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Evaluation of common angling-induced sources of epithelial damage for popular freshwater sport fish using fluorescein

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ABSTRACT

Angling is a popular recreational activity across the globe and a large proportion of fish captured by anglers are released due to voluntary or mandatory catch-and-release practices. The handling associated with hook removal and return of the fish to their environment can cause physical damage to the epidermal layer of the fish which may affect the condition and survival of released fish. This study investigated possible sources of epithelial damage associated with several different handling methods (i.e., landing net types, interactions with different boat floor surfaces, tournament procedures) commonly used in recreational angling for two popular freshwater sport fish species, largemouth bass (*Micropterus salmoides*) and northern pike (*Esox lucius*). Epithelial damage was examined using fluorescein, a non-toxic dye, which has been shown to detect latent epithelial damage. Northern pike exhibited extensive epithelial damage after exposure to several of the induced treatments (i.e., interaction with a carpeted surface, knotted nylon net, and line rolling) but relatively little epithelial damage when exposed to others (i.e., knotless rubber nets, smooth boat surfaces, or lip gripping devices). Largemouth bass did not show significant epithelial damage for any of the treatments, with the exception of fish caught in a semi-professional live release tournament. The detection of latent injuries using fluorescein can be an important management tool as it provides visual examples of potential damage that can be caused by different handling methods. Such visualizations can be used to encourage fish-friendly angler behaviour and enhance the survival and welfare of released fish. It can also be used to test new products that are intended to or claim to reduce injury to fish that are to be released. Future research should evaluate the relationship between different levels of epithelial damage and mortality across a range of environmental conditions.

1. Introduction

Recreational angling is a popular leisure activity world wide, with an estimated 47 billion fish caught annually (Cooke and Cowx, 2004; Arlinghaus et al., 2007). The majority of recreationally angled fish, approximately two-thirds, are released because they are non-desired/non-target species due to voluntary angler actions or harvest regulations (Cooke and Cowx, 2004). Whenever captured fish interact with angling gear there is always some level of injury (Cooke and Sneddon, 2007). At a minimum, there is the physical injury caused by the hook puncture(s). However, injury can also arise from other components of the angling event including the fight (e.g., line rolling), landing (e.g., net damage), and handling (e.g., dropping, holding). In general, most of the existing literature on injury arising from catch-and-release events is focused on the relationships between injury and mortality associated with different hook types (Meka, 2004; Cooke et al., 2003), hook sizes (Cooke et al., 2005) and hooking locations (Pelzman, 1978; Lyle et al., 2007; Fobert et al., 2009). Few studies have investigated injury associated with the use of other angling gear such as landing nets (e.g., Barthel et al., 2003) and mechanical gripping devices (e.g., Danylchuk et al., 2008), or different handling and holding practices (e.g., Steeger et al., 1994; Cooke and Hogle, 2000).

Injuries associated with recreational angling events, such as those caused by landing nets, may not be visually recognizable as they damage the epithelial layer that covers the entire surface of the fish as a barrier to pathogens, UV light, and desiccation (Shephard, 1994). Although these types of injuries do not tend to result in immediate mortality, they may put fish at risk of infection from a variety of different opportunistic pathogens (Ventura and Grizzle, 1987; Svendsen and Bøgwald, 1997; Van West, 2006). These diseased states have the potential to cause sublethal disturbances in physiology, health and behaviour (Cooke and Sneddon, 2007) and in some cases can lead to delayed mortality (Steeger et al.,

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Table 1
Mean ± S.D. and range of total length for northern pike and largemouth bass treated with each handing method.

Treatment	Northern pike		Largemouth bass	
	n	Mean ± S.D. (range)	*n*	Mean ± S.D. (range)
Control			10	341 ± 40 (257–388)
Rubber landing net[a]	11	525 ± 49 (460–603)	9	382 ± 36 (316–432)
Nylon landing net	11	490 ± 112 (375–755)	9	341 ± 62 (257–440)
Holding by the gills control	8	498 ± 29 (456–532)[b]		
Holding by the gills	8	485 ± 42 (401–538)		
Line roll	7	539 ± 77 (418–660)		
Carpeted surface	11	502 ± 58 (420–631)	14	320 ± 66 (240–463)
Boat floor	10	523 ± 54 (421–613)		
Mechanical gripping device	8	501 ± 44 (455–587)		
Dry weigh in			8	306 ± 34 (259–372)
Wet weigh in			8	346 ± 80 (257–457)
Tournament caught fish			11	425 ± 40 (341–484)[c]

[a] Northern pike landed using a rubber landing nets served as controls.
[b] Separate control fish for northern pike held by the gills were used when compared to the rest of the study due to the removal of the opercula.
[c] Tournament caught largemouth bass were significantly larger than those fish used in the remainder of the study.

