Environ Monit Assess (2020) 192:216
https://doi.org/10.1007/s10661-020-8168-7

Are anglers exposed to *Escherichia coli* from an agriculturally impacted river?

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Received: 30 July 2019 / Accepted: 18 February 2020

Abstract The Pine River, in the central, Lower Peninsula region of Michigan, has a long history of contamination. Livestock facilities and manure application sites along the Pine River and its tributaries have led to elevated nutrient levels. In addition to nutrient loading and associated low levels of dissolved oxygen, the presence *Escherichia coli* bacteria have caused environmental and human health concerns. According to the Michigan Department of Health and Human Services, and the Michigan Department of Environment, Great Lakes, and Energy, *E. coli* counts in summer months consistently have exceeded safe levels for human contact since 2005. Though it is recommended that residents do not swim in the Pine River, there are no specific restrictions on recreational fishing which is prevalent. Few studies have evaluated whether or not *E. coli* accumulates in the mucus of fish and, if so, whether that provides a viable route of *E. coli* exposure for anglers. This study first evaluated the presence of fecal coliform and *E. coli* bacteria on hatchery-raised caged fish placed in the river as well as resident fish. Results showed that fecal coliform and *E. coli* bacteria accumulated both on caged and resident fish. This result led to further testing showing *E. coli* to be found on anglers' hands whether or not they handled or interacted with resident fish. This study suggests that fishing in rivers with heavy bacterial loading from agricultural runoff may expose anglers to potentially harmful *E. coli*.

Keywords *Escherichia coli* · Angler · Bacteria · Fish

Introduction

Nutrient and bacterial loading in our nations streams and tributaries have been monitored for over three decades due in large part to concerns over the growth of industrial agriculture (US EPA 1997, 2000). Stream impacts of nitrogen, phosphorus, and bacterial loading (particularly *Escherichia coli*) are primarily agricultural (US EPA 2017; Mallin and Cahoon 2003). Recently, there has been an increased concern with bacterial loading prompting both the US Environmental Protection Agency (EPA) and multiple state agencies to recalculate water quality criteria for recreational use of waters (US EPA 2012). The Michigan Department of Environmental Quality (now Department of Environment, Great Lakes, and Energy) estimates that over 50% of Michigan's streams and tributaries have been impaired due to agricultural inputs. Stream miles impaired by *E. coli* in Michigan's Lower Peninsula increased over 350% between 2008 and 2016 (MDEQ 2017). Bacterial loading of various types in streams and the associated human

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Published online: 05 March 2020

health risks have been of concern for over 50 years (Geldreich and Kenner 1969). Most strains of *E. coli* are not pathogenic; however, *E. coli* are typically the main indicator of microbial health risk since they are easy to quantify and known to accompany other, more pathogenic, microbes. Many times, these associated microbes are flushed into waterways after rain events through underdrains or drain tiles that drain flat topographic regions of the country (Gentry et al. 2007). Over the past 30 years, potential pathogenicity of *E. coli* in streams has been re-evaluated because of the precipitous increase in antibiotic resistance (Ash et al. 2012). Most studies evaluating human health risks from *E. coli* and other microbes associated with agricultural runoff have focused on either the source or the end-point of microbe activity. This primarily includes livestock operations, drinking water, and recreational swimming (Soller et al. 2010; Pruss 2002). Few studies have considered *E. coli* exposure risk due to recreational use of contaminated waterways such as kayaking, canoeing, or angling. Angling presents a particularly concerning case since fish have a mucus layer that could potentially harbor bacteria such as *E. coli* (Benhamed et al. 2014).

This study evaluated two questions: (1) Will fish exposed in an impacted stream have *E. coli* present in their mucus and, if so, (2) will angling create exposure to *E. coli*? The Pine River, located in central Michigan, is known to have significant bacterial and nutrient loading due to livestock production and runoff (Oemke and Borrello 2008). The Pine River is part of the Saginaw River Drainage Basin, one of the largest drainage basins emptying into a freshwater system in the USA, and the site of extensive recreational use which includes fishing, therefore, providing an ideal location to evaluate potential angler exposure.

