	J	F	B	1	3	4	9	B	Dispatch: 8.12.06	Journal: JFB	CE: Jayashree
	Journal Name			Manuscript No.					Author Received:	No. of pages: 18	ME: Nagalakshmi

*Journal of Fish Biology* (2007) **70**, 1–18

doi:10.1111/j.1095-8649.2007.01349.x, available online at <http://www.blackwell-synergy.com>

## The effectiveness of two common sampling methods for assessing imperilled freshwater fishes

M. S. POOS\*<sup>†</sup>, N. E. MANDRAK<sup>‡</sup> AND R. L. McLAUGHLIN\*

\**Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada and* <sup>‡</sup>*Great Lakes Laboratory for Fisheries and Aquatic Sciences, Department of Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, Ontario, L7R 4A6, Canada*

(Received 5 January 2006, Accepted 6 October 2006)

The hypothesis was tested that the most common gear type used to sample fishes in wadeable systems, electrofishing, was more effective than another commonly used gear type, seining, for sampling fish species at risk. Five predictions were tested. At sites where species at risk were detected, (1) the probability of detecting the species at risk, (2) the probability of only one gear type detecting the species at risk and (3) the estimated catch per unit effort of the species at risk, was as high as, or higher, when using electrofishing than when using a seine. (4) The number of sample sites required to detect a species at risk within a watershed and (5) the number of subsections required to detect a species at risk within a site, were as low as, or lower, using electrofishing than the number required using a seine. Based on analyses of these measurements, electrofishing was a more effective gear type than seining for sampling fish species at risk, irrespective of the unit (presence or absence or catch per unit effort) or scale of measurement (watershed or site level). Dissolved oxygen, turbidity, specific conductivity and nitrate concentrations were measured at each site and did not account for the between gear differences. Selection of sampling gear can be a fundamental consideration for the assessment of fish species at risk, where, unlike common species, they may be particularly influenced by small population sizes, restricted geographic ranges and narrow habitat preferences. Resource managers must weigh differences in the risks of injury of fish species at risk against differences in the effectiveness of each gear type when deciding between gear types and the utility of the assessments they represent.

© 2007 Crown copyright

Key words: conservation; recovery plans; resource management; sampling efficiency; species at risk; water chemistry.

### INTRODUCTION

Accurate, precise and economically feasible measurement of the distribution and abundance of fish assemblages is needed for making sound and scientifically defensible decisions regarding effective monitoring and management of aquatic systems (Hendricks *et al.*, 1980; Lyons, 1992; Angermeier & Smogor,

<sup>†</sup>Author to whom correspondence should be addressed at present address: Department of Zoology, University of Toronto, Toronto, Ontario, M5S 3G5, Canada. Tel.: +1 416 946 7230; fax: +1 416 978 8532; email: markpoos@zoo.utoronto.ca

1995; Ensign *et al.*, 2002). This need is especially important for species at risk of extinction, where species assessments are of primary importance to the assignment of conservation designations and decisions on legal listings (Mace, 1994). In particular, imperilled species legislation across the U.S., Europe, Australia and, more recently, in Canada, has reinforced the value of thorough assessments using rigorous sampling protocols for species being considered for, or assigned, conservation designations (Rice, 2005). Without assessments based on rigorous sampling protocols, species vulnerable to extinction could go overlooked or common species that are poorly assessed could be inappropriately assigned a conservation designation (Mace, 1994). To develop conservation actions assisting the recovery of species at risk, resource managers need to know the distribution and abundance of those species at risk.

Selection of sampling gear is important to the assessment of fish assemblages (Yoder & Smith, 1999) and to management decisions based on those assessments (McInerney & Cross, 2000). Currently, electrofishing is most commonly used and regarded as the most effective gear type for sampling stream fish assemblages (Larimore, 1961; Bohlin *et al.*, 1989; Reynolds, 1996; Ensign *et al.*, 2002). This sampling method is the primary (and often only) gear used in assessment and bio-monitoring protocols by management agencies in the U.S. (Rabeni *et al.*, 1997; Barbour *et al.*, 1999), Canada (Stanfield *et al.*, 2001) and Europe (FAME Consortium, 2004; Pont *et al.*, 2006). Further, protocols which rely on both electrofishing and seining, recommend seining only in cases where the size selection of electrofishing may be an issue (Cowx *et al.*, 2001; King & Crook, 2002; Peck *et al.*, 2005) or where sites have high turbidity (Yoder & Smith, 1999). Fish assessments based on these protocols provide the basis for decisions regarding conservation designations and for evaluations of the ecological condition of watersheds. Effectiveness of electrofishing, in terms of the number of individuals and species collected and the effort required to collect them, is an outcome of complex interactions between the biological characteristics of the fishes (Hill & Willis, 1994), physical characteristics of where these fishes live (Simonson & Lyons, 1995; Wang *et al.*, 1996) and technical characteristics of the sampling protocol (Reynolds, 1996). Biological characteristics include differences in the body size and behaviour of the fishes (Ensign *et al.*, 2002). Physical characteristics include factors such as stream flow, conductivity and turbidity (Reynolds, 1996). Technical characteristics of the sampling protocol include factors such as the use of single *v.* multiple passes by electrofishing; the number of netters, or the use of systematic and habitat-based sampling (Bohlin *et al.*, 1989). Despite the importance of these characteristics, researchers are often hindered by economic constraints which facilitate the use of a single-gear sampling method without validation of its effectiveness.

Seining provides an alternative active method of sampling stream fishes. A seine has floats along the top and weights along the bottom. The net is pulled from the ends through the water to sample fishes (Reynolds, 1996). Surveys based on seining have been used for management purposes for >100 years (Bayley & Herendeen, 2000). Moreover, use of seines is becoming increasingly attractive as fisheries managers look for alternatives to electrofishing, because electrofishing can cause injury or mortality of fish species through haemorrhaging and spinal displacement (Sharber & Carothers, 1988; Hollender & Carline,

1994; Carline, 2001; Dwyer *et al.*, 2001; Holliman & Reynolds, 2002; Dolan & Miranda, 2004). Such sampling injuries are particularly important for fish species at risk because of the potential for, and added concern about, negative effects on population growth rate (Nielsen, 1998).

This study tests the hypothesis that electrofishing is more effective than seining for sampling fish species at risk. Electrofishing is often the primary and sometimes only sampling method used for assessment protocols, with the implicit assumption that electrofishing is more effective than other gear types for sampling fishes, including those at risk. This assumption warrants testing as a hypothesis because few studies have compared the effectiveness of electrofishing *v.* seining (Wiley & Tsai, 1983; King & Crook, 2002) and no study has compared their effectiveness in detecting fish species at risk. Moreover, for rare species the hypothesis that electrofishing is more effective than seining, as observed for common species, may be incorrect. For example, in areas where fishes are common, rare fishes may be displaced into a suite of habitats (competition) that impede their susceptibility to electrofishing and alter their behaviour (*e.g.* schooling, activity and movement away from refuges) in ways that impede capture.