1994; Svendsen and Bøgwald, 1997; Howe and Stehly, 1998; Davis, 2005). In fact, stress and bacterial infections have been identified as major sources of mortality for angler caught fish such as black bass (*Micropterus* spp.; e.g., Welborn and Barkley, 1974; Seidenstricker, 1975; Plumb et al., 1975; Archer and Loyacano, 1975).

Fluorescein, a non-toxic dye, has been shown to penetrate damaged epithelium of the cornea and recently the skin of fish (Noga and Udomkusonsri, 2002). When placed under an ultraviolet (UV) light source, areas of damaged epithelium treated with fluorescein produce green light, which can be photographed and the damaged area can be measured using computer software (Noga and Udomkusonsri, 2002; Davis and Ottmar, 2006). This technique improves the current method of injury detection for fish (gross macroscopic evaluation) by allowing latent epithelial damage to be identified and quantified in an objective manner, which has not been previously possible (Colotelo et al., 2009).

This study investigated epithelial damage associated with several different handling methods used commonly in recreational angling for two popular freshwater sport fish species, largemouth bass (*Micropterus salmoides*) and northern pike (*Esox lucius*). Largemouth bass and northern pike differ in morphology and behaviour, resulting in the use of specific and varied handling methods. This analysis used fluorescein to quantify otherwise undetectable epithelial damage. Due to visualization of epithelial damage available with this technique, we also discuss the potential utility of the tool for educating anglers about different handling practices.

2. Methods

2.1. Study area

All fish were angled from Lake Opinicon, located in southeastern Ontario, Canada, via standard angling practices as described below, with the exception of tournament captured largemouth bass. Tournament captured largemouth bass were caught from Big Rideau Lake, also located in southeastern Ontario, Canada, as part of a semi-professional live-release bass fishing tournament.

All experiments took place in late June and early July (2008) at water temperatures of 23–26 °C.

2.2. Handling practices

To avoid inflicting non-experimental epithelial damage following capture, largemouth bass were landed by firmly grasping the lower lip with the thumb and forefinger unless otherwise stated. Northern pike cannot be handled this way because of their dentition and all were landed using a wetted rubber mesh net unless otherwise stated. Once each fish was successfully landed, it was randomly assigned to a treatment group. The bass captured in the tournament were an exception (see below), as they were sampled just prior to release. To avoid any contamination of our results, fish with existing visible signs of injury at time of capture (e.g., old bird wounds or any injury that was partially healed) were excluded from the study.

2.3. Treatments

In total, 87 northern pike (size range: 375–660 mm) and 72 largemouth bass (size range: 240–484 mm) (Table 1) were used in the study. When each fish was successfully landed, it was randomly assigned into one of the treatment groups listed below recognizing that both species were not exposed to all treatments (summarized in Table 1).

2.3.1. Landing net material

Largemouth bass were placed in a rubber or knotted nylon net for 30 s (representing the duration required for hook removal). Northern pike were landed using a rubber mesh landing net and were then placed in a rubber or nylon mesh net for 30 s (representing the duration required for hook removal).

2.3.2. Tournament and weigh-in practices

For the simulation of the weigh-in procedures, individual fish were placed in a weigh-in plastic container (a plastic laundry basket which permitted the rapid flow of water in and out of the container) which was immersed in and removed from water a series of three times, simulating a common weigh-in process at angling tournaments. For each cycle, the container was dipped into the water for 10 s and then removed from the water for 30 s. Following this, the container was either immersed in or removed from water for 30 s to simulate the wet or dry weigh-in process, respectively.

As previously mentioned, tournament captured largemouth bass were intercepted just prior to release at a semi-professional live-release tournament on Big Rideau Lake. Tournament regulations did not standardize the landing gear or handling methods used by the tournament participants and so the epithelial damage detected in this portion of the study was likely the combined result of angling, handling, livewell confinement and the weigh-in procedure. Fish caught in this tournament were confined to a livewell with up to four other fish for up to 6 h. A wet weigh-in format with all five fish being weighed together was used in this tournament.

2.3.3. Holding by gills for hook removal

For the removal of the hook, each fish was held under the operculum, causing the gills of the fish to be in contact with the angler's hands. This handling method is a common way of holding fish such as northern pike which can be difficult to control. Following the fluorescein treatment northern pike were euthanized using an overdose of 120 ppm clove oil anaesthetic (clove oil emulsified in ethanol (1:9); Sigma Aldrich, Toronto, ON) and the opercula were removed prior to photographs being taken (see below).

A separate group of controls were used to compare to those northern pike which were held by the gills for hook removal. The treatment of controls only differed in the handling for hook removal. Control fish were gripped behind the base of the head for hook removal. This method was employed as the authors believed this was the safest method to use for both the fish and the researchers.