Methods

Caged fish pilot study

During the summer of 2016, a pilot study was conducted to evaluate the presence of fecal coliform bacteria, specifically *E. coli*, in the mucus of field-exposed fish. The objective of the caged fish pilot study was to determine if hatchery-raised fish placed in an agriculturally impacted stream would accumulate *E. coli* in their mucus. Due to the limited methods available in the literature, methods were developed and/or adapted from other protocols where specified. Fish cages were constructed with 46 cm and 61 cm polyvinyl chloride pipes (PVC) and 1.25 cm black mesh (FAO 1994). The pipes were connected by one-fourth-inch three-way elbows, and covered in mesh to create a 46-cm × 46-cm × 61-cm cage. Three cages were placed in the Pine River, in locations previously shown to be impacted by agriculture. One cage was placed directly at the confluence of a tributary heavily impacted by a livestock feeding operation (CF) and two downstream (DS-1, DS-2) of the confluence of this tributary in the receiving river. Approximately 20-cm-long bluegill (*Lepomis macrochirus*) were purchased from Stoney Creek Fisheries and Equipment (Grant, MI). Ten fish were placed in a fish cage at all four sites (Fig. 1). Additionally, 20 individuals were split between two 30-gal aquariums and kept in the laboratory as a control. Each cage was staked using a 6-ft iron anchoring rod. Five individuals were swabbed prior to field placement to confirm the absence of *E. coli* bacteria. At 24 h and 48 h intervals following rain events (five total sampling events), five individuals were randomly selected from each cage via a

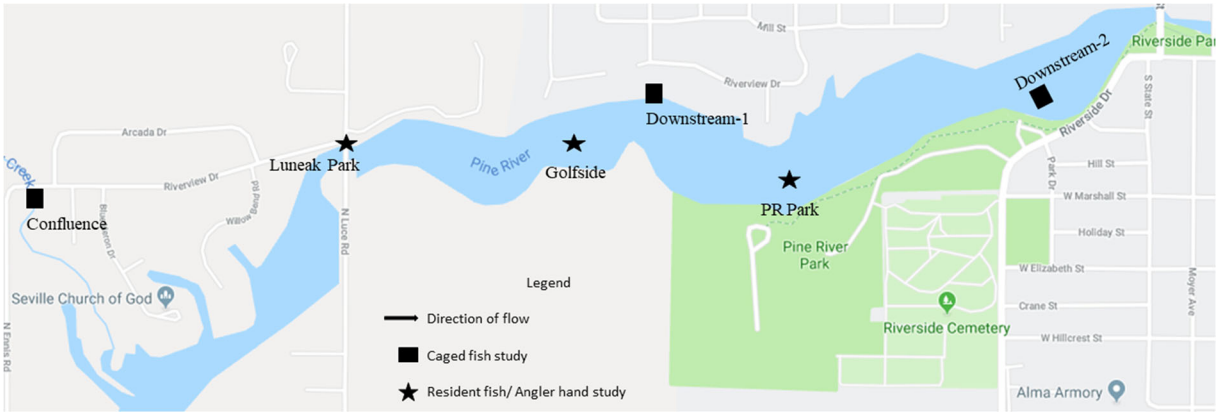

Fig. 1 Map of the field sites from both the caged fish, resident fish, and angler study. The arrows indicate the direction of flow. The squares describe the caged fish study sites, whereas the stars indicate the field sites for the resident fish/angler study

small fishing net and placed in a 5-gal bucket (sterilized with a 5% bleach solution) half full of river water. Fish were brought ashore and swabbed for bacteria. To quantify bacteria in fish mucus, a sterile polyester swab (Falcon, Becton Dickinson and Company, Sparks, MD) was gently passed, continuously rotating, along the lateral line (approximately 10 cm) for a total of three times. Since fish were of a uniform size, the lateral line was used for a consistent swab area. The swab was then placed in a 12- × 75-mm capped polypropylene sterile culture test tube (Fisher #14-956D). During each sampling event and at each site, a sterile swab was dipped and gently swirled three times in the river as a control. Samples were placed in a small Styrofoam cooler for transportation. On day 21, cages were removed from the field. Eight individuals were randomly selected for laboratory filet analysis. These individuals were cleaned and filleted to evaluate potential cross-contamination should anglers be using these fish for consumption. The filets of the fish as well as the internal organs were swabbed using the same swabbing technique. The remaining individuals that were not fileted were euthanized with tricaine methanesulfonate (MS-222) and disposed of according to standard protocols at Alma College.

