Received: 17 May 2017 | Revised: 28 June 2017 | Accepted: 30 June 2017

DOI: 10.1111/jfd.12696

ORIGINAL ARTICLE

WILEY Journal of Fish Diseases

First detection of *Edwardsiella ictaluri* (Proteobacteria: Enterobacteriaceae) in wild Australian catfish

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Funding information
Fisheries Research and Development Corporation (FRDC), Grant/Award Number: 2012/050; Australian Government Research Training Program Scholarship

Abstract

The bacterium *Edwardsiella ictaluri* is considered to be one of the most significant pathogens of farmed catfish in the United States of America and has also caused mortalities in farmed and wild fishes in many other parts of the world. *E. ictaluri* is not believed to be present in wild fish populations in Australia, although it has previously been detected in imported ornamental fishes held in quarantine facilities. In an attempt to confirm freedom from the bacterium in Australian native fishes, we undertook a risk-based survey of wild catfish from 15 sites across northern Australia. *E. ictaluri* was detected by selective culturing, followed by DNA testing, in Wet Tropics tandan (*Tandanus tropicanus*) from the Tully River, at a prevalence of 0.40 (95% CI 0.21–0.61). The bacterium was not found in fishes sampled from any of the other 14 sites. This is the first report of *E. ictaluri* in wild fishes in Australia.

KEYWORDS
catfish, enteric septicaemia, risk-based sampling, *Tandanus tropicanus*

1 | INTRODUCTION

Human population growth, increasing transport capacity and economic globalization have accelerated the rate of introductions of alien species throughout the world, and invasive species are now recognized as a major cause of biodiversity loss and associated changes in ecosystem function (Simberloff, 2011). Invasive species may affect native species directly, through competition or predation, or indirectly, by altering habitat or changing disease dynamics (Lymbery, Morine, Gholipour Kanani, Beatty, & Morgan, 2014). If alien hosts introduce new parasites or pathogens, then these may be transmitted to native hosts, leading to the emergence of new disease in native species (Daszak, Cunningham, & Hyatt, 2000). In a review of 98 cases of co-introductions throughout the world, Lymbery et al.

(2014) found that fishes were by far the most common alien hosts in published studies, making up 55% of the total, with 81% of fish hosts being either potamodromous or diadromous. This may reflect a taxonomic bias in studies, but is also likely to be due to the propensity for freshwater ecosystems to be particularly affected by invasive fishes (Johnson & Paull, 2011).

Alien fish species were first introduced into Australia by European settlers in the late 18th and early 19th century, and there are now at least 35 invasive fish species with established wild breeding populations, of which 22 are imported ornamental species (Lintermans, 2004). An estimated 10–16 million live ornamental fishes are imported into Australia annually, making this the major modern pathway for alien fish introduction (Department of Agriculture, Fisheries & Forestry 2006). Invasive ornamental fishes have introduced a number of co-invading pathogens to Australia, including *Aeromonas salmonicida* (Lehmann & Neumann) (Whittington, Gudkovs, Carrigan, Ashburner, & Thurstan, 1987), *Bothriocephalus acheilognathi* Yamaguti (Dove & Fletcher, 2000) and *Cyprinid herpesvirus 2* (Becker et al., 2014).

The bacterium *Edwardsiella ictaluri* Hawke is the causative agent of enteric septicaemia of catfish (ESC; Hawke, McWhorter, Steigerwalt, & Brenner, 1981) and is responsible for significant losses of farmed catfish in the United States of America (Shoemaker, Klesius, & Evans, 2002). Infection with *E. ictaluri* results in acute, and often fatal, septicaemic disease (Evance, Klesius, Plumb, & Shoemaker, 2011; Shotts, Blazer, & Waltman, 1986) or a chronic infection characterized by meningoencephalitis (Hanson, 2006; Hawke & Khoo, 2004; Newton, Wolfe, Grizzle, & Plumb, 1989). Individuals that survive an acute infection may become asymptomatic carriers for extended periods of up to 200 days and serve as a reservoir of infection (Chen et al., 1994; Klesius, 1992; Mqolomba & Plumb, 1992). The mode of transmission has not been definitively established, but is thought to be primarily from fish to fish via predation, scavenging or oral/nasal uptake of bacteria shed in the faeces (Klesius, 1992, 1994; Xu, Shoemaker, Zhang, & Klesius, 2013).

Acute outbreaks of ESC occur most frequently in water temperatures between 20°C and 30°C (Francis-Floyd, Beleau, Waterstrat, & Bowser, 1987; Shotts & Plumb, 2003). Where *E. ictaluri* is present in catfish farms, prevalence rates are typically high (up to 70%; Klesius, 1992; Wagner, Wise, Khoo, & Terhune, 2002). Studies of *E. ictaluri* in non-cultured populations of fish are rare; however, Hassan et al. (2012) found prevalence varying from 3 to 70% in a three-year longitudinal study of subclinical infection of Ayu sweetfish, *Plecoglossus altivelus* (Temminck & Schlegel), in Japan.

Edwardsiella ictaluri has been isolated from seven families of catfish (Ictaluridae, Bagridae, Clariidae, Pangasiidae, Siluridae, Plotosidae and Ariidae) (Crumlish, Dung, Turnbull, Ngoc, & Ferguson, 2002; Geng et al., 2013; Kasornchandra, Rogers, & Plumb, 1987; OIE 2009; Shotts et al., 1986; Ye, Li, Qiao, & Li, 2009) and from an increasing number of non-catfish species throughout the world (Baxa, Groff, Wishkovsky, & Hedrick, 1990; Hawke et al., 2013; Kent & Lyons, 1982; Keskin, Secer, Izgur, Turkyilmaz, & Mkakosya, 2004; Sakai et al., 2001; Soto et al., 2012; Waltman, Shotts, & Blazer, 1985). It appears to be a host generalist, although several non-catfish species have been found to be resistant to experimental infections (Plumb & Sanchez, 1983).

Edwardsiella ictaluri is believed to be exotic to Australia, although it has been detected in imported ornamental fishes and native catfishes in aquarium facilities. The first report of *E. ictaluri* in Australia was in imported rosy barbs, *Pethia conchonius* (Hamilton) (Humphrey, Lancaster, Gudkovs, & McDonald, 1986). In 2011, *E. ictaluri* was reported in native Australian highfin catfish, *Neoarius berneyi* (Whitley), toothless catfish, *Anodontiglanis dahli* Rendahl, and narrowfront tandan, *Neosilurus ater* (Perugia), held in tanks in the same facility as imported ornamental fishes, suggesting that Australian catfishes (represented by the families Ariidae and Plotosidae) are also susceptible to *E. ictaluri* infection (Animal Health Australia 2012). To date, *E. ictaluri* has not been reported in wild fishes in Australia, although no comprehensive survey has been undertaken. A related species, *Edwardsiella tarda*, is ubiquitous in Australian freshwater environments and has been associated with disease in Australian fishes (Eaves, Ketterer, Anderson, & Beumer, 1990).