Five predictions were used to test whether electrofishing was more effective than seining. First, at sites where species at risk were detected, the probability of detecting a species at risk using electrofishing was expected to be as high as, as or higher, than it was using a seine. Second, at sites where species at risk were detected, the probability of a species at risk being detected with only electrofishing was expected to be as high as, or higher, than it was when using only a seine. Third, at sites where species at risk were detected, the catch estimated using electrofishing was expected to be as high as, or higher, than catch estimates obtained using a seine. Fourth, the number of sample sites required to detect a species at risk within a watershed when using electrofishing was expected to be as low as, or lower, than the number required when using a seine. Finally, at sites where a species at risk was detected, the number of subsections within a site that were required to detect a species at risk using electrofishing was expected to be as low as, or lower, than the number required using a seine. The first three predictions were included as they represent intuitive metrics of accuracy and precision: detectability in general, detectability in cases where one sampling method was inadequate and catch per unit effort. The final two predictions were included to determine whether scale (watershed level or site level) influenced the effectiveness of each sampling method. Such a division can allow resource managers to determine not only the amount of sampling required to detect a species at risk (*i.e.* how many sites are needed to initially detect a species at risk in a watershed), but also to determine the duration of sampling (*i.e.* how many subsections should be sampled within a site to maximize the probability of detecting a species at risk). As sampling methods should be accurate, precise, and economically feasible, the ability to detect imperilled species across scales (*e.g.* how far and how long) is a useful metric for determining precision and economic feasibility. The criterion for the most effective sampling method was determined as the sampling method which was significantly better across the majority of the five predictions and species at risk captured. This criterion was used as it reflects a balance between accuracy,

precision and the economic feasibility of the sampling method, a necessity for all sampling approaches.

## STUDY AREA

The predictions were tested using data collected in the Sydenham River in south-western Ontario, Canada. The Sydenham watershed is being used as a model system to develop scientific tools supporting the assessment needed to defend conservation designations and to monitor the success of recovery plans required by the Canadian Species at Risk Act. This 2725 km<sup>2</sup> watershed is characterized by low relief, with low stream gradients and shallow valleys. Although, the population within the watershed is small (18 000 people), the watershed experiences significant anthropogenic stress with 85% of the area used intensively for agriculture (Dextrase *et al.*, 2003).

The Sydenham watershed has a high number (eight) of fish species at risk relative to other watersheds in Canada (Staton *et al.*, 2003). These species at risk include: the endangered benthivore the northern madtom *Noturus stigmosus* Taylor, the threatened eastern sand darter *Ammocrypta pellucida* (Putnam), threatened spotted gar *Lepisosteus oculatus* Winchell, and four species of special concern, including greenside darter *Etheostoma blennioides* Rafinesque, pugnose minnow *Opsopoeodus emiliae* (Hay), bigmouth buffalo *Ictiobus cyprinellus* (Valenciennes) and blackstripe topminnow *Fundulus notatus* (Rafinesque). Many of these species are demersal, including: the spotted sucker, bigmouth buffalo, northern madtom, eastern sand darter and greenside darter, and their declines are thought to be due to increases in agriculture and associated sediment loads (Dextrase *et al.*, 2003).

## MATERIALS AND METHODS

### SAMPLE SITES

Fifty sites were sampled across the Sydenham River watershed in 2003 (Fig. 1). Forty-two sites were spread across the watershed, at least 2 km apart, to achieve uniform coverage. Eight sites were selected to overlap with ongoing surveys of mussels (Metcalf-Smith *et al.*, 2003), benthic macroinvertebrates (M. Andreae, pers. comm.), and previous surveys of fish species at risk (Dextrase *et al.*, 2003). Non-wadeable river sections near Shetland Conservation Area (CA) and in the lower portions of the watershed were not sampled (Fig. 1).

Each site was defined as either a pool-riffle sequence *c.* 60 m in length ( $n = 15$ ) or, where there were no clearly defined pool-riffle sequences (*e.g.* due to channelization for agriculture), a 60 m length of stream ( $n = 35$ ). This followed the recommendation of Bohlin *et al.* (1989) to use sites of roughly equal length. Each site was further subdivided into 10 subsections of equal length using 11 transects laid out perpendicular to the bank. The subdivision of each site was needed to accurately divide fish sampling into two scales: site level (based on the accumulation of subsections) and watershed level (based on the accumulation of sites).

### FISH SAMPLING

Fishes were collected using two different methods: single-pass backpack electrofishing (pulsed DC current at 200–225 V, hertz = 60, pulse length = 3 ms) with two netters, or

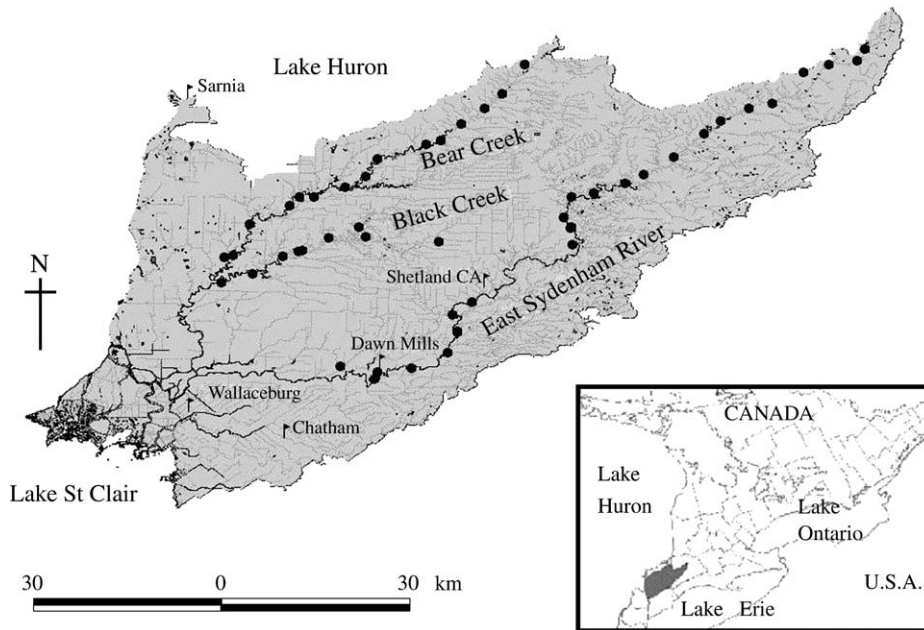


FIG. 1. Sites sampled in the Sydenham River watershed in 2003.  $n = 50$  sites.

a bag seine (8.2 m  $\times$  2 m, 7.5 mm mesh, bag 2 m  $\times$  2 m  $\times$  2 m) pulled by two people with a third lifting the net over obstructions. Fishes were collected in each subsection sequentially, identified to species, counted, and returned to the river, with the exception of those retained as voucher specimens. Both gear types were deployed when habitat was appropriate (with water depths  $< 2$  m and stream widths  $> 0.5$  m). On average, a site was  $722 \pm 54$  m<sup>2</sup> (mean  $\pm$  s.e., range 208 to 1954 m<sup>2</sup>). The order of gear deployment was determined randomly. Approximately 1 h after the last sample of fishes was returned to the water, the site was re-sampled using the other gear type. Electrofishing was carried out in an upstream direction at a rate of 1 m<sup>2</sup> per 5 s (Stanfield *et al.*, 2001), systematically and continuously covering each subsection sequentially from side to side for the entire site. The total sampling effort depended on the area of the site. A minimum of 2000 s (mean  $\pm$  s.e.  $4257 \pm 130$  s) was shocked at each site, exceeding the recommendation of 1500 s of electrofishing to quantify the structure of fish assemblages (Yoder & Smith, 1999). The added time was used to address the rarity of fish species at risk (Mace, 1994), and the possibility that they would require more sampling to be detected. Seining was completed in a downstream direction using five to eight seine hauls to systematically sample the entire area of two subsections sequentially and continuously for the entire site. Some variation occurred in the number of hauls due to obstructions and physical features of the individual sites. In lower sections of the river (below Dawn Mills, Fig. 1), where the seine could not stretch the entire stream width ( $n = 4$ ), care was taken so to not disturb the un-sampled areas and seining was systematically repeated. Gillnets (5 cm mesh) were used as block nets on the upstream end of the site during electrofishing and the downstream end of the site during seining, unless a natural obstruction (*e.g.* shallow water) was present. One voucher specimen of each species was kept for each site.