Each test tube containing a swab was filled with 2.0 ml of deionized water, vortexed for 5 s to expel and suspend bacteria from the sterile swab and transferred to a bottle of Coliscan® Easygel (Microbiology Laboratories LLC, Goshen, IN) and gently swirled. The solution was poured on to a sterile Easygel® pretreated petri dish. The plates were then placed into an incubator in accordance with Coliscan® Easygel instructions; microbial enumeration was conducted following the US Environmental Protection Agency protocol (Bordner and Winter 1978) and reported in colony-forming units (CFUs). Both fecal coliform and *E. coli* were identified and distinguished from each other using differences in color. The *E. coli* were a dark blue while other fecal coliform were pink. Findings from the caged fish pilot were used to establish the study parameters for the next phase.

Resident fish study

On July 21, 2017, researchers and volunteers were divided into two teams to assess the presence of bacteria on resident fish. Locations were selected based on previous data that confirmed the presence of fecal coliform bacteria and *E. coli* in both caged fish and water (Fig. 1). As risk to anglers was of particular concern, a variety of fish species, such as sunfish, blue gill suckers, and largemouth bass, were collected via angling. Anglers fished from both boat and the shore to account for potential variation in water depth. Each team consisted of a designated swabber and two or three anglers. Individuals angled using a fishing pole, fishing line, bait, and tackle. Only regulation-sized fish were sampled per Michigan Department of Natural Resource angling regulations (MDNR 2016). When fish were retrieved, a sterile disposable plastic stencil (3 cm × 10 cm) was placed on the fish and centered along the lateral line. The entire surface inside the stencil was uniformly sampled by passing a sterile polyester swab (Falcon, Becton Dickinson and Company, Sparks, MD) from top to bottom left to right with continuous rotation of the swab. After swabbing fish as described above, swabs were placed into 12 × 75 mm capped polypropylene sterile culture test tubes (Fisher #14-956D) that had been pre-loaded with 1 ml of sterile phosphate-buffered saline (PBS) until transported to the laboratory for extraction. PBS was used as a modification from Downey et al. (2012). Each stencil was only used once. Bacteria were quantified as described below. A small corner of the caudal fin of each fish was clipped using nail clippers on each fish before release to ensure that no fish were caught more than once.

Angler exposure study

Angler's hands were also swabbed at the start and end of the resident fish study. These results indicated that *E. coli* were present on hands only after angling activities, and no fecal coliform was detected prior to fishing. Therefore, an additional study was completed on August 2, 2017, to quantify bacteria on the hands of anglers. To ensure the absence of bacteria prior to angling, before departure, the angler's hands were swabbed by tracing their fingers and palms with a sterile polyester swab (Falcon, Becton Dickinson and Company, Sparks, MD). Anglers were divided into three different locations: Golfside, Riverview, and Luneak Park. At Golfside fish were caught from a boat and at Riverview and Luneak Park fish were caught from the shore. There were two anglers in each group, each assigned a different treatment. These treatments consisted of hands swabbed after catching their first and last fish (treatment 1) or hands swabbed every 30 min, regardless of if the angler caught a fish (treatment 2). All fishing was conducted for 2 h. Samples

were collected from the angler's hands based on their treatment. The sample was taken by tracing the fingers and palms of the angler with continuous rotations of the polyester swab. The swab was immediately inserted into a 12- × 75-mm capped polypropylene sterile culture test tube (Fisher #14-956D) that was pre-loaded with 1 ml of PBS. The labeled vials were stored in a Styrofoam vial holder inside of a small Styrofoam cooler for transportation. After 2 h of fishing, all anglers sterilized their hands using disposable wipes soaked in a bleach solution. Anglers then dipped their hands directly into the river and their palms were subsequently swabbed (treatment 3).

Bacteria enumeration

Bacteria enumeration methods for both the resident fish and angler treatments were adapted from Downey et al. (2012). Samples in the culture test tubes were vortexed for 2 min to expel and suspend bacteria into the PBS. The swab was removed, and the solution was transferred to a bottle of Coliscan® Easygel (Microbiology Laboratories LLC, Goshen, IN) and gently swirled. The solution was poured on to a sterile Easygel® pretreated petri dish. The plates were then placed into an incubator at 37 °C for 24 h in accordance with Coliscan® Easygel instructions; microbial enumeration was conducted following US Environmental Protection Agency protocol (Bordner and Winter 1978) and reported in CFUs. As described above, *E. coli* and other fecal coliform were distinguished based on color.