We report here the results of an active surveillance programme, funded by the Australian Government Department of Agriculture, through the Fisheries Research and Development Corporation, to provide evidence to support the claim that *E. ictaluri* is not present wild fish populations in Australia. We designed a targeted, risk-based survey for *E. ictaluri* in wild catfish in rivers in northern Australia. The aim of the survey was to estimate the probability that catfish in northern Australia are free from the bacterium. *Edwardsiella ictaluri* was detected, however, in catfish from one of the sampled rivers.

2 | MATERIALS AND METHODS

2.1 | Survey design

A complete description of the methodology for survey design is available as Supporting Information (Appendix S1). The survey targeted high-risk populations of fish. We assumed that, if the bacterium is exotic to Australia, then the most likely source of entry is through the release of infected ornamental fishes, and proximity of wild fishes to human population centres is therefore the major risk factor. Because disease associated with *E. ictaluri* typically occurs in warmer waters (Buller, 2014), we restricted the survey to populations of catfish in tropical and subtropical northern Australia. We assumed, in the absence of information to the contrary, that all catfish species in northern Australia are equally likely to harbour *E. ictaluri* and that there are no age- or sex-dependent differences in susceptibility of naïve fish (Peterson & Davis, 2012; Plumb & Hanson, 2011). We further assumed that if any fishes were harbouring the bacterium, the majority would be asymptomatic or recovered carriers, and therefore, our sampling techniques, which target actively swimming fish, would have an approximately equal chance of catching either these fishes or fishes without the bacterium. Sampling is therefore assumed to be random after the population has been stratified by risk factors.

We used the stochastic, scenario tree approach of Martin, Cameron, and Greiner (2007) to develop a model of the survey process, defining all ways in which a positive outcome (isolation of *E. ictaluri*) can be obtained at user-defined design prevalences. In developing the model, we assumed a conservative test sensitivity of 0.80 for selective culturing of *E. ictaluri* (c.f. Bebak, Shoemaker, Arias, & Klesius, 2011) from fish tissues, and a test specificity of 1, because any positive culture results were to be confirmed by PCR and DNA sequencing. The model, implemented in Microsoft Excel with the PopTools add-in, allowed the survey sensitivity (the probability of detecting the bacterium given that it is present) to be calculated. From this, it was possible to calculate the probability that the survey region is free from *E. ictaluri*, given negative survey results. The final sampling design, based on the model, involved a mean sample size of 18 fish from each of 15 sites (Table 1; Figure 1), providing a probability of 95% that wild populations of catfish in northern Australia are free of *E. ictaluri* at an overall prevalence of 1% (among-river and within-river design prevalences of 10% each), given negative survey results.

2.2 | Sampling

Most fishes were captured in fyke nets of 2-mm woven mesh, with width, length and depth varying depending on the characteristics of the site. In a small number of sites, fyke nets could not be set and fish were captured using gill nets, seine nets, backpack electrofishing and/or line fishing. At least 20 fishes were sampled from each site except the Ross River catchment, Bloomfield River and Ashburton River, where between 16 and 19 fish were collected. All fish were collected under Animal ethics permit RW2618/13 approved by the Murdoch University Research Ethics committee. Sampled fishes were transported live in aerated river water to temporary, sanitised laboratory holding facilities and kept in fresh, dechlorinated water, with aeration through a sponge filter, until they were killed for examination. Transportation time varied from 10 min to 4 hr, depending on the site. In most sites, fishes were held for less than three hours before being killed, although in four of the more remote sites, they were held for one to two weeks. After each sampling session, all equipment were disinfected in chloroxylenol solution (Dettol; 0.3%), then washed in clean water and air-dried before being used at another site.

Fishes were killed by immersion in an anaesthetic bath of isoeugenol (AquiS) at 175 mg/L for 20 min (or at least 10 min after cessation of opercular movement). Weight, body length measurements and any external or internal gross abnormalities were recorded. Fish condition was estimated from the residuals of the regression of weight on body length. Following euthanasia, fishes were dissected, and kidney, spleen and intestinal tissues (without gut contents) were removed. A portion of each tissue was inoculated onto agar plates for bacterial isolation. The remainder of the kidney, spleen and intestine, along with samples from axial skeletal muscle, eye and brain, were fixed in 10% formalin for histopathology. All fish dissections and inoculation procedures were performed under conditions to minimize dust contamination: in a laboratory if one was available within four hours of the sampling site or in temporary laboratory facilities erected in the field. To prevent cross-contamination, the necropsy

TABLE 1 Sample sites, locations and fish species captured from each site

Site ID	River	Latitude (°S)	Longitude (°E)	Fish species collected (*n*)
BLR	Logan River	27.7609	153.0670	*Neoarius graeffei* (Kner & Steindachner) (20)
BBR	Brisbane River	27.5447	152.7837	*Neoarius graeffei* (20)
TCM	Mary River	26.3319	152.7020	*Tandanus tandanus* (18), *Neosilurus hyrtlii* Steindachner (1), *Neoarius graeffei* (1)
SPM	Mary River	26.0342	152.5106	*Tandanus tandanus* (9), *Neoarius graeffei* (11)
BYB	Burnett River	25.2304	152.0116	*Neoarius graeffei* (20)
MPR	Pioneer River	21.1540	148.7266	*Tandaus tandanus* (20)
TRR	Ross River	19.3232	146.7360	*Neosilurua ater* (15), *Neosilurus hyrtlii* (1)
CTU	Tully River[a]	17.8818	145.8412	*Tandanus tropicanus* (20)
CBA	Barron River[a]	17.2611	145.5378	*Tandanus tandanus* (20)
CBI	Bloomfield River	15.9868	145.2882	*Tandanus tropicanus* (19)
DRC	Rapid Creek	12.3955	130.8722	*Neosilurus hyrtlii* (30)
NTD	Daly River	13.6780	130.6439	*Neoarius graeffei* (20), *Neoarius leptaspis* (Bleeker) (2), *Neosilurus ater* (1)
KLK	Ord River	15.7932	128.7177	*Neoarius graeffei* (14), *Neoarius midgleyi* (Kailola & Pierce) (13)
KSC	Fitzroy River	17.9924	124.2023	*Neosilurus hyrtlii* (10), *Neoarius graeffei* (7), *Neosilurus ater* (3)
PAR	Ashburton River	21.7777	114.9817	*Neoarius graeffei* (18)

[a]In these sites, a number of fyke samples were necessary to capture the required number of fishes and the coordinates refer to the modal locality.