2.3.4. Line rolling

Angled fish had the hook removed, and were then wrapped in a 28 cm multistrand nylon coated steel wire leader (20 lb test; Bass Pro Shops, Springfield, MO) to simulate line rolling, which can occur when angling northern pike. The fish was submerged in water with the leader taught around them for 30 s. Since line rolling typically occurs during the landing of the fish, we cannot eliminate the potential that any northern pike rolled in the line during the angling event, however, any fish that were visibly wrapped in the line at landing were eliminated from the study. Also, once fish struck the lure the line was immediately pulled tight for the duration of the landing.

2.3.5. Interaction with boat floor carpeted surfaces

After fish were angled, they were placed in a plastic container which was lined with artificial polypropylene outdoor carpeting, similar to that found in some fishing boats, or on the smooth metal surface of a boat floor. Fish were unrestrained on this surface for 30 s.

2.3.6. Mechanical gripping device

For hook removal and handling of the fish, a Berkley® Big Game Lip Grip (Berkley, Pure Fishing Canada, Portage La Prairie, MB) mechanical device which grips the lower lip of a fish was used (see Danylchuk et al., 2008). These tend to be used for fish which are difficult to handle because of size or dentition.

2.3.7. Control fish

A group of control fish were also examined for both species. Largemouth bass controls were removed from the water by grabbing the lower lip and immediately placed in the anaesthetic solution for epithelial damage detection. Northern pike controls were angled using standard angling gear and were landed using a rubber landing net. Upon capture, they were immediately placed in the anaesthetic solution for epithelial damage detection (see below).

2.4. Epithelial damage detection

Following treatment, all fish were placed in a 50 ppm clove oil anaesthetic (clove oil emulsified in ethanol, 1:9; Sigma Aldrich, Toronto, ON) and remained there until fish reached stage four of anaesthesia, as noted by a loss of equilibrium and coordinated fin movements (Summerfelt and Smith, 1990). Fish were submerged in a 0.2 mg/mL solution of fluorescein (Fluorescein, Disodium Salt; Aldon Corp., Avon, NY) in distilled water for 6 min and were then placed in an anaesthetic bath containing 50 ppm clove oil (clove oil emulsified in ethanol at 1:9) for 6 min to rinse and keep fish anaesthetized (Noga and Udomkusonsri, 2002). The exception to this was northern pike included in the holding by the gills treatment group and associated controls. These northern pike were euthanized in a 120 ppm clove oil solution (clove oil emulsified in ethanol at 1:9).

The fish were photographed in complete darkness, against a black background, using a digital SLR ELIXIM Pro EX-F1 camera (Casio Computer Co., Ltd., Tokyo, Japan) at ISO 100, F6.7, and a 20 s exposure. The camera was positioned 80 cm directly above the fish and the shortwave (254 nm) UV light source (Mineralight® UVGL-48; UVP Inc., Upland, CA) at a 45° angle to the fish, 60 cm above, so that the entire organism was illuminated by the UV light. The entire right and left sides of each fish were photographed. Northern pike assigned to the control and mechanical gripping device treatment groups were also photographed on the underside. Following treatment, fish were placed in a container of fresh lake water until equilibrium was regained, after which the fish were released.

Fluorescein causes a positive reaction with epithelial damage through a chemical reaction resulting in the production of green light. Photographs of the right, left and underside (where applicable) of the fish were analyzed, using ImageJ software (http://rsb.info.nih.gov/ij/; National Institute of Health, Bethesda, MD), by tracing the areas of green and measuring the number of pixels. This process was done twice by the same observer and the average number of green pixels was calculated and used for statistical analysis. The proportion of epithelial damage on each side of the fish was calculated by dividing the total number of green pixels by the total number of pixels encompassed by the fish. The proportion of epithelial damage for the left and right sides of each fish were then summed and used for statistical analysis. For northern pike which were photographed on their underside (those landed using a rubber landing net and those handled using a mechanical gripping device) the total proportion of epithelial damage was calculated for only that side of the fish and compared using statistical analysis.

2.5. Statistical analysis

Comparison of similar handling methods for each species was done using *t*-test (for comparison of two handling methods) or one-way ANOVAs followed by a Tukey's *post hoc* test when necessary (for comparison of three or more handling methods). Data transformations were conducted as needed to meet the assumptions of normality and homogeneity of variance required for parametric tests. SPSS software was used for all statistical tests and significance was assessed at $\alpha = 0.05$ (Zar, 1984).

3. Results

There were no significant differences between total lengths of northern pike in each treatment group (Table 1; One-way ANOVA, $F_{8,77} = 0.725$, $p = 0.669$), however, tournament captured largemouth bass were significantly larger than largemouth bass used in all other treatment groups (Table 1; One-way ANOVA, $F_{6,62} = 5.624$, $p < 0.001$).