Statistical analysis

For the resident fish, a two-sample *t* test was used to determine any significance between the number of bacteria on individuals caught from a boat or from the shore. All other comparisons among treatments were made with a one-way ANOVA using SPSS 23 (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp., Armonk, NY).

Results and discussion

Presence of bacteria on caged and resident fish mucus

All fish, including controls and those placed in the field, tested negative for *E. coli* prior to placement, indicating that the fish were free of *E. coli* prior to field exposure. Additionally, control individuals had no *E. coli* on days 10 and 21 of the study. Among the three field sites, beginning with the CF site and moving downstream, *E. coli* was detected 16%, 40%, and 56.5% of the five sampling times, respectively (Fig. 2). *E. coli* CFUs were highest at the DS-2 site suggesting an increase in CFUs of *E. coli* moving downstream, away from the confluence. Among the three field sites, beginning with the CF site and moving downstream, *E. coli* was detected 40%, 0%, and 20% on the sterile swab dipped into the river to access presence/absence of coliform bacteria. This suggests that the presence of *E. coli* and other coliform bacteria

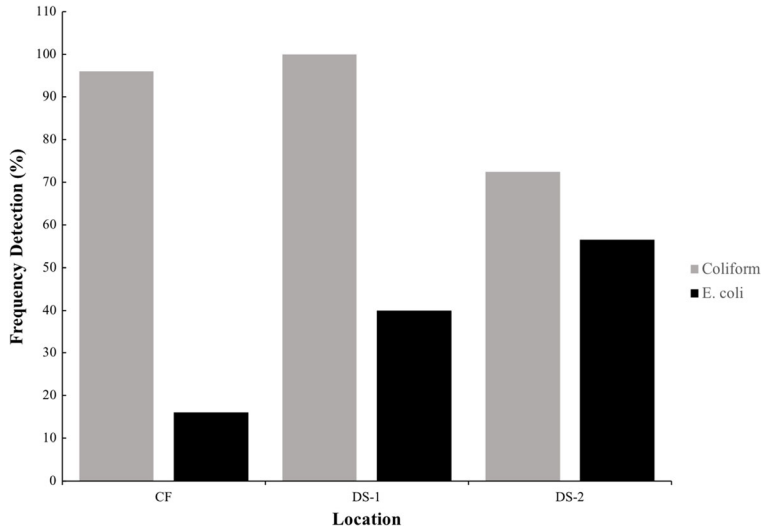

Fig. 2 Frequency of fecal coliform and *E. coli* detection on caged fish sampled five times throughout a 21-day in situ study

in the water does not necessarily correspond to the presence of bacteria in mucus and vice versa. This is further supported by differences in detection frequency between water and mucus in the angler study.

At all three sites, *E. coli* was detected at least once following rain events (Figs. 2 and 3). Although there is limited information in the literature detecting the presence of bacteria on caged fish, microbes persisting in the mucoid exterior slime of fish has been documented. *Erysipelothrix rhusiopathiae*—a gram-positive rod first isolated by Koch in 1878—is known to grow and survive in fish mucus over long periods of time (Wood 1975). During a 21-day exposure, *E. coli* was detected in mucus of individual fish placed into a stream contaminated with *E. coli* which suggested there would be a presence of *E. coli* on resident fish.