LOW RESOLUTION FIG

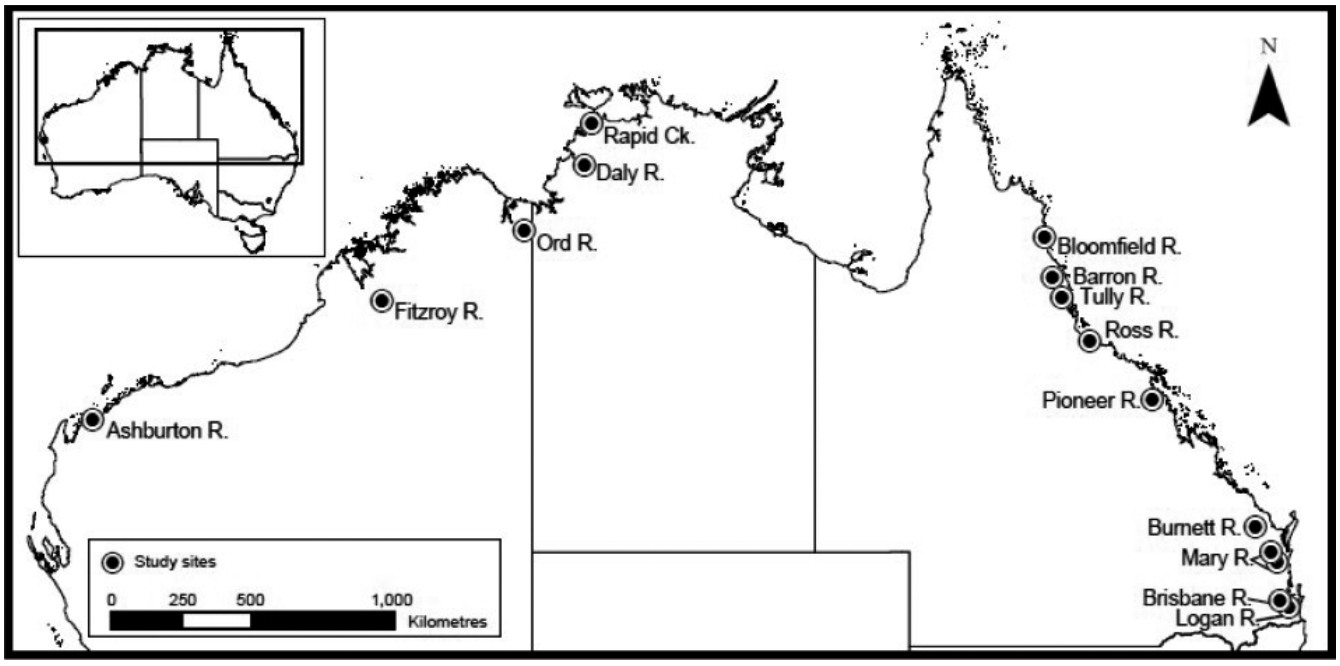

FIGURE 1 Location of sampling sites for detection of *E. ictaluri* in northern Australia

table, dissecting board and all instruments were cleaned with 70% ethanol between fish dissections. Once the abdominal cavity was opened, and between collections of different tissue samples, all dissecting instruments were again cleaned with ethanol and allowed to air-dry. Inoculation loops were used only once.

For bacterial isolation, a sample of pooled kidney and spleen tissues and a sample of intestinal tissue from each individual fish were separately homogenized by crushing with a sterile inoculation loop or needle within a sterile Eppendorf tube, and each inoculated onto blood agar (BA; 3% horse blood agar, PathWest Laboratory Medicine WA) and *E. ictaluri* medium (EIM) plates, prepared according to Shotts and Waltman (1990). Selective culturing of homogenized kidney tissue has been found to be the most reliable method of determining infection status in both clinical and subclinical infections, with a diagnostic sensitivity and specificity of 0.92 and 0.91, respectively, under laboratory conditions (Bebak et al., 2011). Inoculated plates were couriered in insulated boxes under ambient temperatures to the Animal Health Laboratories, Department of Agriculture and Food, Western Australia. Transit time from sample collection to arrival was typically 12 hr.

2.3 | Isolation and identification of *E. ictaluri*

Inoculated plates (both blood agar and EIM) were incubated at 24°C and examined daily for three days. Bacteria were identified using the MALDI-TOF (matrix-assisted laser desorption ionization–time-of-flight mass spectrometer) Biotyper (Bruker Daltonics, Billerica, MA, USA) (Carbonnelle et al., 2011). Isolates identified to species level as being *E. ictaluri* from the MALDI-TOF database were further confirmed using conventional biochemical tests according to Buller (2014) and molecular techniques. All biochemical tests and extraction of DNA were performed using growth from a pure subculture on blood agar. Replicate biochemical sets were incubated at 24°C and 37°C.

For molecular testing, DNA was extracted from the subculture using the PrepMan Ultra Reagent (Applied Biosystems, Foster City, CA, USA) and tested using species-specific primers IVS (5′- TTA AAG TCG AGT TGG CTT AGG G-3′) and IRS (5′-TAC GCT TTC CTC AGT GAG TGT C -3′); and genus-specific primers 16S-flank (5′-TAT CTA ATC CTG TTT GCT CCC C-3′) and 23S-F (5′-GAC GTT GAT AGG CTG GGT GT -3′) (Williams & Lawrence, 2010). DNA from an isolate of *Edwardsiella tarda* was also tested using these primers. The PCR mix consisted of Promega master mix reagent (Promega Australia) with a final concentration of 0.5 μmol/L for each primer, 2 μl of DNA in a 25 μl reaction volume. Amplification involved an initial denaturation step at 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 45 s, elongation at 72°C for 2.5 min, with a final step of elongation at 72°C for 5 min.

Identification was confirmed on one positive culture by performing 16S rDNA sequencing using universal 16S rRNA gene primers 530F and 1392R (Lane, 1991) and sequencing the amplified products obtained from the IVS/IRS primers and from the 16S flank/23S-F primers. Amplified product was purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sent to the Australian Genome Research Facility for sequencing. Sequences were aligned to related 16S rDNA sequences in GenBank using Blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A consensus sequence was prepared from the overlapping amplicon sequences using CAP3 (Huang & Madan, 1999). Positive samples (both culture and DNA) were sent to the Fish Diseases Laboratory at the Australian Animal Health Laboratory for independent confirmation using species-specific PCR (IVS and IRS primers) and 16S rDNA sequencing.

2.4 | Histopathology

For any fishes which tested positive for *E. ictaluri*, and for a subsample of fishes which tested negative, fixed tissues were processed using standard histology techniques. Formalin-fixed bony tissues were demineralized in 5% nitric acid for one hour before routine histo-processing and embedding in paraffin wax. Five-micrometre sections were stained with haematoxylin and eosin and examined by light microscopy on an Olympus BX41. Each of two fish pathologists (EK and SG-K) examined every tissue section independently for a minimum of 15 min.