## WATER CHEMISTRY

Water chemistry variables, such as dissolved oxygen, specific conductivity, turbidity and nitrate concentration, were taken at each sample site. Water quality variables were

measured using a HydroLab DataSonde 4a multi-probed sensor. The sensor measured specific conductivity ( $\pm 0.001 \text{ mS cm}^{-1}$ ), turbidity ( $\pm 5 \text{ NTU}$ ), dissolved oxygen ( $\pm 0.2 \text{ mg l}^{-1}$ ), and nitrate concentration ( $\pm 0.2 \text{ mg l}^{-1}\text{-N}$ ). These measurements were made c. 20 m above the upstream end of the sample site continuously for the duration of sampling. Measurements were averaged ( $\pm \text{s.e.}$ ) over the sampling duration for each site sampled. Pooled species at risk abundance at each site was used to test the effects of water chemistry on each gear type and whether there was an interaction. A multiple linear regression (PROC GLM) was run in SAS version 9.1. The multi-probed sensor malfunctioned at one site and that data were not included in the analysis.

## DATA ANALYSIS

A comparative approach was used, in which the five predictions for electrofishing and seining were tested. For this approach, all sites where species at risk were not found were eliminated (*i.e.* the number of sites included in the study design, the sampling universe, was defined to be only those sites where the species was known to be present from their capture by at least one gear). This step was necessary to eliminate zero inflation bias (Martin *et al.*, 2005). Although, those zeros were considered to be 'true' observations given the extent of sampling as compared to most other standard protocols (Yoder & Smith, 1999; Stanfield *et al.*, 2001), comparisons were optimized to minimize the effect of false negatives, and to maximize between gear differences.

### Prediction 1

At sites where species at risk were detected, the probability of detecting any individual of a species at risk (at a site) using the electrofisher was expected to be as high as or higher than it was using a seine.

The probability of detection (as a proportion) was defined as the number of sites where a species at risk was detected with a given gear type divided by the total number of sites where this species was detected with any gear type. Contingency table analysis comparing detection (detected or not-detected) with gear type (electrofishing or seining) was used to determine if the probability of detection differed between gear types. Tables were calculated for each species separately, and for all species at risk after pooling. Statistical significance was assessed using a *G*-test (Zar, 1999).

### Prediction 2

At sites where species at risk were detected, the probability of detecting any individual of a species at risk (at a site) with only the electrofisher was expected to be as high as, or higher, than it was when using only a seine.

This probability was defined as the number of sites where a specific gear type was the only gear to detect a species at risk divided by the number of sites where that species at risk was detected. This prediction differs from the first as it considers which sampling methodology detected a species when the other did not. Such a comparison is useful as it determines which sampling methodology captured unique specimens, which would have otherwise been missed. Contingency table analysis comparing detections unique to each gear type (detected or not-detected) with gear type (electrofishing or seining) was used to determine if the probabilities differed between gear types. Tables were calculated for each species separately, and for species at risk after pooling. Statistical significance was assessed using a *G*-test (Zar, 1999).

### Prediction 3

At sites where species at risk were detected, the catch per unit effort estimates using electrofishing (at a site) were expected to be as high as, or higher, than catch per unit effort estimates obtained using a seine.

The catch per unit effort at each site was estimated by dividing the number of individuals (*i.e.* abundance) of a given species at risk collected using a specific gear type by

the area (m<sup>2</sup>) of the site. Differences in catch per unit effort estimates obtained using the gear types were tested using a Wilcoxon matched pairs signed rank test. This distribution free test was used because catch per unit effort estimates were not normally distributed (Zar, 1999).

#### *Prediction 4*

The number of sample sites required to detect a species at risk within a watershed when using electrofishing was expected to be as low as, or lower, than the number required when using a seine.

This prediction was tested by modelling how the probability of detecting a given species at risk within the watershed changed as the cumulative number of sites sampled increased (*i.e.* a cumulative probability-of-detection curve). For each species at risk, separate cumulative probability-of-detection curves were generated for electrofishing and seining separately, and the two gears combined. For a given site, a sampling gear was assigned a value of 1 if a species at risk was detected using it, and a value of 0 if it was not. Each curve was generated by randomizing the sequence of detections from the field survey 1000 times and estimating the mean probability of detection in relation to the number of sites sampled. The estimated number of sites required to obtain a probability of detection equal to 0.95 was compared among accumulation curves. Mean area sampled by electrofishing, seining and the combination of electrofishing and seining were calculated for comparison, given that using both gears would require extra effort at a site.

#### *Prediction 5*

At sites where species at risk were detected, the number of subsections within a site that were required to detect a species at risk using electrofishing was expected to be as low as, or lower, than the number required using a seine.

This prediction was tested by modelling how the probability of detecting a given species at risk within a site changed as the cumulative number of subsections sampled increased. For each species at risk, separate cumulative probability-of-detection curves were generated for electrofishing and seining, but not together because subsections were sampled two at a time while seining. For a given subsection, a sampling gear was assigned a value of 1 if a species at risk was detected using it, and a value of 0 if it was not. The curve was generated by randomizing the observed sequence of detections from the field survey 1000 times and estimating the mean probability of detection in relation to the number of subsections. A stratified randomization process was used to quantify the number of subsections need to detect a species at risk with a given site across all sites. Each randomization was blocked by site and species were counted as detected after the first capture. As the randomization process for subsections was blocked by site, maximal and minimal boundaries were included to reduce the potential error of constraining the randomization to sites with greatly disproportionate probabilities of detection. This was done by simulating data which represented maximal (simulated data completely fixed for half of the subsections and equally random for the other half of subsections) and minimal (simulated data equally random for all subsections) boundaries for each species accumulation curve. The simulated randomization boundaries provide a surrogate measure of the error of blocking the randomization by site, a requirement unique to randomizing subsections and not sites. The estimated number of subsections required to obtain a probability of detection equal to 0.95 was compared among accumulation curves.

## RESULTS

### FISH SAMPLING

Four of the eight species at risk expected to be in the watershed were collected. They were eastern sand darter, greenside darter, blackstripe topminnow

and spotted sucker. The remaining four species at risk previously reported in the Sydenham River (Staton *et al.*, 2003), pugnose minnow, northern madtom, bigmouth buffalo and spotted gar, were not collected in this study. Two of these species (the northern madtom and spotted gar) are thought to be extirpated from the river, while the other two (bigmouth buffalo and pugnose minnow) are thought to be in non-wadeable sections, which were not sampled (Dextrase *et al.*, 2003). No species at risk were captured in the block nets.

## WATER CHEMISTRY

Sample sites were characterized by, on average ( $\pm$ S.E.), specific conductivity of  $430.1 \pm 11.52 \mu\text{S cm}^{-1}$ , turbidity of  $95.2 \pm 11.3$  NTU, dissolved oxygen of  $7.3 \pm 0.33 \text{ mg l}^{-1}$ , and nitrate concentration of  $7.73 \pm 1.24 \text{ mg l}^{-1}\text{-N}$ , with northern branches of Black Creek and Bear Creek with higher turbidity, conductivity and nitrate concentrations than the East Sydenham River (Fig. 1).