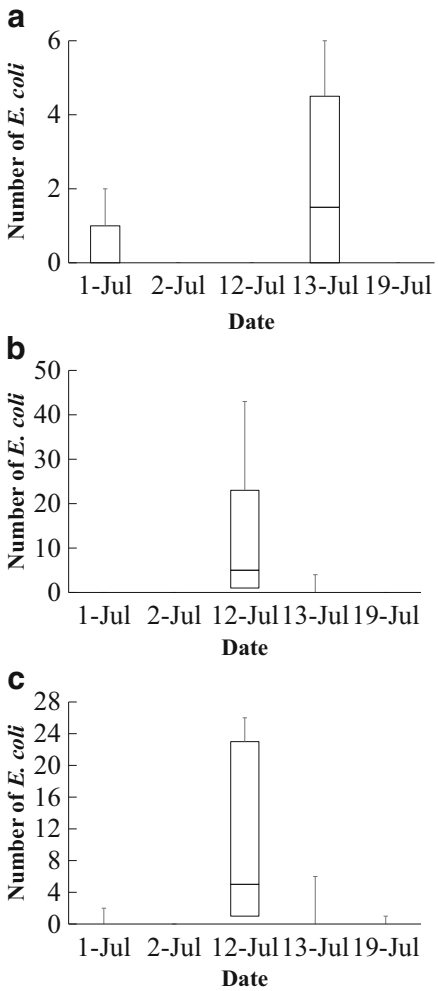

Fig. 3 Box-plot diagrams depicting the number of *E. coli* detected on caged fish. **a** The number of *E. coli* found on fish located in the confluence. **b** The number of *E. coli* found at the next site downstream (DS-1). **c** The number of *E. coli* found at the furthest downstream site (DS-2). The lower (Q1) and upper (Q3) quartiles represent observations outside of the 9–91 percentile range; the error bars represent standard deviation per sampling day at each location

Of the resident fish caught both offshore in a boat and onshore, 88% of the fish tested positive for fecal coliform; of those fish, 73% tested positive for *E. coli.* The frequency of detection of *E. coli* was 67% in fish angled from a boat, whereas the frequency of detection of fish angled from the shore was slightly lower at 60% (Fig. 4). Although fish caught from the shore had a lower frequency of detection compared to those from the boat, the CFUs of *E. coli* were significantly higher (p value 0.04789; Fig. 5). The reason for this difference is unknown, but this may explain the presence of bacteria on the caged fish, since fish were confined near the shore. Additionally, this difference in CFUs is important in relation to angler exposure. In the Pine River, a large portion of anglers fish from the shore, especially behind the dam at the boat launch. Therefore, if fish caught from the shore have significantly higher *E. coli* CFUs, angler exposure risk could also be higher.

Due to the high variability in the data, there was no significant effect of fish size or species on CFUs. This may indicate that the accumulation of bacteria in mucus is driven by properties of the water rather than the fish (Table 1). Future studies, however, evaluating the relationship between the amount of bacteria in the water and in fish mucus are required to better understand this relationship. As the majority of fish caught in this study were sunfish, the lack of difference is likely also driven by our small sample size.

Angler exposure

Of the three angler exposure treatments, 50% of anglers had *E. coli* present on hands after the first and last catch of the day, and 31% of anglers had *E. coli* present on hands at 30 min intervals, independent of the number of fish caught (Fig. 6). However, after exposure to river water via hand dip, 78% of anglers had *E. coli* present on hands. *E. coli* was present in river water regardless of site (Table 1). While there was not a statistically significant difference in CFUs among the three treatments, these results do suggest different potential exposure routes for *E. coli* to anglers (Fig. 7). In 2015, the State

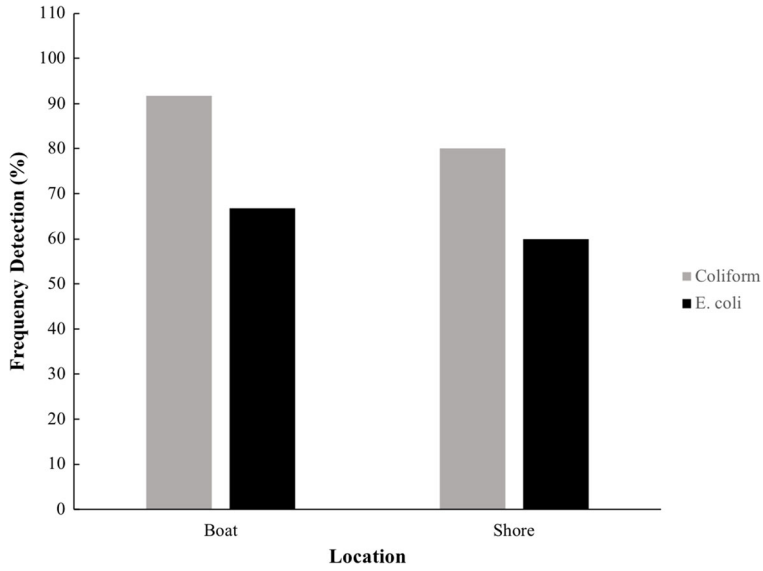

Fig. 4 Frequency of fecal coliform and *E. coli* detection on fish caught from a boat and the shore