2.5 | Data analysis

Fishes which tested positive for *E. ictaluri* by bacterial culture and DNA analysis for either tissue sample (pooled kidney/spleen or intestine) were classed as infected. By infected, we mean only that the bacterium is present in the fish, not necessarily that it is multiplying (of which we have no knowledge) or that it is causing disease. Prevalence of infection was calculated as the proportion of infected fish at a site, with 95% confidence intervals estimated using Jeffrey's method (Brown, Cai, & DasGupta, 2001). This assumes a binomial distribution, which requires that each sampling event (i.e., each fish captured at a site) is independent and has the same probability that an infected fish will be found. Differences in between infected and uninfected fish in length, weight and condition score were examined with t tests; the residuals were all normally distributed.

To estimate the probability that each sampled site in which no infected fishes were found was truly free from the bacterium, we used Bayesian inference to derive a post-survey (posterior) probability distribution for the number of infected river systems in northern Australia, given that infection was found in one of our surveyed sites (see Results). The resulting probability distribution (Appendix S2) was then substituted for the among-river design prevalence in our stochastic survey model and the model used in 10,000 iterations to estimate median probability (with 95% confidence interval) of the site being uninfected.

3 | RESULTS

Gram-negative, oxidase-negative rods, identified as *E. ictaluri* using MALDI-TOF, were cultured from eight of 20 fish sampled at one site: Bullyard Creek, a tributary of the Tully River in northern Queensland. In seven of these fish, the bacterium was cultured from pooled kidney/spleen samples and in one fish, from both kidney/spleen and intestinal samples. All isolates identified as *E. ictaluri* from culture produced amplicons at the expected molecular weight in the species-specific (2,000 bp) and genus-specific (1,300 bp) PCR amplifications. DNA from an isolate of *E. tarda* did not produce an amplicon in the species-specific PCR, but produced an amplicon of approximately 1,300 bp with the genus primers. 16S rDNA sequence analysis achieved maximum 100% identity over 100% of the query coverage against *E. ictaluri*. The partial 3591 nt consensus sequence of the ribosomal RNA subunit (23S, IVS, IRS and 16S) revealed a 99.7% match for *Edwardsiella ictaluri* (Genbank accession CP001600.2).

All *E. ictaluri* isolates grew on blood agar as 0.3 mm colonies at 24 hr and increased in size to 0.7 mm at 48 hr with a slight greening of the agar. All isolates were positive for beta haemolysis, ornithine decarboxylase (ODC), lysine decarboxylase, reduction of nitrate, methyl red (MR), fermentation of glucose, maltose and mannose, and growth and motility (tube method) at 24°C and 37°C. Results were negative for arginine dihydrolase, Voges–Proskauer reaction, urease, indole, citrate utilization, aesculin hydrolysis, gelatin hydrolysis, ortho-nitrophenol-β-galactosidase and fermentation of arabinose, inositol, lactose, mannitol, salicin, sorbitol, sucrose, trehalose and xylose when tested in conventional biochemical media. Gas production was variable, with 44% of isolates positive for gas production when tested using a Durham tube in the glucose test. All isolates grew on deoxycholate agar and xylose deoxycholate agar, but not on brilliant green agar (BGA). Results for all tests were the same at both incubation temperatures.

All 20 fish sampled in the Tully River catchment were Wet Tropics tandan, *Tandanus tropicanus* Welsh, Jerry and Burrows, and the prevalence of infection at this site was 0.40 (95% CI 0.21–0.61). No fish (either infected or uninfected) from this site exhibited any behaviours that have been previously associated with ESC disease, such as reduced movement, swimming in circles or hanging motionless in the water column. There was no difference between infected and uninfected fish in either length ($t_{18} = 0.38$, $p = .71$), weight ($t_{18} = 0.07$, $p = .95$) or condition ($t_{18} = 1.25$, $p = .22$). Infected fish had no evidence of gross abnormalities, either externally or on examination of internal organs. Histological examination of infected tissues found no evidence of pathological lesions associated with bacterial septicaemia.

Bacterial cultures of tissue samples from the 323 fishes collected from 14 other sites throughout northern Australia were negative for *E. ictaluri*. The probability that these sites truly contained no infected fishes is shown in Table 2; in six of these sites, the probability was greater than 95%.

4 | DISCUSSION

The principal finding from this survey was the detection of *E. ictaluri* in one wild fish population in Australia. Wild fishes in continental Australia can no longer be assumed to be free from *E. ictaluri*, with the bacterium being detected at a prevalence of 0.40 in *T. tropicanus* catfish in the Tully River catchment. The Tully River system drains an area of 1,684 km^2 in the Australian Wet Tropics bioregion in northern Queensland. Although very little is known of the biology of the host species, *T. tropicanus*, a number of studies on other Australian plotosid catfishes have found high site fidelity, with localized movements (<2 km) for foraging or spawning, related to discharge levels (Beatty, Morgan, McAleer, & Ramsay, 2010; Koster et al.,

TABLE 2 Probability that sites which tested negative for *E. ictaluri* contain no infected catfish. Output from 10,000 iterations of a stochastic model of the survey

Site ID	River	Median probability	Lower 95% confidence limit	Upper 95% confidence limit
BLR	Logan River	0.8696	0.8470	0.8875
BBR	Brisbane River	0.9962	0.9932	0.9978
TCM	Mary River	0.9803	0.9713	0.9860
SPM	Mary River	0.9803	0.9713	0.9860
BYB	Burnett River	0.7440	0.7239	0.7613
MPR	Pioneer River	0.9859	0.9784	0.9905
TRR	Ross River	0.8699	0.8471	0.8880
CBA	Barron River	0.9935	0.9891	0.9959
CBI	Bloomfield River	0.8177	0.7949	0.8366
DRC	Rapid Creek	0.9145	0.8946	0.9295
NTD	Daly River	0.8602	0.8375	0.8784
KLK	Ord River	0.9934	0.9890	0.9958
KSC	Fitzroy River	0.8292	0.8063	0.8480
PAR	Ashburton River	0.8056	0.7830	0.8245

2014; Reynolds, 1983). Longer-range movements do sometimes occur; however, Marshall et al. (2016) found that *Tandanus tandanus* (Mitchell) may move 70 km or more in upstream and downstream directions in response to high-flow events. While it is possible that the infection is limited in extent within the lowlands of the Tully River catchment (below a large dam and a natural escarpment), further studies on the movement behaviour of *T. tropicanus* would be required to determine the potential for spread of *E. ictaluri* within the Tully River and its tributaries.

4.1 | Origin of infection

With the current data, it appears unlikely that *E. ictaluri* is present at six other sites in northern Australia, but for the remaining eight sites that were sampled, we cannot rule out the possibility that the bacterium is present at prevalences below the sensitivity of methods employed in the survey. The current survey, which was designed to determine the probability of freedom from infection across the whole of northern Australia, did not have sufficient power to infer that the bacterium was absent from all of the rivers where it was not found. It is therefore possible that the bacterium we detected represents a more widespread native strain, or a strain that has been present for some time, rather than one which has been recently introduced and confined to the Tully River.