3.1. Landing net material

Landing net material elicited significant epithelial damage relative to control fish for northern pike (Fig. 1; *t*-test, $t_{21} = 5.325$, $p < 0.001$), however, there was no significant difference in epithelial damage among control fish and different net materials for largemouth bass (Mean ± S.E., control 0.86 ± 0.74%, rubber landing net 0.17 ± 0.08%, knotted nylon landing net 0.77 ± 0.33%; One-way ANOVA, $F_{2,25} = 0.387$, $p = 0.683$). When comparing the proportion of damage detected for the epithelial disturbance caused by the knotted nylon net on northern pike and largemouth bass, northern pike

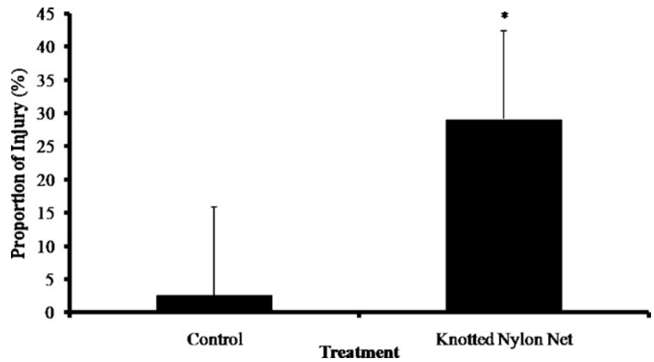

Fig. 1. The proportion of injury (±S.E.) detected on northern pike treated with landing nets made of rubber (controls; $n = 12$) or knotted nylon ($n = 11$). Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

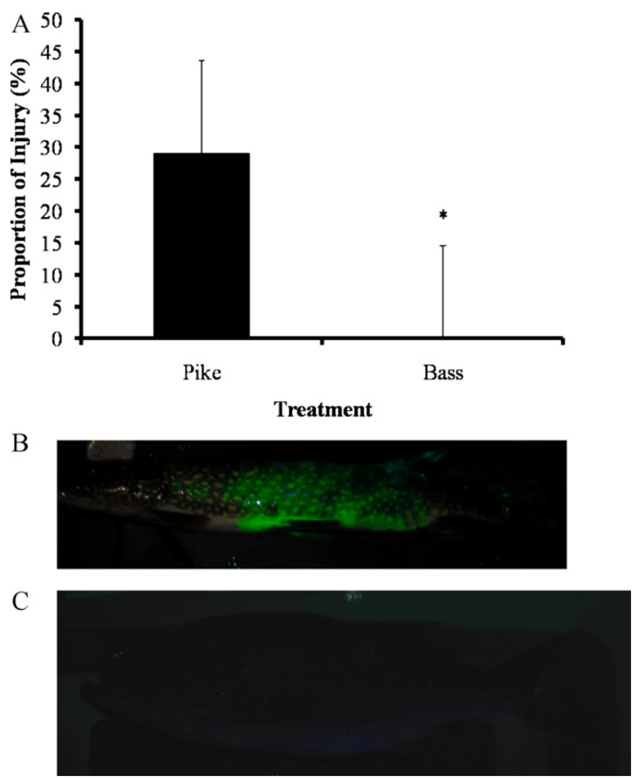

Fig. 2. (A) The proportion of injury (±S.E.) detected on northern pike ($n = 11$) and largemouth bass ($n = 9$) when treated with a knotted nylon landing net. (B) Photograph of northern pike handled with a knotted nylon landing net for 30 s and treated with fluorescein. (C) Photograph of largemouth bass handled with a knotted nylon landing net for 30 s and treated with fluorescein. Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

exhibited significantly higher levels of detectable damage (Fig. 2; *t*-test, $t_{17} = 7.600$, $p < 0.001$).

3.2. Tournament and weigh-in practices

Largemouth bass exposed to simulated weigh-in practices did not result in significant detectable damage relative to controls, however, fish sampled from a semi-professional tournament experienced epithelial damage that was higher than both controls and the individual components of the tournament (Fig. 3; One-way ANOVA; $F_{3,33} = 2.953$, $p = 0.047$; with Tukey's HSD *post hoc*). The overall impact of a largemouth bass being exposed to a live release tournament elicited higher levels of epithelial damage than was detected from any other treatments on this species in this study.

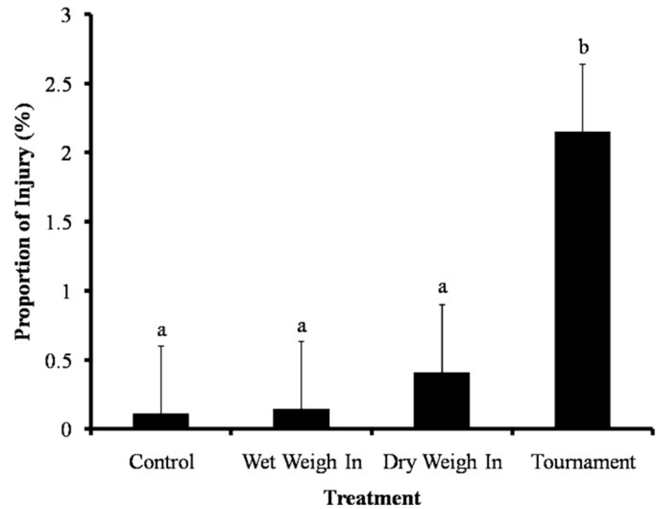

Fig. 3. The proportion of injury (±S.E.) detected on largemouth bass when treated by wet ($n = 8$) and dry ($n = 8$) weigh in procedures as well as overall tournament effects ($n = 11$) relative to control ($n = 10$). Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