None of the water chemistry variables influenced the abundance estimates of species at risk (multiple linear regression,  $n = 49$ ,  $P > 0.05$ ). The overall nine variable model was significant for determining the abundance of species at risk (multiple linear regression,  $n = 49$ ,  $P < 0.05$ ), but sampling gear was the only significant variable in the model (multiple linear regression,  $n = 49$ ,  $P < 0.001$ ). There was no interaction between dissolved oxygen, specific conductivity, turbidity and nitrate concentration and sampling method used to estimate the abundance of fish species at risk (multiple linear regression,  $n = 49$ ,  $P > 0.05$ ).

## DATA ANALYSIS

### *Prediction 1*

The probability of detecting a fish species at risk was as high, or higher, for electrofishing than it was for seining. Overall, electrofishing detected species at risk at 95% of the sites where they were ultimately found to occur with either method. This was significantly higher than seining ( $G$ -test,  $n = 63$ ,  $P < 0.001$ ), which detected fish species at risk at 67% of the sites where they were ultimately found to occur. For individual species, electrofishing was significantly better than seining at detecting the greenside darter (100% *v.* 69.7%;  $G$ -test,  $n = 33$ ;  $P < 0.001$ ) and the spotted sucker (100% *v.* 0%;  $G$ -test,  $n = 6$ ;  $P < 0.001$ ). Further, there was a strong difference between electrofishing and seining at detecting the eastern sand darter, (83% *v.* 33%), but this difference between gear types was not statistically significant at the conventional  $\alpha = 0.05$  ( $G$ -test,  $n = 6$ ,  $P = 0.07$ ), probably because of the low number of sites where the eastern sand darter was detected. Finally, the detection of the blackstripe topminnow was identical for electrofishing and seining. It was detected by both gears at 89% (16/18) of the sites where it was ultimately found to occur.

### *Prediction 2*

The probability that a fish species at risk was detected using only the electrofisher was as high, or higher, than for a seine only. Overall, electrofishing detected fish species at risk at an additional 30% of the sites where seining did



not. This was significantly higher ( $G$ -test,  $n = 63$ ,  $P < 0.001$ ) than seining, which detected fish species at risk at an additional 4% of sites where electrofishing did not. For individual species at risk, electrofishing detected the greenside darter (21.2% v. 0%;  $G$ -test,  $n = 33$ ,  $P < 0.01$ ) and the spotted sucker (100% v. 0%;  $G$ -test,  $n = 6$ ,  $P < 0.001$ ) significantly more often than seining. Electrofishing and seining both detected the blackstripe topminnow at 11% of sites when the other gear did not ( $G$ -test,  $n = 18$ ,  $P > 0.05$ ). Finally, electrofishing detected the eastern sand darter at 67% of sites when seining did not; compared to the 17% of the sites when seining detected the eastern sand darter and electrofishing did not. Although this difference is important, it was not statistically significant ( $G$ -test,  $n = 6$ ,  $P = 0.08$ ), possibly because of the small sample sizes.

### Prediction 3

The catch per unit effort of fish species at risk was as high as, or higher than, using electrofishing than when seining. Overall, electrofishing caught significantly more species at risk than seining (Wilcoxon matched pairs signed rank test;  $n = 63$ ,  $P < 0.001$ ). For the greenside darter and the spotted sucker, electrofishing caught significantly more per unit area than with seining (Wilcoxon matched pairs signed rank test,  $n = 33$ ,  $P < 0.001$ , and  $n = 6$ ,  $P < 0.05$ , respectively). For eastern sand darter and blackstripe topminnow, the catch per unit effort tended to be higher for electrofishing than for seining but did not differ significantly between electrofishing and seining (Wilcoxon matched pairs signed rank test;  $n = 6$ ,  $P > 0.5$ , and  $n = 18$ ,  $P > 0.05$ , respectively).

### Prediction 4

In all cases considered, the number of sample sites required to detect a fish species at risk in the watershed was as low as, or lower, using electrofishing than when using the seine (Fig. 2). For the blackstripe topminnow, the number of sample sites required to achieve a 95% probability of detection was estimated to be eight for both gear types, although they differed in the area required for sampling (4619 for electrofishing v. 5257 for the seine; Fig. 2). When sample area (effort) was considered, electrofishing required sampling less area than seining (4610 v. 5257 m<sup>2</sup> respectively), on average, to achieve 95% probability of detection (Table I). This was also the case for the eastern sand darter (24 sites and 26 457 m<sup>2</sup> for electrofishing v. 38 sites and 57 485 m<sup>2</sup> for seining) and the greenside darter (three sites and 2474 m<sup>2</sup> for electrofishing v. five sites and 4164 m<sup>2</sup> for seining), while the spotted sucker was detected only with electrofishing (19 sites and 13 273 m<sup>2</sup>; Table I).

When the two gear types were considered together, the number of sites needed to achieve a 95% probability of detection remained effectively the same as the number required with electrofishing alone. For example, the eastern sand darter and greenside darter were found in the same number of sites (24 and three respectively) with the two gears combined as they were with just electrofishing. The only exception was the blackstripe topminnow, where the number of sample sites required to achieve 95% detection was slightly lower when combining gear types than when electrofishing (six sites for both gears combined