Although some variation in plasmid DNA has been found between Vietnamese and US isolates of *E. ictaluri* (Rogge et al., 2013), phylogeographic data are not currently adequate to enable an accurate assessment of the origin of the bacterium. There is also some evidence of phenotypic variation among isolates of *E. ictaluri* from different geographic locations, although again this is not sufficiently marked to allow definitive inferences of origin. Isolates from the Tully River catchment grew well and were motile at 37°C, unlike isolates from the USA and those previously detected in quarantined imports in Australia (Hawke et al., 1981; Humphrey et al., 1986). Tully River isolates also grew well at 3% NaCl, whereas *E. ictaluri* strains from yellow catfish, *Pelteobagrus fulvidraco* (Richardson), cultured in China did not grow in the presence of >2% NaCl (Liu, Li, Zhou, Wen, & Ye, 2010). Previous reports of growth on BGA medium were not found with the Tully River isolates or with an isolate cultured and identified at Animal Health Laboratories, DAFWA, from quarantined fish imported from Indonesia. Isolates detected in Vietnamese catfish, *Pangasionodon hypophthalmus* (Sauvage), were negative for ornithine decarboxylase (ODC) (Crumlish, Thanh, Koesling, Tung, & Gravningen, 2010), whereas Tully River isolates were positive for ODC. Most isolates are generally reported as negative for fermentation of mannitol; however, isolates from rainbow trout, *Oncorhynchus mykiss* (Walbaum), cultured in Turkey were positive for mannitol (Keskin et al., 2004).

If the presence of *E. ictaluri* in the Tully River catchment results from a recent introduction, rather than a native strain, then the most probable source is through the accidental or deliberate release of infected alien fish. The only alien species captured during sampling in the Tully River catchment was the common platy, *Xiphophorus maculatus* (Günther). This was also the only alien species recorded in the Tully River catchment according to the review of Kroon, Phillips, Burrows, and Hogan (2015) and has been present in the river since at least 1994 (Hogan & Graham, 1994). While neither *X. maculatus* nor any other poeciliid species have been recorded as hosts of *E. ictaluri*, the bacterium appears to be a host generalist and there is no reason to believe that *X. maculatus* cannot act as a carrier. Alternatively, *E. ictaluri* may have been introduced into the Tully River catchment by another alien fish species that either has not established breeding populations or is at such low numbers that it has not been detected. In addition to *X. maculatus*, five other alien fish species have been found in the Johnstone and Murray River catchments, adjacent to the Tully River catchment (Kroon et al., 2015):

the poeciliids eastern gambusia, *Gambusia holbrooki* (Girard), guppy, *Poecilia reticulata* (Peters), green swordtail, *Xiphophorus helleri* (Heckel), the cichlids Mozambique tilapia, *Oreochromis mossambicus* (Peters) and spotted tilapia, *Pelmatolapia mariae* (Boulenger). Although *E. ictaluri* has not been reported from the tilapia species present in the Johnstone and Murray River catchments, it has been isolated from Nile tilapia, *Oreochromis niloticus* (L.) (Soto et al., 2012), and it is thus likely that other tilapia species may also act as carriers.

4.2 | Implications of the invasion

The consequences of infection to native fish species are difficult to predict; however, as infection is often associated with high mortality rates, *E. ictaluri* may represent a threat to Australia's unique freshwater fish fauna. Of 256 described species of freshwater fishes in Australia, 190 (74%) are endemic (Unmack, 2013). This unique fauna is also under threat, with 74 species (28%) listed as threatened under state or national legislation (Lintermans, 2013). Introduced disease may therefore represent a significant additional threat to freshwater fish biodiversity. Very little information is available on the pathogenicity of *E. ictaluri* to native fish species, although clinical signs of disease were apparently seen in the native catfishes *A. dahli*, *N. ater* and *N. berneyi* found to be infected in an aquarium facility (Animal Health Australia 2012). In studies overseas, morbidity and mortality rates have varied widely, depending on fish species and environmental conditions (Buller, 2014).

The presence of *E. ictaluri* in wild Australian fish may also have economic consequences. The ornamental fish industry in Australia was valued at $350 M in 2005 (Tilzey, 2005), with up to 15,000,000 fish imported and 700,000 exported per year (O'Sullivan, Clark, & Morison, 2008); this trade may be affected if Australia does not have disease-free status. Aquaculture, currently Australia's fastest growing primary industry (Food & Agricultural Organisation 2014), may also be impacted. Salmonids (principally *O. mykiss* and *Salmo salar* L.) and barramundi, *Lates calcarifer* (Bloch), are among the most valuable cultured finfish species (Stephan & Hobsbawn, 2014). *Lates calcarifer* and numerous salmonid species are susceptible to *E. ictaluri* (Baxa et al., 1990; Gibson-Kueh, Crumlish, & Ferguson, 2004; Keskin et al., 2004).

4.3 | Management options

Assuming that *E. ictaluri* is an introduced pathogen and not a native Australian strain of the bacterium, then management options may need to be considered. *Edwardsiella ictaluri* is able to survive for long periods (at least 95 days) in the benthos (Plumb & Quinlan, 1986). Given the potential for survival in the environment, eradication of the bacterium is not a viable approach. Any management actions that are undertaken should therefore be aimed at minimizing the potential for spread from infected to uninfected rivers. As a first step, this requires an accurate estimation of the geographic distribution of *E. ictaluri* in Australia. If further sampling determined with sufficient confidence that *E. ictaluri* is confined to the lower Tully River catchment, then management actions would depend on a risk assessment of the pathways by which the bacterium may spread, the probability of exposure of fishes following spread and the consequences of infection to exposed fishes (OIE 2015).

The most likely route for further spread of *E. ictaluri* is by the movement of infected fishes beyond the Tully River catchment. This may occur through human agency, if infected fishes are removed and subsequently released in other catchments, or through natural movement of infected fishes via flood plumes along the coast or connectivity of floodplain wetlands. Spread of *E. ictaluri* by sources other than infected fishes may also be possible. Taylor (1992) detected *E. ictaluri* in 53% of 137 piscivorous birds in the USA, although most isolates could not be cultured. While the viability of the bacterium in birds is not known, the transfer of infection from the Tully River catchment to neighbouring catchments through regurgitated crop contents or faeces cannot be ruled out. As *E. ictaluri* can survive in the benthos for extended periods of time (Plumb & Quinlan, 1986), it is also possible that the bacterium could be transferred by mechanical means, for example, on the feet of wading birds, or on boots, angling equipment, boats or research monitoring equipment.