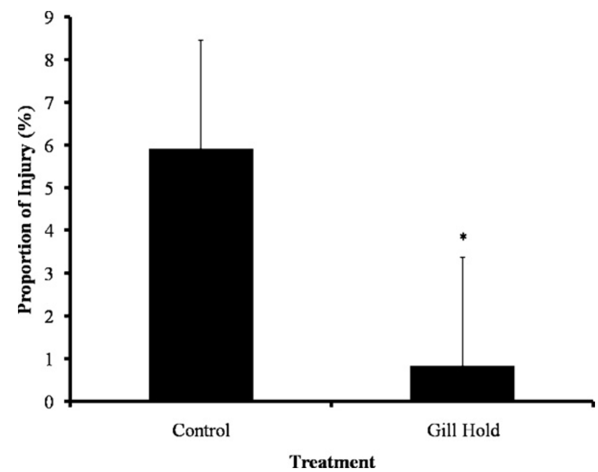

Fig. 4. The proportion of injury (±S.E.) detected on northern pike which were held by the gills ($n = 8$) and treated as controls ($n = 8$). Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

3.3. Holding by the gills for hook removal

There was a significant difference in the level of epithelial damage detected when comparing northern pike held by the gills for hook removal and those which were treated as controls. Control fish had a significantly higher level of epithelial damage than those held by the gills (Fig. 4; *t*-test, $t_{14} = 2.447$, $p = 0.028$).

3.4. Line rolling

Northern pike which were exposed to line rolling showed a significantly higher proportion of epithelial damage than those fish which were not exposed to experimentally induced line rolling (Fig. 5; *t*-test, $t_{17} = 2.167$, $p = 0.045$). The majority of the damage detected was in the vicinity of the pelvic and pectoral fins.

3.5. Interaction with boat floor surfaces

Northern pike were placed on either a smooth metal boat floor or a carpeted surface, and the carpeted surface showed a significantly higher proportion of epithelial damage than the smooth

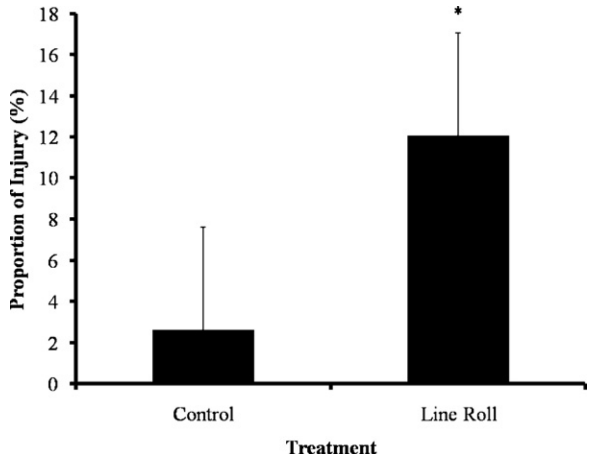

Fig. 5. The proportion of injury (±S.E.) detected on northern pike wrapped in a steel leader ($n = 7$) and treated as controls ($n = 12$). Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

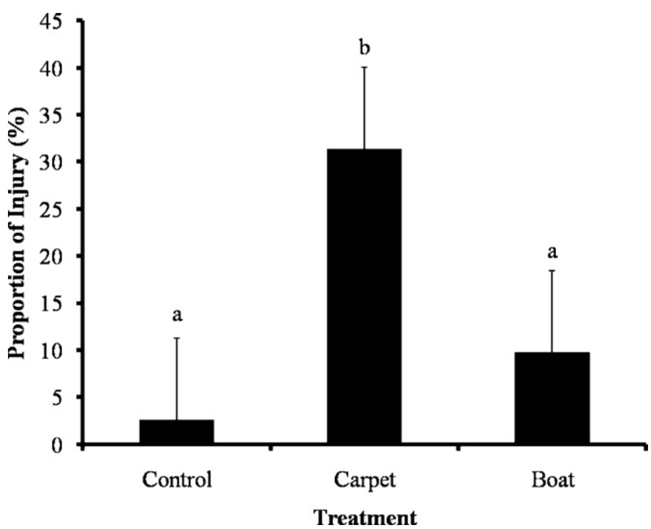

Fig. 6. The proportion of injury (±S.E.) detected on northern pike which interacted with a carpeted surface ($n = 11$) and smooth metal boat floor ($n = 10$). ($N = 12$ for control fish.) Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