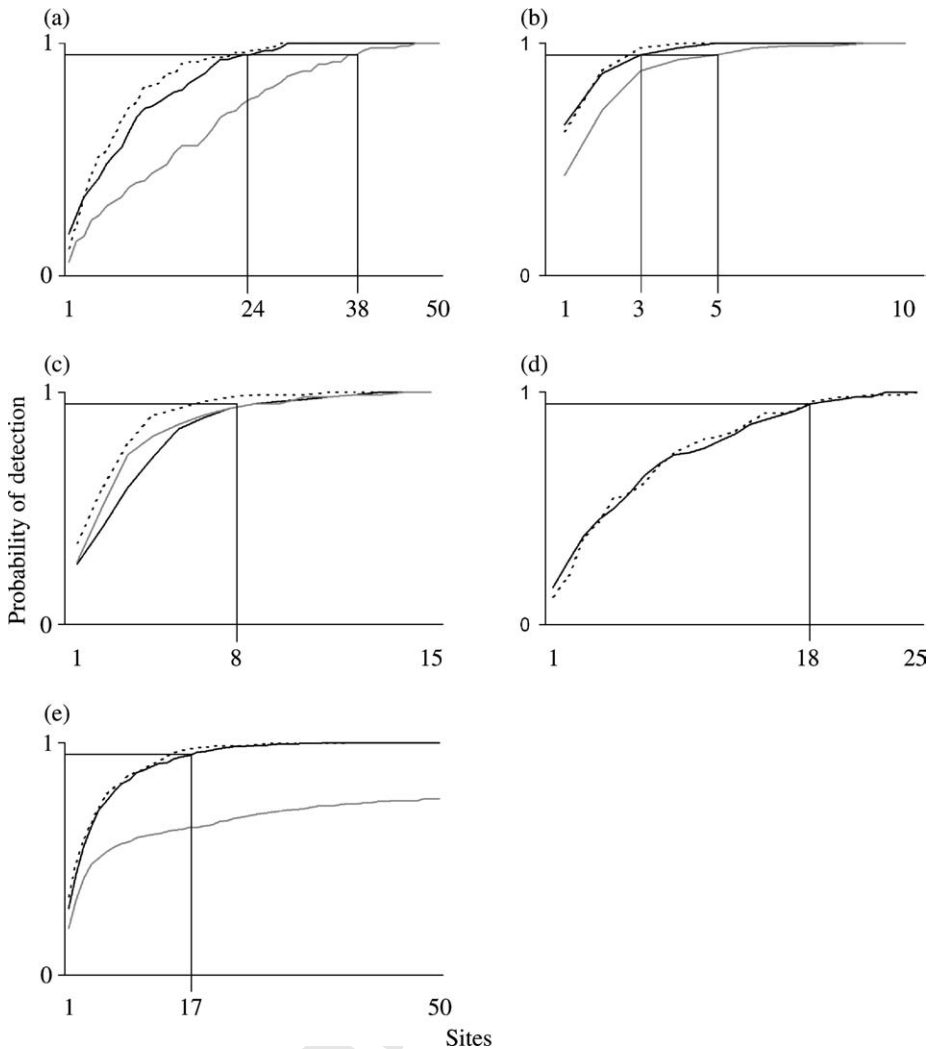


FIG. 2. Estimated number of sample sites needed to detect the occurrence of: (a) eastern sand darter, (b) greenside darter, (c) blackstripe topminnow, (d) spotted sucker and (e) fish species at risk (pooled across 50 sampled sites) with probability 0.95 on the Sydenham River, Ontario in 2003. Curves are provided for electrofishing, seining, and electrofishing and seining together. The vertical line and corresponding value represent the number of sites required to reach 95% probability of detection for the watershed.

v. eight for electrofishing and seven for seining); however, combining gear types required almost twice the sampling effort in terms of area (7404 v. 4619 m<sup>2</sup> for electrofishing and 5257 m<sup>2</sup> for seining; Table I).

#### Prediction 5

The number of subsections required to detect a fish species at risk with probability 0.95 at a site where it was detected and known to occur was the same

TABLE I. Total area (m<sup>2</sup>), number of sites (*n*), and the number of subsections within sites required to initially detect the eastern sand darter, greenside darter, blackstripe topminnow, spotted sucker and all fish species at risk (pooled) across 50 sampled sites on the Sydenham River, Ontario in 2003 with 95% probability using electrofishing, seining and both gears sequentially together

	Electrofishing			Seining			Both	
	Sites ( <i>n</i> )	Subsections ( <i>n</i> )	Area (m <sup>2</sup> )	Sites ( <i>n</i> )	Subsections ( <i>n</i> )	Area (m <sup>2</sup> )	Sites ( <i>n</i> )	Area (m <sup>2</sup> )
Eastern sand darter	24	9	26 457	38	7	57 485	24	57 838
Greenside darter	3	7	2474	5	8	4164	3	4968
Blackstripe topminnow	8	6	4619	8	7	5257	6	7404
Spotted sucker	19	10	13 273	nc	nc	nc	19	26 546

nc, no individuals collected.

using electrofishing or seining. Two species at risk, the greenside darter and the blackstripe topminnow, were caught within one subsection of each other (seven subsections for electrofishing *v.* eight for seining), while the spotted sucker was caught using only electrofishing (Fig. 3). The variability in the effectiveness of electrofishing and seining for the eastern sand darter was too high to draw any conclusions. Although this species was found (at 95% probability of detection) at two less subsections using seining than electrofishing (seven *v.* nine respectively), this finding may not represent a significant difference in sampling as there was a large deviation in the minimum (boundary = 2 subsections) and maximum (boundary = 10 subsections) boundaries of detection in the six sites where they were sampled with the seine (Fig. 3).

## DISCUSSION

Given the criterion of effectiveness (*i.e.* accuracy, precision and economic feasibility), electrofishing was more effective than seining for sampling fish species at risk in the Sydenham River. In general, all five of the predictions regarding effectiveness were supported (Table II). For example, at a site where a fish species at risk was detected, the probability of detecting it, the probability of a single gear type detecting it, and the number of individuals detected per sample area, were all as high, or higher, for electrofishing as they were for seining. In addition, the number of sites and subsections within a site needed to detect (at 95% probability) a fish species at risk was as low, or lower, for electrofishing than it was for seining. Seining was not significantly better than electrofishing for any of the predictions across any of the imperilled species sampled (Table II).

The conclusion regarding the greater effectiveness of electrofishing is consistent with the findings of several studies that independently showed electrofishing was more effective than seining in detecting fish species in general across varying types of habitats (Dauble & Gray, 1980; Wiley & Tsai, 1983; Pygott *et al.*, 1990). Electrofishing alone, however, may not be appropriate in all

