. They are, however, good variables to consider when designing controlled experiments to determine which risk factors actually predispose a pond to ESC-associated mortalities. The model and methodology developed for this study may be useful for the investigation of additional economically important catfish diseases. This study showed some commonly recorded production variables (feed consumption, pond size and depth, nitrite levels, and stocking events) were associated with ESC-associated mortalities and if monitored could help identify "at risk" ponds prior to ESC outbreaks.

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