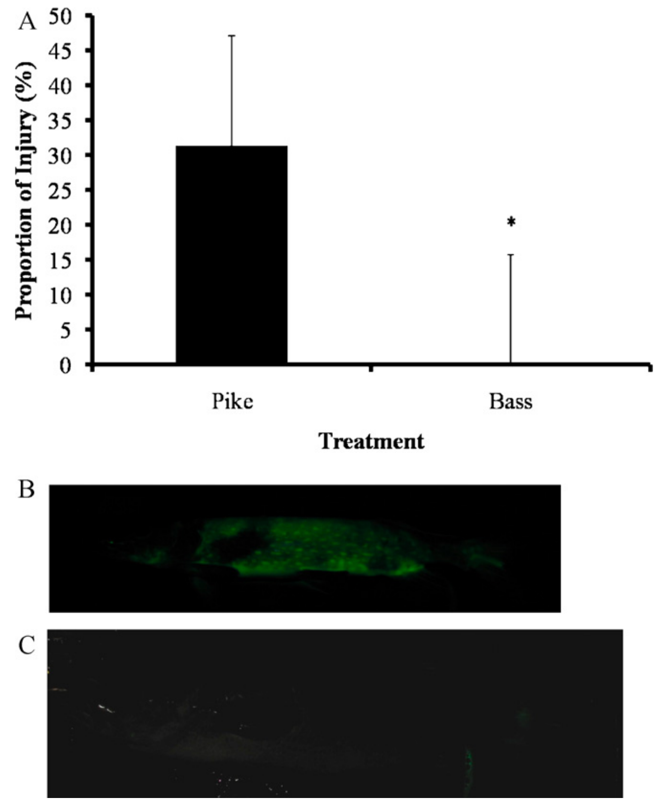

Fig. 7. (A) The proportion of injury (±S.E.) detected on northern pike ($n = 11$) and largemouth bass ($n = 17$) that interacted with a carpeted surface. (B) Photograph of northern pike placed on a carpeted surface for 30 s and treated with fluorescein. (C) Photograph of largemouth bass placed on a carpeted surface for 30 s and treated with fluorescein. Dissimilar letters indicate significant differences ($P < 0.05$) in the proportion of fluorescein detected.

metal boat floor as well as the controls which did not interact with a boat surface (Fig. 6; One-way ANOVA, $F_{2,30} = 12.301$, $p < 0.001$). There was no significant difference in epithelial damage detected between largemouth bass that interacted with a carpeted surface (Mean ± S.E., 2.4 ± 1.8%) and those which did not interact with any surface (Mean ± S.E., 0.9 ± 0.7%) (t-test, $t_{25} = 0.659$, $p = 0.516$). There was a significant difference in the proportion of damage detected on the two species when treated with the same potential epithelial damage source, with northern pike demonstrating a higher proportion of detected damage than largemouth bass (Fig. 7; t-test, $t_{25} = 5.569$, $p < 0.001$).

3.6. Mechanical gripping device

Northern pike that were handled with a mechanical gripping device for hook removal (Mean ± S.E., 0.0276 ± 0.101) did not show a significantly higher proportion of epithelial damage on the lower jaw than those handled as controls (Mean ± S.E., 2.6 ± 10.7) (t-test, $t_{19} = 0.099$, $p = 0.922$).

4. Discussion

Interaction between fish skin and different netting materials has been shown to result in different levels of visible injury (i.e., fin fray, scale loss; e.g., Cooke and Hogle, 2000; Barthel et al., 2003; De Lestang et al., 2008), yet there have been no attempts to rigorously and objectively quantify the level of epithelial damage. Fluorescein serves as a tool that enables the quantification of epithelial damage (Davis and Ottmar, 2006; Dauble et al., 2007) and the current study utilized that ability to document and quantify epithelial damage. This study revealed that northern pike landed using a knotted nylon net had higher levels of epithelial damage detected with fluorescein when compared with those landed using a rubber net (29.1% and 2.6%, respectively; Fig. 1). Interestingly, there was no significant latent damage detected under the same comparison with largemouth bass. Using a more subjective approach with bluegill (*Lepomis macrochirus*), Barthel et al. (2003) revealed that knotted netting materials elicited higher levels of injury (i.e., fin fraying and scale loss) and subsequent mortality than rubber nets. Given that largemouth bass and northern pike differed in their response to nets indicates that not all species may be equally sensitive to dermal disturbance. In general, the netting assessments are consistent with the growing body of evidence that desirable netting materials are soft and non-abrasive, and that in general the use of nets should be limited to when it is necessary to control the fish to prevent injury to the fish or the angler.