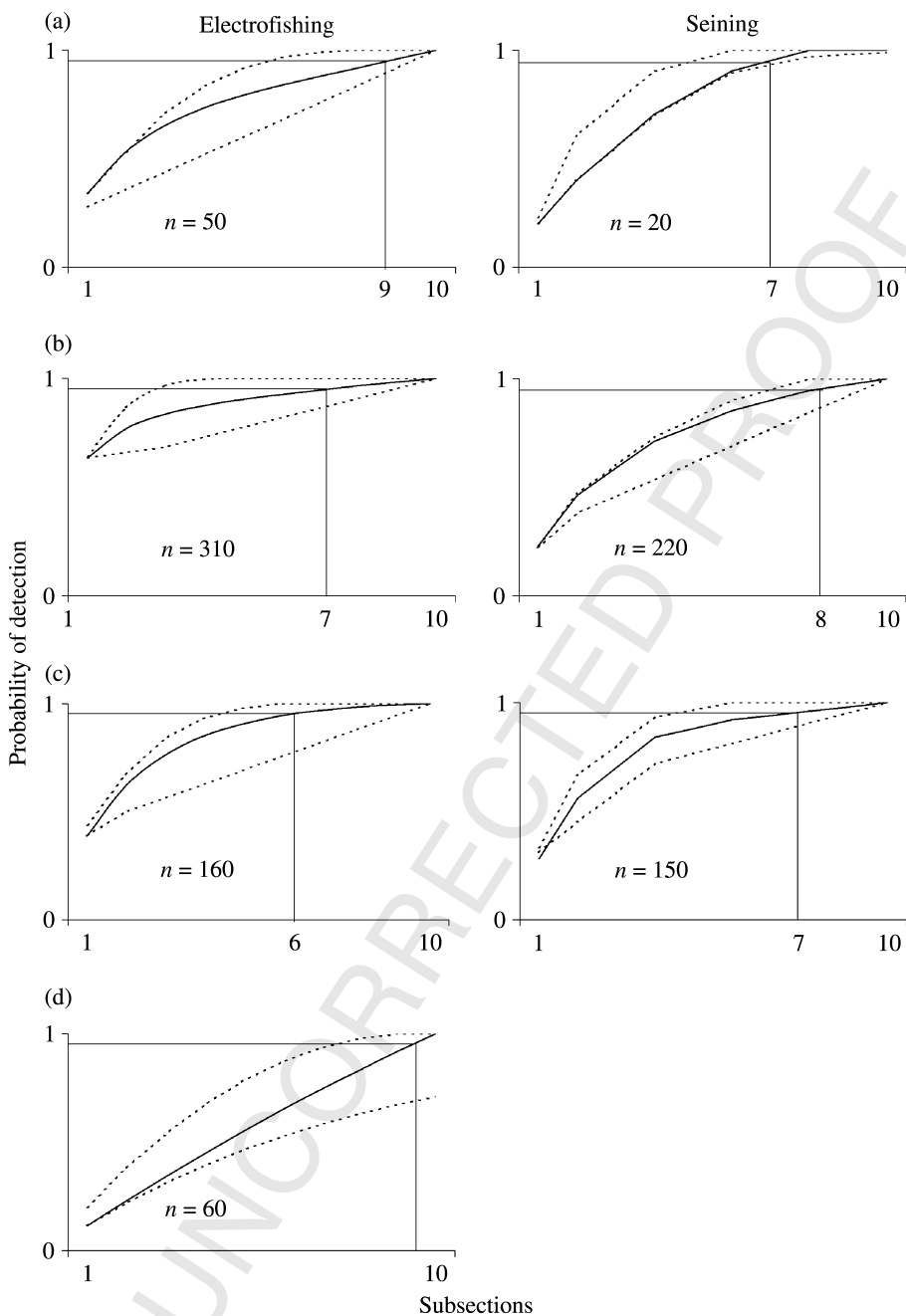


FIG. 3. Estimated number of subsections within a sample site in the Sydenham River, Ontario in 2003 ( $n$  = number of sub-samples). Separate detection curves required to detect a species at risk within a site with 95% probability of detection using electrofishing and seining are provided for: (a) eastern sand darter, (b) greenside darter, (c) blackstripe topminnow and (d) spotted sucker. The vertical line and corresponding value represent the number of subsections required to reach 95% probability of detection for the site. The dashed lines indicate simulated minimal and maximal boundaries for probability of detection.

TABLE II. Outcome of the five predictions used to compare the effectiveness of electrofishing and seining for sampling the eastern sand darter, greenside darter, blackstripe topminnow, spotted sucker and fish species at risk (pooled) across 50 sampled sites on the Sydenham River, Ontario in 2003. The five predictions tested were: at sites where species at risk were detected, (1) the probability of detecting the species at risk, (2) the probability of only one gear type detecting the species at risk and (3) the estimated catch of the species at risk, was as high as, or higher, when using electrofishing than when using seining. (4) The number of sample sites required to detect a species at risk within a watershed and (5) the number of subsections required to detect a species at risk within a site, were as low as, or lower, using electrofishing than the number required using seining

	Prediction 1	Prediction 2	Prediction 3	Prediction 4	Prediction 5
Eastern sand darter	=	=	=	E	=
Greenside darter	E	E	E	E	E
Blackstripe topminnow	=	=	=	=	E
Spotted sucker	E	E	E	E	E
All Species at Risk (Pooled)	E	E	E	E	N/A
Overall conclusion	E	E	E	E	E

E, electrofishing performed significantly better than seining; =, no statistical difference between the two; N/A, a situation which was not applicable.

situations. Effectiveness of electrofishing can be influenced by environmental conditions at each sample site (Reynolds, 1996). For example, the blackstripe topminnow was found in sites with significantly higher specific conductivity (mean  $\pm$  s.e.  $526.2 \pm 11.50$  mS cm<sup>-1</sup>, *t*-test, d.f. = 1.27,  $P < 0.05$ ) and in significantly higher turbidity (mean =  $182.6 \pm 16.91$  NTU, *t*-test, d.f. = 127,  $P = 0.001$ ) than the other species at risk (conductivity:  $492.1 \pm 10.51$  mS cm<sup>-1</sup>, turbidity:  $91.62 \pm 8.501$  NTU). These factors can reduce electrofishing success (Hill & Willis, 1994), because fishes captured by the electric field may not be seen and trapped by the netters (Pygott *et al.*, 1990). Alternatively, high turbidity could aid the effectiveness of seining by limiting the avoidance response of fish species at risk to the oncoming seine (Weinstein & Davis, 1980; Glass & Wardle, 1989). Such an example highlights the value of testing the suitability of a gear type or sampling protocol for the particular condition, species and geographic area, as done in this study. The results indicate that electrofishing was as effective as, or better, than seining across the variety of water chemistry variables and wadeable habitat types sampled.

Effectiveness of electrofishing can also be influenced by different characteristics of fishes. Larimore (1961) concluded that the greenside darter was easily captured using electrofishing and he attributed this success to the relatively large body size and bright colouration of this species relative to other fish species in the study system. He also concluded that the relatively small size and surface swimming behaviour of the blackstripe topminnow made it difficult to stun and collect with electrofishing. The difference in the characteristics may also explain the relative success of the seine for sampling the blackstripe

topminnow, as compared to the other species at risk sampled. Another related species from the same taxonomic family, the mummichog *Fundulus heteroclitus* (L.), is captured effectively using seines (Allen *et al.*, 1992). The tendency for *Fundulus* spp. to swim up in the water column ahead of the approaching seine, may make them more susceptible to capture with a seine than demersal species that try to swim under, or around, the net (Lyons, 1986; Allen *et al.*, 1992; Holland-Bartels & Dewey, 1997; Snyder, 2003). Targeted sampling for specific imperilled species may require specialized sampling protocols which take into consideration species specific differences to maximize their effectiveness. Alternatively, standardized sampling protocols need to be accurate, precise and cost efficient across a variety of species and habitat types. The results from this study suggest that electrofishing is well suited for standardized sampling protocols for imperilled species, although validation of this claim is required for other systems and species.

When choosing gear types, biologists need to give greater consideration to the trade-off between differences in the risks of injury to fish species at risk associated with alternative gear types and differences in the sampling effectiveness of those gears. Recent literature has advocated seining over electrofishing in an attempt to reduce potential injuries and mortalities of fishes (Nielsen, 1998; Snyder, 2003). Species at risk are a particular concern, because they are often found at low abundances. Although, the mortality caused by each sampling method was not measured, changing from electrofishing to seining will probably entail a reduction in precision and accuracy in estimating the distribution and abundance of fish species at risk. In addition, injuries incurred by seined fishes remain poorly understood. The associated mortality of seining may be much higher than perceived as seines capture fishes in bulk, thus increasing the handling time for each fish as compared to electrofishing. Furthermore, recent research has shown that electrofishing for fishes at risk may induce low, but acceptable, levels of mortality in the study of endangered fish species (Ruppert & Muth, 1997; Holliman *et al.*, 2003a, b). Therefore, in situations where injury of individual fish is a concern, biologists will need to weigh differences in the risks of injury of fish species at risk against differences in the effectiveness of each gear type when deciding between gear types. It may be better to develop electrofishing protocols that are less injurious to species at risk than to outright switch to seining. For example, this study shows that switching to seining would require sampling 14 more sites for sampling the eastern sand darter, two more sites for the greenside darter, and would not detect the spotted sucker (Table I).

The selection of sampling gear is easily overlooked, yet rigorous, quantitative comparisons of gear types represent one way in which science can support existing imperilled species legislation and assist efforts to conserve native species. The selection of gear types may influence estimates of the distribution and abundance of fishes due to differences in their sampling effectiveness (Holland-Bartels & Dewey, 1997) and, in turn, this may influence management decisions. Unreliable estimates of the distribution and abundance of fish species at risk can result in failure to list species that need protection, or result in the inappropriate assignment of designations to species that do not need protection (Hilton-Taylor *et al.*, 2000). In addition, recovery plans are mandated by

imperilled species legislation in both the U.S. and Canada (Mace, 1994; Dextrase *et al.*, 2003). Monitoring the distribution and abundance of species listed as 'at risk' is fundamentally important for assessing the success of those recovery plans.