Once moved to a new catchment, the bacterium then needs to be transferred to uninfected fishes. If *E. ictaluri* is spread by the movement of infected fishes, then these would need to come into contact with naïve fishes in the receiving catchment. There is also, however, circumstantial evidence that environmental sources of the bacterium (i.e., in the benthos) may be the major route of infection when acute disease is not present (Hassan et al., 2012), so fishes in the receiving catchment may be infected directly from transferred mud or water. Finally, the consequences of infection to native fish species are difficult to predict at present. Although clinical signs of disease have been previously reported for three native catfish species in captivity (Animal Health Australia 2012), no details are available. In the current survey, we found no evidence of behavioural changes, loss of condition or pathological lesions in infected *T. tropicanus*.

4.4 | Conclusions and recommendations

Wild fish populations in Australia can no longer be considered to be free from *E. ictaluri*, although it is not yet clear whether the bacterium is confined to one river or is more widespread through northern Australia. As a first step, additional sampling should be undertaken to more precisely determine the geographic range of *E. ictaluri* in Australia. If *E. ictaluri* is widespread, then the only management actions required may be to passively monitor fish health in affected rivers. If the bacterium is confined to the lower Tully River catchment, then a risk assessment would be required to determine whether additional management activities are required. There are some key information gaps that need to be filled before an effective risk assessment can be undertaken. Of particular importance is determining whether the *E. ictaluri* detected in this study has been recently introduced or is a native strain, and investigating the susceptibility and tolerance to infection by *E. ictaluri* of Australian native fish species.

ACKNOWLEDGEMENTS

This study is part of the Fisheries Research and Development Corporation (FRDC) project 2012/050 "*Edwardsiella ictaluri* survey in wild catfish populations," which is supported by funding from the Fisheries Research and Development Corporation on behalf of the Australian Government. This work was also supported by an Australian Government Research Training Program Scholarship. Thanks to the many people who assisted greatly with collection and processing of samples: Terry Miller, Gavin Dally (Museum and Art Gallery of the Northern Territory), Bertus Hanekom, JR Albert (Nyikina-Mangala Rangers) and James Keleher (Murdoch University).

REFERENCES

Animal Health Australia (2012). *Animal health in Australia 2011*. Canberra, ACT: Animal Health Australia.

Baxa, D. V., Groff, J. M., Wishkovsky, A., & Hedrick, R. P. (1990). Susceptibility of nonictalurid fishes to experimental infection with *Edwardsiella ictaluri*. *Diseases of Aquatic Organisms*, *8*, 113–117. https://doi.org/10.3354/dao008113

Beatty, S. J., Morgan, D. L., McAleer, F. J., & Ramsay, A. R. (2010). Groundwater contribution to baseflow maintains habitat connectivity for *Tandanus bostocki* (Teleostei: Plotosidae) in a south-western Australian river. *Ecology of Freshwater Fish*, *19*, 595–608. https://doi.org/10.1111/j.1600-0633.2010.00440.x

Bebak, J., Shoemaker, C., Arias, C., & Klesius, P. (2011). Assay performance during validation of freezing channel catfish *Ictalurus punctatus* (Rafinesque) infected with a gram-negative bacterium. *Aquaculture Research*, *42*, 169–176. https://doi.org/10.1111/j.1365-2109.2010.02551.x

Becker, J. A., Tweedie, A., Rimmer, A., Landos, M., Lintermans, M., & Whittington, R. J. (2014). Incursions of *Cyprinid herpesvirus 2* in goldfish populations in Australia despite quarantine practices. *Aquaculture*, *432*, 53–59. https://doi.org/10.1016/j.aquaculture.2014.04.020

Brown, L. D., Cai, T. T., & DasGupta, A. (2001). Interval estimation for a binomial proportion. *Statistical Science*, *16*, 101–133. https://doi.org/10.1214/ss/1009213286

Buller, N. B. (2014). *Bacteria and fungi from fish and other aquatic animals; A practical identification manual*, 2nd ed. Wallingford: CABI.

Carbonnelle, E., Mesquita, C., Bille, E., Day, N., Dauphin, B., Beretti, J. L., … Nassif, X. (2011). MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clinical Biochemistry*, *44*, 104–109. https://doi.org/10.1016/j.clinbiochem.2010.06.017

Chen, M. F., Henry-Ford, D., Kumlin, M. E., Key, M. L., Light, T. S., Cox, W. T., & Modin, J. C. (1994). Distribution of *Edwardsiella ictaluri* in California. *Journal of Aquatic Animal Health*, *6*, 234–241. https://doi.org/10.1577/1548-8667(1994) 006<0234:DOEIIC>2.3.CO;2

Crumlish, M., Dung, T. T., Turnbull, J. F., Ngoc, N. T. N., & Ferguson, H. W. (2002). Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypothalamus* (Sauvage), cultured in the Mekong Delta, Vietnam. *Journal of Fish Diseases*, *25*, 733–736. https://doi.org/10.1046/j.1365-2761.2002.00412.x

Crumlish, M., Thanh, P. C., Koesling, J., Tung, V. T., & Gravningen, K. (2010). Experimental challenge studies in Vietnamese catfish, *Pangasionodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. *Journal of Fish Diseases*, *33*, 717–722. https://doi.org/10.1111/j.1365-2761.2010.01173.x

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science*, *287*, 443–449. https://doi.org/10.1126/science.287.5452.443

Dove, A. D. M., & Fletcher, A. S. (2000). The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. *Journal of Helminthology*, *74*, 121–127. https://doi.org/10.1017/S0022149X00000160

Eaves, L. E., Ketterer, P. J., Anderson, I. G., & Beumer, J. P. (1990). The isolation and identification of *Edwardsiella tarda* from a diseased native Australian eel (*Anguilla reinhardtii*). *Australian Veterinary Journal*, *67*, 336–337. https://doi.org/10.1111/j.1751-0813.1990.tb07819

Evance, J. J., Klesius, P. H., Plumb, J. A., & Shoemaker, C. A. (2011). *Edwardsiella* septicaemias. In P. K. Woo & D. W. Bruno (Eds.), *Fish diseases and disorders* (pp. 512–569). London: CABI.

Food and Agricultural Organisation of the United Nations (2014). National aquaculture sector overview: Australia. http://www.fao.org/fishery/countrysector/naso_australia/en. Accessed August 13, 2014.

Francis-Floyd, R. M. H., Beleau, P. R., Waterstrat, P. R., & Bowser, P. R. (1987). Effect of water temperature on the clinical outcome of infection with *Edwardsiella ictaluri* in channel catfish. *Journal of the American Veterinary Medical Association*, *191*, 1413–1416.