Fish captured during tournaments are exposed to a range of stressors (i.e., captured via hook, typically landed using nets, held in a live-well for up to 8 h, and weighed in and handled by multiple individuals before they are released; Suski et al., 2004) and mortality arising from such events can be high (Wilde, 1998; Cooke et al., 2002). While there have been many studies that have examined the fate of fish released from tournaments, most focus on the contraction of pathogens (e.g., largemouth bass virus, *Aeromonas hydrophila, Saprolegnia spp.*) rather than the sources of injuries which make fish susceptible (e.g., Steeger et al., 1994; Wilde, 1998; Schramm et al., 2004). The current study focused on the weigh-in processes and the overall impact of the tournament for the individual fish. The weigh-in process has been shown to be an important event with respect to physiological status of the fish upon release at a tournament. Previous studies have revealed that when fish are provided with water of adequate quality, the weigh-in at the end of the day determines the physiological status of the fish upon their return to the lake or river (Suski et al., 2004). In the current study, fish handled according to the wet and dry weigh-in procedures did not differ significantly in the proportion of epithelial damage detected when compared with control fish (0.1%, 0.4% and 0.9%, respectively; Fig. 3). Fish captured in the semi-professional live-release tournament did have significantly higher proportions of epithelial damage (2.2%), when compared with controls and the two weigh-in procedures tested (Fig. 3). Disease has been highlighted as an important issue surrounding bass tournaments (e.g., Steeger et al., 1994; Schramm et al., 2004; Grant et al., 2005). Potential injuries associated with tournaments are important given that disease transmission is affected by the presence of injury, immunosuppression associated with physiological stress, and confinement in livewells (Schramm et al., 2004). Regardless of the proportion of epithelial damage detected, any efforts to reduce injury, even if quite minor, could be of immense benefit to tournament-caught fish (Siepker et al., 2007). Although there was no significant difference in the proportion of epithelial damage observed in the two weigh-in treatments in the current study, wet weigh-in procedures have other benefits for fish captured in live-release tournaments. Wet weigh-in procedures were developed as a means of reducing stress associated with the air exposure during the weigh-in (see Tufts and Morlock, 2004). From an injury perspective, fish are not able to "recover" from injuries experienced in early components of the tournament. Therefore, injuries observed at the time of release (as was done in the current study) are representative of the entire capture and handling event, and would be predicted to be greater than the individual components (e.g., interaction with landing net, interaction with boat surface) tested separately. Indeed, the only injury that was statistically different from control levels in the current study was the tournament as a whole rather than its various components. This cumulative effect of the tournament requires further research to determine the level of injury incurred from the different components of the tournament process (e.g., landing net, live well confinement).

Holding fish by the gills for hook removal and photographs is a common handling practice for large fish, or those difficult to hold such as northern pike. There is suggestion that this handling method is detrimental to the fish as the gills provide a thin barrier to the blood stream where gases are exchanged and damage to this tissue could result in inability of efficient transfer of these gases (Hughes, 1984). As this tissue is very thin, it also suggests that this area would be more susceptible to epithelial damage. Interestingly, use of fluorescein failed to document significant epithelial damage arising from this handling method. Indeed, the control fish had significantly higher levels of injuries to the gills when compared with fish held by the gills for hook removal (Fig. 4). It is unclear whether the gills are simply not injured from this handling method or if fluorescein is ineffective at documenting epithelial damage on the gills. Dedual and Shorland (2006) demonstrated that luminol (a chemiluminescent chemical used to detect latent blood) was effective in highlighting areas of gill damage associated with handling of trout by the gills for hook removal. Given that the gills are an active surface where gases and ions are transferred and where water is passed even while anaesthetized, it is not unreasonable to suggest that fluorescein may be cleared more rapidly on gill tissue than on the epithelium on skin of the fish. Anecdotally, we did not observe any visible signs of gill bleeding by pike.

While angling for large fish such as northern pike, steel leaders are commonly used to prevent the line from being cut by their dentition. When these predatory fish strike, they may roll their bodies in an attempt to free themselves and they may become tangled in the line. Fish are often caught with visible scars indicating the fish has previously been tangled in a line. An important feature of the leader is that it can reduce break-offs where lures are left in the mouth of fish which creates welfare impacts on fish (Arlinghaus et al., 2007). Interestingly, the current study found significant damage resulting from the fish being manually wrapped in a coated steel leader (12.0%; Fig. 5), however, it is unclear if different fishing line types or leader materials could be used to reduce epithelial damage arising from line wrapping, however, future research is warranted in this area.

A popular flooring material used in boats is all-weather carpeting, which provides traction under wet conditions, and adds protection to the boat frame. When angling, it is a common occurrence for fish to interact with the flooring of the boat (e.g., when dropped or placed on the floor to facilitate hook removal) and the material on that surface may impact the epithelial damage caused to the fish before it is released. This study revealed that interacting with a carpeted surface caused the highest proportion of epithelial damage of all treatments for both largemouth bass and northern pike (2.4% & 31.4%, respectively; Fig. 6). Conversely, when the fish interacted with a flat metal surface, common in jon boats, there was relatively little epithelial damage (9.8% for northern pike; Fig. 6). These data emphasize that fish should be handled in a manner that reduces the chances of them being dropped in the boat, regardless of the surface, and that it is important to pick the fish up quickly if dropped, particularly in boats with rough interior surfaces. Ideally fish would be handled over water to minimize injury risk.