The approach employed here tested five independent predictions considering multiple sampling metrics (presence or absence and catch per unit effort) and multiple spatial scales (watershed and site level) and demonstrated that electrofishing was more effective than seining for sampling fish species at risk under a variety of environmental conditions. Although many studies have shown that electrofishing is the single most effective sampling method for characterizing fish assemblages in wadeable systems (Vincent, 1971; Hendricks *et al.*, 1980; Gammon *et al.*, 1981; Reynolds, 1996; Yoder & Smith, 1999), none have tested whether electrofishing was more effective against other gear types for sampling fish species at risk. Examinations specifically focusing on species at risk are important and needed because species at risk typically occur at lower abundances and in more restricted habitat types (Mace, 1994), which can make them more challenging to detect and sample than non-listed species. Sampling methods should reflect the species at risk biology, and by necessity, need to be accurate, precise and economically feasible so that species at risk can be effectively detected, enumerated, and properly assessed by conservation agencies.

Funding was provided by the Department of Fisheries and Oceans Canada Species at Risk Program (SARCEP) and the federal Interdepartmental Recovery Fund (IRF) to NEM and a Natural Sciences and Engineering Research Council Discovery Grant to R.L.M. Thanks to the Department of Fisheries and Oceans, Environment Canada and the Royal Ontario Museum for contributing resources and assisting with the completion of this study, M. Finch, J. Keenlside, M. Parslow and S. Foley for their dedicated field sampling, A. Dextrase, S. Staton, J. Barnucz, T. Heiman, J. Clark, F. Neave and T. den Haas for their technical advice, D. A. Jackson for statistical advice and assistance, and, D. L. G. Noakes, R. J. Wootton and anonymous reviewers for their constructive comments on earlier drafts of this manuscript. Research protocols were approved by the University of Guelph Animal Care Committee (Animal Use Protocol #03R055).

## References

- Allen, D. M., Service, S. K. & Ogburn-Matthews, M. V. (1992). Factors influencing the collection efficiency of estuarine fishes. *Transactions of the American Fisheries Society* **121**, 234–244.
- Angermeier, P. L. & Smogor, R. A. (1995). Estimating number of species and relative abundances in stream-fish communities: effects of sampling effort and discontinuous spatial distributions. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 936–949.
- Barbour, M. T., Gerritsen, J., Snyder, B. D. & Stribling, J. B. (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edn. *EPA Report. EPA 841-B-99-002*. Washington, DC: U.S. Environmental Protection Agency, Office of Water.
- Bayley, P. B. & Herendeen, R. A. (2000). The efficiency of a seine net. *Transactions of the American Fisheries Society* **129**, 901–923.
- Bohlin, T., Hamrin, S., Heggberget, T. G., Rasmussen, G. & Saltveit, S. J. (1989). Electrofishing – theory and practice with special emphasis on salmonids. *Hydrobiologia* **173**, 9–43.

- Carline, R. F. (2001). Effects of high frequency pulsed DC electrofishing on a wild brown trout population. *North American Journal of Fisheries Management* **21**, 571–579.
- Cowx, I. G., Nunn, A. D. & Harvey, J. P. (2001). Quantitative sampling of 0-group fish populations in large lowland rivers: point abundance sampling by electrofishing versus micromesh seine netting. *Archiv für Hydrobiologie* **151**, 369–382.
- Dauble, D. D. & Gray, R. H. (1980). Comparison of a small seine and a backpack electroshocker to evaluate nearshore fish populations in rivers. *Progressive Fish-Culturist* **42**, 93–95.
- Dextrase, A. J., Staton, S. K. & Metcalfe-Smith, J. L. (2003). Recovery strategy for species at risk in the Sydenham River: an ecosystem approach. *Recovery of Nationally Endangered Wildlife (RENEW) National Recovery Plan No. 25*. Ottawa: Government of Canada.
- Dolan, C. R. & Miranda, L. E. (2004). Injury and mortality of warm water fishes immobilized by electrofishing. *North American Journal of Fisheries Management* **24**, 118–127.
- Dwyer, W. P., Shepard, B. B. & White, R. G. (2001). Effect of backpack electroshock on westslope cutthroat trout injury and growth 110 and 250 days post treatment. *North American Journal of Fisheries Management* **21**, 646–650.
- Ensign, W. E., Temple, A. J. & Neves, R. J. (2002). Effects of fright bias on sampling efficiency of stream fish assemblages. *Journal of Freshwater Ecology* **17**, 127–139.
- FAME Consortium (2004). *Manual for the Application of the European Fish Index – EFI: A Fish-based Method to Assess the Ecological Status of European Rivers in Support of the Water Framework Directive*. Vienna: University of Natural Resources and Applied Life Sciences.
- Gammon, J. R., Spacie, A., Hamelink, J. L. & Kaesler, R. L. (1981). Role of electrofishing in assessing environmental quality of the Wabash River. In *Ecological Assessments of Effluent Impacts on Communities of Indigenous Aquatic Organisms*, pp. 307–324. Philadelphia, PA: American Society for Testing and Materials.
- Glass, C. W. & Wardle, C. S. (1989). Comparison of the reactions of fish to a trawl gear, at high and low light intensities. *Fisheries Research* **7**, 249–266.
- Hendricks, M. L., Hocutt, C. H. & Stauffer, J. R. (1980). Monitoring of fish in lotic habitats. In *Biological Monitoring of Fish* (Hocutt, C. H. & Stauffer, J. R., eds), pp. 205–232. Lexington, MA: Lexington Books.
- Hill, T. D. & Willis, D. W. (1994). Influence of water conductivity on pulsed AC and pulsed DC electrofishing catch rates for largemouth bass. *North American Journal of Fisheries Management* **14**, 202–207.
- Hilton-Taylor, C., Mace, G. M., Capper, D. R., Collar, N. J., Stuart, S. N., Bibby, C. J., Pollock, C. & Thomsen, J. B. (2000). Assessment mismatches must be sorted out: they leave species at risk. *Nature* **404**, 541–542.
- Holland-Bartels, L. E. & Dewey, M. R. (1997). The influence of seine capture efficiency on fish abundance estimates in the upper Mississippi River. *Journal of Freshwater Ecology* **12**, 101–111.
- Hollender, B. A. & Carline, R. F. (1994). Injury to wild brook trout by backpack electrofishing. *North American Journal of Fisheries Management* **14**, 643–649.
- Holliman, F. M. & Reynolds, J. B. (2002). Electroshock-induced injury in juvenile white sturgeon. *North American Journal of Fisheries Management* **22**, 494–499.
- Holliman, F. M., Reynolds, J. B. & Kwak, T. J. (2003a). Electroshock-induced injury and mortality in the spotfin chub, a threatened minnow. *North American Journal of Fisheries Management* **23**, 962–966.
- Holliman, F. M., Reynolds, J. B. & Kwak, T. J. (2003b). A predictive risk model for electroshock-induced mortality of the endangered Cape Fear shiner. *North American Journal of Fisheries Management* **23**, 905–912.
- King, A. J. & Crook, D. A. (2002). Evaluation of a sweep net electrofishing method for the collection of small fish and shrimp in lotic freshwater environments. *Hydrobiologia* **472**, 223–233.
- Larimore, R. W. (1961). Fish population and electrofishing success in a warm-water stream. *Journal of Wildlife Management* **25**, 1–12.