Geng, Y., Wang, K. Y., Li, C. W., Ren, S. Y., Zhou, Z. Y., Liu, X. X., … Chen, D. F. (2013). Isolation and characterisation of *Edwardsiella ictaluri* from southern catfish, *Silurus soldatovi merionalis*, (Chen) cultured in China. *Journal of the World Aquaculture Society*, *44*, 273–281. https://doi.org/10.1111/jwas.12025

Gibson-Kueh, S., Crumlish, M., & Ferguson, H. W. (2004). A novel 'skinny pot-belly' disease in Asian seabass fry, *Lates calcarifer* (Bloch). *Journal of Fish Diseases*, *27*, 731–735. https://doi.org/10.1111/j.1365-2761.2004.00594.x

Hanson, L. A. (2006). Enteric septicaemia of catfish. In ????. ???? (Ed.), *World organisation for animal health's manual of diagnostic tests for aquatic animals*, 5th ed. (pp. 228–235). Paris: World Organisation for Animal Health.

Hassan, E. S., Mahmoud, M. M., Kawato, Y., Nagai, T., Kawaguchi, O., Iida, Y., … Nakai, T. (2012). Subclinical *Edwardsiella ictaluri* infection of wild ayu *Plecoglossus altivelis*. *Fish Pathology*, *47*, 64–73. https://doi.org/10.3147/jsfp.47.64

Hawke, J. P., Kent, M., Rogge, M., Baumgartner, W., Wiles, J., Shelley, J., … Peterson, T. S. (2013). Edwardsiellosis caused by *Edwardsiella ictaluri* in laboratory populations of zebrafish *Danio rerio*. *Journal of Aquatic Animal Health*, *25*, 171–183. https://doi.org/10.1080/08997659.2013.782226

Hawke, J. P., & Khoo, L. H. (2004). Infectious diseases. In C. S. Tucker & J. A. Hargreaves (Eds.), *Biology and culture of channel catfish* (pp. 387–443). Amsterdam: Elsevier.

Hawke, J. P., McWhorter, A. C., Steigerwalt, A. G., & Brenner, D. J. (1981). *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. *International Journal of Systematic Bacteriology*, *31*, 396–400. https://doi.org/10.1099/00207713-31-4-396

Hogan, A., & Graham, P. (1994). *Tully-Murray floodplain fish distributions and fish habitat*. Report to the Consultants, Tully-Murray Sugar Industry Infrastructure Package. Walkamin, Qld: Freshwater Fisheries and Aquaculture Centre, Department of Primary Industry.

Huang, X., & Madan, A. (1999). CAP3: A DNA sequence assembly program. *Genome Research*, *9*, 868–877. https://doi.org/10.1101/gr.9.9.868

Humphrey, J. D., Lancaster, C., Gudkovs, N., & McDonald, W. (1986). Exotic bacterial pathogens *Edwardsiella tarda* and *Edwardsiella ictaluri* from imported ornamental fish *Betta splendens* and *Puntius conchonius*, respectively: Isolation and quarantine significance. *Australian Veterinary Journal*, *63*, 369–371. https://doi.org/10.1111/j.1751-0813.1986.tb02900.x

Johnson, P. T., & Paull, S. H. (2011). The ecology and emergence of diseases in fresh waters. *Freshwater Biology*, *56*, 638–657. https://doi.org/10.1111/j.1365-2427.2010.02546.x

Kasornchandra, J., Rogers, W. A., & Plumb, J. A. (1987). *Edwardsiella ictaluri* from walking catfish, *Clarias batrachus* L., in Thailand. *Journal of Fish Diseases*, *10*, 137–138. https://doi.org/10.1111/j.1365-2761.1987.tb00729.x

Kent, M. L., & Lyons, J. M. (1982). *Edwardsiella ictaluri* in the green knife fish, *Eigenmannia virescens*. *Fish Health News*, *2*(ii), ????–????.

Keskin, O., Secer, S., Izgur, M., Turkyilmaz, S., & Mkakosya, R. S. (2004). *Edwardsiella ictaluri* infection in rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Veterinary and Animal Sciences*, *28*, 649–653.

Klesius, P. H. (1992). Carrier state of channel catfish infected with *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*, *4*, 227–230. https://doi.org/10.1577/1548-8667(1992) 004<0227:CSOCCI>2.3.CO;2

Klesius, P. K. (1994). Transmission of *Edwardsiella ictaluri* from infected dead to uninfected channel catfish. *Journal of Aquatic Animal Health*, *6*, 180–182. https://doi.org/10.1577/1548-8667(1994) 006<0180:TOEIFI>2.3.CO;2

Koster, W. M., Dawson, D. R., Clunie, P., Hames, F., McKenzie, J., Moloney, P. D., & Crook, D. A. (2014). Movement and habitat use of the freshwater catfish (*Tandanus tandanus*) in a remnant floodplain wetland. *Ecology of Freshwater Fish*, *24*, 443–455. https://doi.org/10.1111/eff.12159

Kroon, F., Phillips, S., Burrows, D., & Hogan, A. (2015). Presence and absence of non-native fish species in the Wet Tropics region, Australia. *Journal of Fish Biology*, *86*, 1177–1185. https://doi.org/10.1111/jfb.12614

Lane, D. J. (1991). 16S/23S rRNA sequencing. In E. Stackebrandt & M. Goodfellow (Eds.), *Nucleic acid techniques in bacterial systematics* (pp. 115–175). Chichester: Wiley.

Lintermans, M. (2004). Human-assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research*, *38*, 481–501. https://doi.org/10.1080/00288330.2004.9517255

Lintermans, M. (2013). Conservation and management. In P. Humphries & K. Walker (Eds.), *Ecology of Australian freshwater fishes* (pp. 283–316). Melbourne, Vic.: CSIRO Publishing.

Liu, J. Y., Li, A. H., Zhou, D. R., Wen, Z. R., & Ye, X. P. (2010). Isolation and characterization of *Edwardsiella ictaluri* strains as pathogens from diseased yellow catfish (*Pelteobagrus fulvidraco* (Richardson) cultured in China. *Aquaculture Research*, *41*, 1835–1844. https://doi.org/10.1111/j.1365-2109.2010.02571.x

Lymbery, A. J., Morine, M., Gholipour Kanani, H., Beatty, S. J., & Morgan, D. L. (2014). Co-invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, *3*, 171–177. https://doi.org/10.1016/j.ijppaw.2014.04.002

Marshall, J. C., Menke, N., Crook, D. A., Lobegeiger, J. S., Balcombe, S. R., Huey, J. A., … Linke, S. (2016). Go with the flow: The movement behaviour of fish from isolated waterhole refugia during connecting flow events in an intermittent dryland river. *Freshwater Biology*, *8*, 1242–1258. https://doi.org/10.1111/fwb.12707

Martin, P. A. J., Cameron, A. R., & Greiner, M. (2007). Demonstrating freedom from disease using multiple complex data sources. 1: A new methodology based on scenario trees. *Preventive Veterinary Medicine*, *79*, 71–97. https://doi.org/10.1015/j.prevetmed.2006.09.008

Mqolomba, T. N., & Plumb, J. A. (1992). Longevity of *Edwardsiella ictaluri* in the organs of experimentally infected channel catfish, *Ictalurus punctatus*. *Aquaculture*, *101*, 1–6. https://doi.org/10.1016/0044-8486(92)90226-B

Newton, J. C., Wolfe, L. G., Grizzle, J. M., & Plumb, J. A. (1989). Pathology of experimental enteric septicaemia in channel catfish, *Ictalurus punctatus* (Rafinesque), following immersion-exposure to *Edwardsiella ictaluri*. *Journal of Fish Diseases*, *12*, 335–347. https://doi.org/10.1111/j.1365-2761.1989.tb00322.x

OIE (2009). *Manual of diagnostic tests for aquatic animals*, 6th ed. Paris: OIE.