Department of Community Health verified that the *E. coli* concentrations in the Pine River exceed the limit for safe human contact. Following these findings, a precautionary sign was posted to inform individuals of the dangers of recreating in waterways with high bacterial loads. The results from our study reveal an additional route of *E. coli* exposure through angling. Moving forward, States' Health Departments should consider adding verbiage to include cautions regarding human contact with fish via angling in bacterially impaired rivers. While the authors could not locate a study evaluating the transfer of *E. coli* from fish to humans, there are known occupational pathogens such as *Mycobacterium marinum* and *Erysipelothrix rhusiopathiae*, both causative agents of fish handler's disease (Decostere et al. 2004; Reboli and Farrar 1989; Brooke and Riley 1999). Infection occurs after contact with infected individuals and their wastes and byproducts as well as any organic matter contaminated by the latter (Wood 1975).

Additionally, eight fish selected from the caged fish pilot study were fileted and swabbed for presence of fecal coliform and *E. coli* and each of the eight individuals had at least 1 CFU of *E. coli* present on the filet. This suggests that another potential route of exposure to

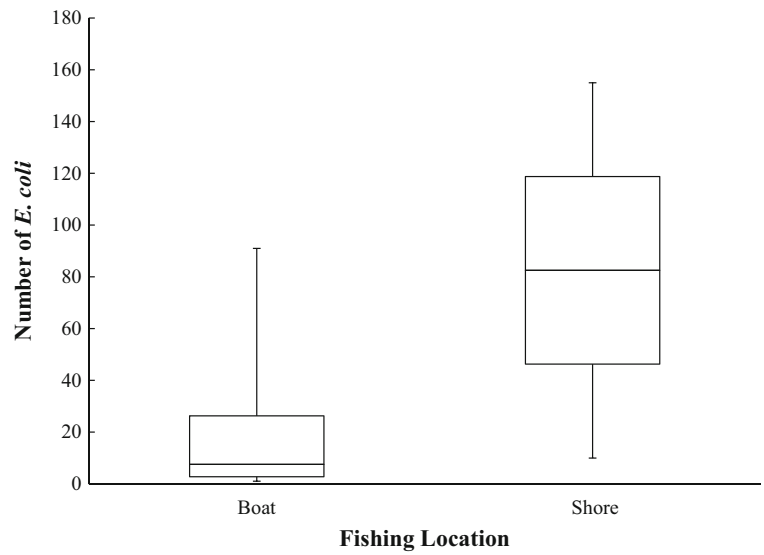

Fig. 5 Number of *E. coli* found on fish samples caught from the shore and boat when *E. coli* was detected. Excludes values that exceed 500 colony-forming units. There were significantly more *E. coli* colony-forming units on fish caught from the shore than from the boat. The error bars represent the standard deviation per location. The lower (Q1) and upper (Q3) quartiles represent observations outside of the 9–91 percentile range. The middle line represents the median

Table 1 Colony-forming units found in water samples taken during the resident fish and angler studies

Site	24 July			2 August	
	Fecal coliform	*E. coli*	Site	Fecal coliform	*E. coli*
Riverview	2	1	Boat Golfside	20	11
Riverview	5	0	Boat Golfside	16	13
Riverview	0	0	Boat Golfside	26	17
Luneak	7	1	Shore Luneak	9	3
Luneak	3	1	Shore Luneak	14	10
Luneak	21	11	Shore Luneak	2	0

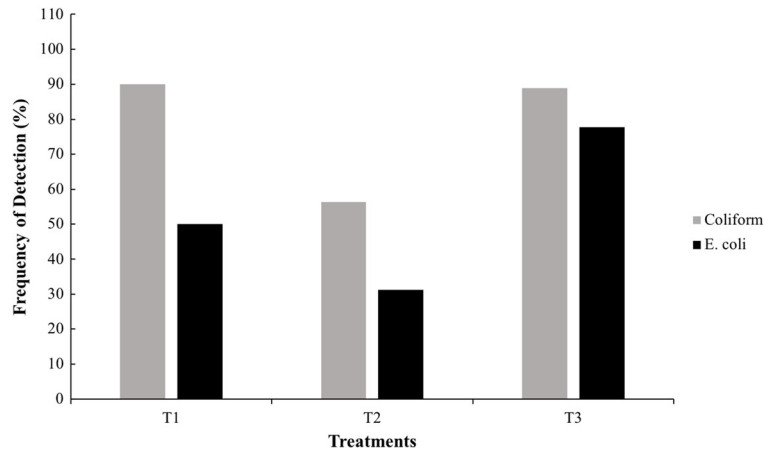

Fig. 6 Frequency of fecal coliform and *E. coli* detection on angler's hands during each of the three treatments. Treatments were as follows: T1 anglers' hands were swabbed after their first and last catch; T2 anglers' hands were swabbed at 30-min intervals; and T3 anglers' hands were dipped into the river