- Lyons, J. (1986). Capture efficiency of a beach seine for seven freshwater fishes in a north-temperate lake. *North American Journal of Fisheries Management* **6**, 288–289.
- Lyons, J. (1992). The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. *North American Journal of Fisheries Management* **12**, 198–203.
- Mace, G. M. (1994). Classifying threatened species: means and ends. *Philosophical Proceedings of the Royal Society of London Series B* **344**, 91–97.
- Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., Tyre, A. J. & Possingham, H. P. (2005). Zero tolerance ecology: improving ecological inference by modeling the source of zero observations. *Ecology Letters* **8**, 1235–1246. doi:10.1111/j.1461-0248.2005.00826.x
- McInerney, M. C. & Cross, T. K. (2000). Effects of sampling time, intraspecific density, and environmental variables on electrofishing catch per effort of largemouth bass in Minnesota lakes. *North American Journal of Fisheries Management* **20**, 328–336.
- Metcalf-Smith, J. L., DiMaio, J., Station, S. K. & DeSolla, S. R. (2003). Status of the freshwater mussel communities of the Sydenham River, Ontario, Canada. *American Midland Naturalist* **150**, 37–50.
- Nielsen, J. L. (1998). Scientific sampling effects: electrofishing California's endangered fish populations. *Fisheries* **23**, 6–12.
- Peck, D. V., Herlihy, A. T., Hill, B. H., Hughes, R. M., Kaufmann, P. R., Klemm, D. J., Lazorchak, J. M., McCormick, F. H., Peterson, S. A., Ringold, P. L., Magee, T. & Cappaert, M. R. (2005). Environmental monitoring and assessment program – surface waters western pilot study: field operations manual for Wadeable Streams. *EPA Report EPA 600/R-05/xxx*. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development.
- Pont, D., Huguény, B., Beier, U., Goffaux, D., Melcher, A., Noble, R., Rogers, C., Roset, N. & Schmutz, S. (2006). Assessing river biotic condition at a continental scale: a European approach using functional metrics and fish assemblages. *Journal of Applied Ecology* **43**, 70–80. doi:10.1111/j.1365-2664.2005.01126.x
- Pygott, J. R., O'Hara, K., Cragg-Hine, D. & Newton, C. (1990). A comparison of the sampling efficiency of electric fishing and seine netting in two contrasting canal systems. In *Developments in Electric Fishing* (Cowx, I. G., ed.), pp. 130–139. Oxford: Fishing News Books.
- Rabeni, C. F., Sarver, R. J., Wang, N., Wallace, G. S., Weiland, M. & Peterson, J. T. (1997). *Development of Regionally Based Biological Criteria for Streams of Missouri*. Columbia, MO: Missouri Department of Natural Resources.
- Reynolds, J. B. (1996). Electrofishing. In *Fisheries Techniques* (Murphy, B. R. & Willis, D. W., eds), pp. 221–253. Bethesda, MD: American Fisheries Society.
- Rice, J. C. (2005). Understanding fish habitat ecology to achieve conservation. *Journal of Fish Biology* **67** (Suppl. B), 1–22. doi:10.1111/j.1095-8649.2005.00933.x
- Ruppert, J. B. & Muth, R. T. (1997). Effects of electrofishing fields on captive juveniles of two endangered cyprinids. *North American Journal of Fisheries Management* **17**, 314–320.
- Sharber, N. G. & Carothers, S. W. (1988). Influence of electrofishing pulse shape on spinal injuries in adult rainbow trout. *North American Journal of Fisheries Management* **8**, 117–122.
- Simonson, T. D. & Lyons, J. (1995). Comparisons of catch per effort and removal procedures for sampling stream fish assemblages. *North American Journal of Fisheries Management* **15**, 419–427.
- Snyder, D. E. (2003). Electrofishing and its harmful effects on fish. In *Information and Technology Report (ITR) USGS/BRD/ITR-2003-0002*. Denver, CO: U.S. Government Printing Office.
- Stanfield, L., Jones, M., Stoneman, M., Kilgour, B., Parish, J. & Wichert, G. (2001). *Stream Assessment Protocol for Southern Ontario*. Picton: Ontario Ministry of Natural Resources.
- Station, S. K., Dextrase, A. J., Metcalfe-Smith, J. L., DiMaio, J., Nelson, M., Parish, J., Kilgour, B. & Holm, E. (2003). Status and trends of Ontario's Sydenham River

- ecosystem in relation to aquatic species at risk. *Ecological Monitoring and Assessment* **88**, 283–310.
- Vincent, R. (1971). River electrofishing and fish population estimates. *Progressive Fish-Culturist* **33**, 163–169.
- Wang, L., Simonson, T. D. & Lyons, J. (1996). Accuracy and precision of selected stream habitat estimates. *North American Journal of Fisheries Management* **16**, 340–347.
- Weinstein, M. P. & Davis, R. W. (1980). Collection efficiency of seine and rotenone samples from tidal creeks, Cape Fear River, North Carolina. *Estuaries* **3**, 98–105.
- Wiley, M. L. & Tsai, C. F. (1983). The relative efficiencies of electrofishing vs. seines in Piedmont streams of Maryland. *North American Journal of Fisheries Management* **3**, 243–253.
- Yoder, C. O. & Smith, M. A. (1999). Using fish assemblages in a state of biological assessment and criteria program: essential concepts and considerations. In *Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities* (Smith, T. P., ed.), pp. 17–63. Boca Raton, FL: CRC Press.
- Zar, J. H. (1999). *Biostatistical Analysis*. Upper Saddle River, NJ: Prentice Hall.

# Author Query Form

---

**Journal: Journal of Fish Biology**

**Article : jfb\_1349**

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication. Many thanks for your assistance.

---

Query No.	Query	Remark
1	Please note that the year '2005' in the text citation 'FAME Consortium, 2005' has been replaced with 'FAME Consortium, 2004' as per the list. Please check.	
2	Please check whether 'pugnose minnow <i>Opsopoeus emiliae</i> ' has to be changed to 'pugnose minnow <i>Opsopoeodus emiliae</i> ' in the sentence 'These species at risk...'. <i>Opsopoeodus emiliae</i>	

---

# MARKED PROOF

## Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

<i>Instruction to printer</i>	<i>Textual mark</i>	<i>Marginal mark</i>
Leave unchanged	... under matter to remain	Ⓟ
Insert in text the matter indicated in the margin	∧	New matter followed by ∧ or ∧ <sup>Ⓢ</sup>
Delete	/ through single character, rule or underline or ┌───┐ through all characters to be deleted	Ⓞ or Ⓞ <sup>Ⓢ</sup>
Substitute character or substitute part of one or more word(s)	/ through letter or ┌───┐ through characters	new character / or new characters /
Change to italics	— under matter to be changed	↙
Change to capitals	≡ under matter to be changed	≡
Change to small capitals	≡ under matter to be changed	≡
Change to bold type	~ under matter to be changed	~
Change to bold italic	≈ under matter to be changed	≈
Change to lower case	Encircle matter to be changed	≡
Change italic to upright type	(As above)	⊕
Change bold to non-bold type	(As above)	⊖
Insert 'superior' character	/ through character or ∧ where required	Υ or Υ under character e.g. Υ or Υ
Insert 'inferior' character	(As above)	∧ over character e.g. ∧
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	ʹ or ʸ and/or ʹ or ʸ
Insert double quotation marks	(As above)	“ or ” and/or ” or ”
Insert hyphen	(As above)	⊥
Start new paragraph	┌	┌
No new paragraph	┐	┐
Transpose	┌┐	┌┐
Close up	linking ○ characters	○
Insert or substitute space between characters or words	/ through character or ∧ where required	Υ
Reduce space between characters or words		↑