OIE (2015). *Aquatic animal health code*. Paris: OIE.

O'Sullivan, D., Clark, E., & Morison, J. (2008). *The Australian ornamental fish industry in 2006/07*. ???, Australia: Report for the Fisheries Research & Development Corporation and the Department of Agriculture, Fisheries & Forestry.

Peterson, B. C., & Davis, K. B. (2012). Effects of gender and sex hormones on disease susceptibility of channel catfish to *Edwardsiella ictaluri*. *Journal of the World Aquaculture Society*, *43*, 733–738. https://doi.org/10.1111/j.1749-7345.2012.00588.x

Plumb, J. A., & Hanson, L. A. (2011). Catfish bacterial diseases. In J. A. Plumb & L. A. Hanson (Eds.), *Health maintenance and principal microbial diseases of cultured fish* (pp. 275–313). Ames, IA: Wiley-Blackwell.

Plumb, J. A., & Quinlan, E. E. (1986). Survival of *Edwardsiella ictaluri* in pond water and bottom mud. *Progressive Fish Culturist*, *48*, 212–214. https://doi.org/10.1577/1548-8640(1986) 48<212:SOEIIP>2.0.CO;2

Plumb, J. A., & Sanchez, D. J. (1983). Susceptibility of five species of fish to *Edwardsiella ictaluri*. *Journal of Fish Diseases*, *6*, 261–266. https://doi.org/10.1111/j.1365-2761.1983.tb00075.x

Reynolds, L. F. (1983). Migration patterns of five fish species in the Murray-Darling river system. *Australian Journal of Marine and Freshwater Research*, *34*, 857–871. https://doi.org/10.1071/MF9830857

Rogge, M. L., Dubytska, L., Jung, T. S., Wiles, J., Elkamel, A. A., Rennhoff, A., … Thune, R. L. (2013). Comparison of Vietnamese and US isolates of *Edwardsiella ictaluri*. *Diseases of Aquatic Organisms*, *106*, 17–29.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., … Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology, Evolution, and Systematics*, *32*, 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Shoemaker, C. A., Klesius, P. H., & Evans, J. J. (2002). In ovo methods for utilizing the modified live *Edwardsiella ictaluri* vaccine against enteric septicaemia in channel catfish. *Aquaculture*, *203*, 221–227. https://doi.org/10.1016/S0044-8486(01)00631-7

Shotts, E. B., Blazer, V. S., & Waltman, W. D. (1986). Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, *43*, 36–42. https://doi.org/10.1139/f86-005

Shotts, E. B., & Plumb, J. A. (2003). Enteric septicaemia. In AFS-FHS (Ed.), *FHS blue book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens* (pp. ????–????). Bethesda, MD: AFS-FHS.

Shotts, E. B., & Waltman, W. D. II (1990). A medium for the selective isolation of *Edwardsiella ictaluri*. *Journal of Wildlife Diseases*, *26*, 214–218. https://doi.org/10.7589/0090-3558-26.2.214

Simberloff, D. (2011). How common are invasion-induced ecosystem impacts? *Biological Invasions*, *13*, 1255–1268. https://doi.org/10.1007/s10530-011-9956-3

Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology*, *20*, 1349–1357. https://doi.org/10.1111/j.1523-1739.2006.00524.x

Soto, E., Griffin, M., Arauz, M., Riofrio, A., Martinez, A., & Cabrejos, M. E. (2012). *Edwardsiella ictaluri* as the causative agent of mortality in cultured Nile tilapia. *Journal of Aquatic Animal Health*, *24*, 81–90. https://doi.org/10.1080/08997659.2012.675931

Stephan, M., & Hobsbawn, P. (2014). *Australian fisheries and aquaculture statistics 2013*. Fisheries Research and Development Corporation project 2010/208. Canberra, ACT: ABARES.

Taylor, P. W. (1992). Fish-eating birds as potential vectors of *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health*, *4*, 240–243. https://doi.org/10.1577/1548-8667(1992) 004<0240:FEBAPV>2.3.CO;2

Tilzey, R. (2005). *A strategic approach to the management of ornamental fish in Australia*. Canberra, ACT: Bureau of Rural Sciences, Commonwealth of Australia.

Unmack, P. J. (2013). Biogeography. In P. Humphries & K. Walker (Eds.), *Ecology of Australian freshwater fishes* (pp. 25–48). Melbourne, Vic.: CSIRO Publishing.

Wagner, B. A., Wise, D. J., Khoo, L. H., & Terhune, J. (2002). The epidemiology of bacterial disease in food-size channel catfish. *Journal of Aquatic Animal Health*, *14*, 263–272. https://doi.org/10.1577/1548-8667(2002) 014<0263:TEOBDI>2.0.CO;2

Waltman, W. D., Shotts, E. B., & Blazer, V. S. (1985). Recovery of *Edwardsiella ictaluri* from danio (*Danio devario*). *Aquaculture*, *46*, 63–66. https://doi.org/10.1016/0044-8486(85)90176-0

Whittington, R. J., Gudkovs, N., Carrigan, M. J., Ashburner, L. D., & Thurstan, S. J. (1987). Clinical, microbiological and epidemiological findings in recent outbreaks of goldfish ulcer disease due to atypical *Aeromonas salmonicida* in south-eastern Australia. *Journal of Fish Diseases*, *10*, 353–362. https://doi.org/10.1111/j.1365-2761.1987.tb01082.x

Williams, M. L., & Lawrence, M. L. (2010). Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR. *Letters in Applied Microbiology*, *50*, 153–157. https://doi.org/10.1111/j.1472-765X.2009.02770.x

Xu, D. H., Shoemaker, C. A., Zhang, Q., & Klesius, P. H. (2013). Naturally infected channel catfish (*Ictalurus punctatus*) concurrently transmit *Ichthyophthirius multifiliis* and *Edwardsiella ictaluri* to naïve channel catfish. *Aquaculture*, *376–379*, 133–136. https://doi.org/10.1016/j.aquaculture.2012.021

Ye, S., Li, H., Qiao, G., & Li, Z. (2009). First case of *Edwardsiella ictaluri* infection in China farmed yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, *292*, 6–10. https://doi.org/10.1016/j.aquaculture.2009.03.036

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