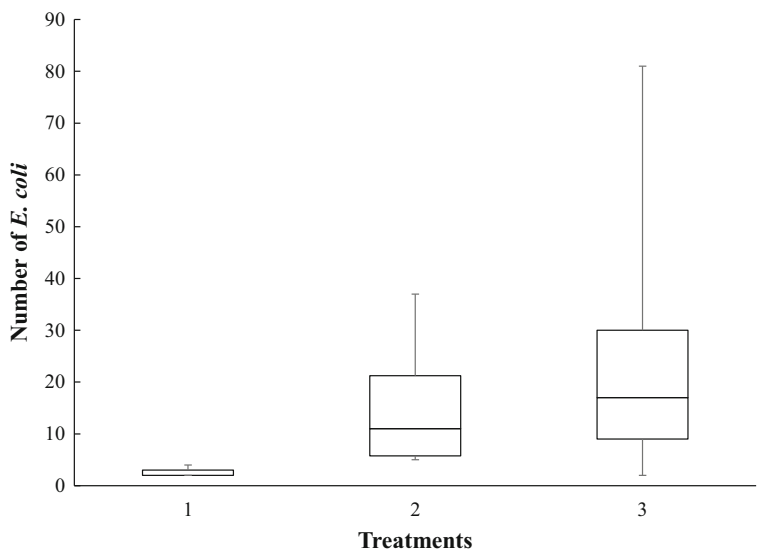

Fig. 7 Number of *E. coli* on three hand treatments when *E. coli* was detected. Treatment 1 includes angler hands that were swabbed after catching and handling the first and last catch of the fishing day. Treatment 2 includes angler hands that were swabbed in 30-min intervals for 2 h, regardless of handling a fish or not. Treatment 3 includes clean hands that were dipped directly into the river. Excludes values that exceed 500 colony-forming units. The error bars represent the standard deviation per treatment. The lower (Q1) and upper (Q3) quartiles represent observations outside of the 9–91 percentile range. The middle line represents the median

anglers is via cross-contamination while cleaning and preparing the fish for consumption. This route of exposure could result in direct or indirect oral exposure to *E. coli* via unclean hands, cross-contaminated knives/cutting boards, or improperly cooked fish.

Conclusions

While previous studies have demonstrated the presence of *E. coli* in the Pine River, angler exposure to *E. coli* was unknown. The current study determined that *E. coli* can be detected in fish mucus of both caged and resident fish, indicating heavy bacterial loading in streams which results in accumulation of potentially harmful bacteria in fish mucus. Regardless of number and fish species caught, it was determined that recreational angling activities resulted in the presence of *E. coli* on angler hands. This suggests a recreational pathway of exposure to *E. coli*—a pathway similar to documented occupational biohazards in fishing industries. Furthermore, there is a potential for cross-contamination during fish cleaning as well as oral exposure by contaminated hands. It is important to note, however, that while anglers have a high likelihood of exposure to these bacteria, the human health risk posed to anglers through direct contact with elevated levels of *E. coli* in fish mucus is unknown. As industrial agricultural practices continue to impair river systems, further biological monitoring, epidemiological evaluations, and risk assessments are pertinent in order to preserve ecological integrity and protect human health.

Acknowledgments The authors would like to thank Alma College for their monetary contributions to support environmental research. The authors would also like to acknowledge Dr. Jeff and Mrs. Ginna Holmes for their generous contribution and continual support of environmental research within the Mid-Michigan community as well as throughout the state. We would also like to thank Grace Sutherland and Hannah King for their assistance in the field and data collection. We would also like to extend our gratitude to the Healthy Pine River community group for their tireless efforts and support in river conservation.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted (Alma College Institutional Animal Care and Use Committee).

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