Mechanical gripping devices have been introduced as an alternative to landing nets for use in fish which are difficult to hold on to and to enhance fish condition when they are released by limiting scale loss and epithelial damage (Danylchuk et al., 2008). Danylchuk et al. (2008) reported extensive damage to the lower jaw of bonefish (*Albula vulpes*) which were handled using the mechanical gripping device. External and internal injuries have also been reported on barramundi (*Lates calcarifer*) handled with lip gripping devices (Gould and Grace, 2009). However, our study did not detect significant epithelial damage on northern pike which were handled using the mechanical gripping device. More research is needed to understand the full range of injuries arising from use of these devices as there is evidence of skeletal damage resulting from the use of these devices (Gould and Grace, 2009). If these devices can prevent dropping the fish and avoid use of nets, there may be benefit of using lip gripping devices if they do not yield significant mouth injuries as observed by Danylchuk et al. (2008). As noted here, there was negligible detectable injury on the body of fish from using these devices so they may have merit for some species such as northern pike.

It is evident that northern pike and largemouth bass do not respond in the same manner to the treatments applied in this study. In the treatments examining the impacts of carpeted surfaces and knotted nets, northern pike had significantly higher proportions of epithelial damage than largemouth bass (Figs. 2 and 7). The difference in detectable epithelial damage rates suggests that there is difference in the susceptibility to epithelial disturbance between the two species or in the way the fluorescein treatment reacts with their specific blood constituents. Indeed, the extent of epithelial damage could be influenced by the fish's body morphology, behaviour while interacting with angling gear, and epithelial anatomy. All fish are covered by a protective epithelial layer, but the thickness and composition may differ between species and life stage which may affect both their sensitivity to epithelial damage, but also the efficacy of fluorescein as a tool for the quantification of such injury (Shephard, 1994). Disruption to this epithelial layer, as indicated by a positive reaction with fluorescein, creates susceptibility to infection, regardless of quantity of epithelial damage detected. However, there may be intra- and inter-specific variation in how fish respond to the same proportion of epithelial damage. Although there were differences in the proportion of epithelial damage detected in largemouth bass and northern pike, the sublethal and lethal consequences may not indeed differ. It is unknown what the threshold of epithelial damage is for each species, which reinforces the need for research to focus on the long-term consequences of epithelial injuries. Further research is also needed to identify which species are the best candidates for the fluorescein treatment, as it may not be appropriate for all species. The threshold of tolerable epithelial damage may vary depending on species and this can influence gear choice and handling practices used. Davis and Ottmar (2006) found that walleye pollock (*Theragra chalcogramma*) were the only species, of four tested, which showed a sigmoid curve relating epithelial damage detected and delayed mortality rates, suggesting an increased susceptibility to epithelial damage.

An important point to consider when testing handling methods or gear and their influence on fish epithelium is the age of injury. Older injuries, those occurring prior to treatment, may increase the amount of injury detected. It is important that fluorescein, or any other injury quantification process, detects and quantifies the specific injury being tested. Experimentally inflicted epithelial damage was shown to no longer be detectable at 24 h after infliction for bluegill (Colotelo, 2009). This directly relates to rate of epithelial healing which can vary based on species skin characteristics and temperature and should be considered when examining sources of epithelial injury.

5. Management implications and future considerations

Fluorescein is capable of quantitatively and objectively differentiating between levels of epithelial damage arising from different gear types which provides direction to managers and anglers for reducing the impacts of fishing on individuals that are released. Photographs of fish handled using different techniques can be used to encourage fish-friendly handling practices for anglers (Figs. 2b and c and 7b and c). Such an approach was used by government researchers in New Zealand to illustrate handling injuries in rainbow trout (*Oncorhynchus mykiss*) and was published in a magazine that was accessible to stakeholders (Dedual and Shorland, 2006). Management agencies and even anglers (with appropriate training and permits) could conduct their own visual assessment of the injury caused by different handling practices and this will encourage conservation oriented practices. Also, fluorescein can be used to validate improvements in handling practices and test conservation-oriented products (e.g., gloves promoted to reduce mucous damage to fish, nets promoted as safer for fish). Collectively, this study reveals that fluorescein is an effective tool for quantifying epithelial damage in fish. Moreover, common activities and gears employed by anglers yield different levels of epithelial damage that could be reduced or eliminated through education and/or innovations in gear design. Such research is consistent with the need to maintain the welfare status of fish (Cooke and Sneddon, 2007) and has the potential to reduce mortality among fish that are angled and released. In addition, the use of fluorescein to investigate potential sources of epithelial damage is also highly relevant among other situations in which fish are handled (i.e., aquaculture, ornamental trade, research, zoos and aquaria) and discarded (i.e., commercial fisheries, research) (Colotelo et al., 2009).

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