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Effectiveness of Stream Sampling Methods in Capturing Non-native Rusty Crayfish (*Orconectes rusticus*) in Ontario

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Habitat alteration and species introductions have contributed to the decline of native crayfish in Ontario. Although lake populations of crayfish in Ontario are monitored, there is no corresponding program for streams. We used removal-based sampling to evaluate the efficacy of three sampling techniques (backpack electrofishing, hand capture, and seining) to characterize native and non-native crayfish populations in six streams in the Kawartha Lakes region and five streams in the Muskoka/Haliburton Lakes region. All types were effective at detecting non-native Rusty Crayfish (*Orconectes rusticus*). Rusty Crayfish were collected from 65% of samples, constituted 90% of the total catch, and were the only species present in 30% of streams. Compared with other methods, electrofishing was more likely to capture additional crayfish species. Removal-based sampling was not a reliable approach for estimating capture probability and population size. Failure of the removal model was due to increases in the number of crayfish captured after the first pass or too few individuals collected. Mean capture probabilities for electrofishing (0.30) and hand capture (0.31) did not result in reliable population estimates. Compared with seining, electrofishing and hand capture resulted in more sexually active males (fewer sexually inactive males) and more small (< 25 mm carapace length) individuals. For each method, there were differences in capture probability among length classes. A combination of electrofishing and seining (with multiple passes) would maximize species detection, permit sampling of a range of habitat types, and be easily integrated into existing stream fish surveys.

Key Words: invasive species; monitoring; Rusty Crayfish; capture success; streams; *Orconectes rusticus*; Ontario; electrofishing; hand capture; seining; Appalachian Brook Crayfish; *Cambarus bartonii*; Calico Crayfish; *Orconectes immunis*; Allegheny Crayfish; *Orconectes obscurus*; Northern Clearwater Crayfish; *Orconectes propinquus*; Virile Crayfish; *Orconectes virilis*

Introduction

Almost half of the freshwater crayfish species in North America are considered imperilled, making crayfish second only to freshwater mussels in terms of conservation concern (Butler *et al*. 2003; Taylor *et al.* 2007). Worldwide, habitat alteration and non-native crayfish are considered to be the key threats to native crayfish species (Taylor *et al*. 2007). In Ontario, urbanization, draining of wetlands, and acid rain have had a negative effect on crayfish habitat (Guiasu 2007, 2009; Edwards *et al*. 2009). Also contributing to the decline of native crayfish in Ontario is the spread of Smallmouth Bass (*Micropterus dolomieu*) and the non-native Rusty Crayfish (*Orconectes rusticus*) (Edwards *et al.* 2009; Phillips *et al.* 2009).

The Rusty Crayfish has moved or been transported (via bait buckets) into Canadian waters from the northern limits of its natural range in the Ohio River basin of the United States (Rosenburg *et al*. 2010*). In other parts of the Laurentian Great Lakes basin, the major methods of Rusty Crayfish introduction include the release from bait buckets by recreational anglers, the intentional release by aquarium hobbyists, and their introduction by lake users to control nuisance weeds (Olden *et al*. 2011). Where introduced, the Rusty Crayfish has caused dramatic changes to aquatic ecosystems including the replacement of native crayfish, damage to macrophyte beds, and shifts in macroinvertebrate and fish assemblages (Phillips *et al*. 2009).

The Rusty Crayfish was first reported in Canada during the 1960s in Lake of the Woods in northwestern Ontario and a small number of south-central Ontario lakes (Crocker and Barr 1968). It has subsequently been captured in numerous lakes and rivers in other regions of the province (Berrill 1978; Momot 1996; Edwards *et al*. 2009). Although trends in the status of native and non-native crayfish have been monitored across hundreds of south-central Ontario lakes (Edwards *et al.* 2009; Somers and Reid 2010*), a corresponding program has not been developed for Ontario streams and rivers. Given the labour-intensive nature of removing the Rusty Crayfish, a highly fecund species, the likelihood of successful eradication or control is largely dependent on early detection (Hamr 2010; Lieb *et al*. 2011a). However, the absence of a standardized, coordinated monitoring program prevents a defensible assessment of the status of native crayfish and the impact of Rusty Crayfish (and another non-native species, Allegheny Crayfish, *O. obscurus*) in flowing waters and limits opportunities for timely remedial action at newly invaded locations.

Selection of sampling method is an important part of the design of monitoring programs. In this study, we assessed the effectiveness of three methods of stream sampling (backpack electrofishing, hand capture, and seining) in capturing Rusty Crayfish. These methods were selected because of their past use to survey stream crayfish assemblages (Jezerinac 1991; Guiasu *et al*. 1996; Sibley and Nöel 2002; Heath *et al*. 2010), their current use to monitor stream fish in Ontario (Stanfield 2005*; Portt *et al*. 2008*), and their suitability for sampling in habitats where wading is possible. Baited traps are often used to sample crayfish in Ontario (Guiasu *et al*. 1996; Somers and Reid 2010*). However, traps were not evaluated in this study as they require repeat site visits, are vulnerable to vandalism or theft (Bernardo *et al*. 2011), and their deployment may be impractical in shallow or fast-flowing water.

A removal-based sampling strategy (depletion) was used to assess the effectiveness of each method in capturing and characterizing the abundance of native crayfish and Rusty Crayfish and differences in probability of capture related to size and reproductive form. Removal-based strategies have been used successfully to assess the efficiency of techniques for sampling stream-dwelling crayfish (Rabeni *et al*. 1997; Alonzo 2001) and fish (Amiro 1990; Reid *et al*. 2009). Unlike mark-recapture methods, they also do not require multiple sampling visits.

Study Area

The study was undertaken along six streams in the Kawartha Lakes region (44°18'N, 78°19'W) — Fleetwood, Jackson, Jennings, Meade, Riverview, and Thompson creeks — and five streams in the Muskoka/Haliburton Lakes region (45°9'N, 79°4'W) — Dickie Lake outlet and Blairhampton, Cinder, Coca-Cola, and Moot Lake creeks.

Sampling locations — ten sites in the Kawartha region and five in the Muskoka/Haliburton region — were selected using recent Rusty Crayfish collection records (EDDMapSOntario 2014*). They represent a range of water temperatures (mean 16.2°C, range 6°–25°), conductivity (mean 353 µs/cm, range 8–990 µs/cm), channel width (mean 5.3 m, range 1–13.6 m), and streambed materials. Seven of the ten crayfish species reported to occur in Ontario are found in these regions. Of those not found, two (Devil Crayfish, *Cambarus diogenes*, and Digger Crayfish, *Fallicambarus fodiens*) are obligate burrowers (Crocker and Barr 1968); thus, the effectiveness of sampling methods for these species must be tested separately (e.g., Ridge *et al*. 2008).

Methods

Fifteen removal-based samples were acquired for each sampling method. Electrofishing and hand-capture were undertaken along reaches of riffle and shallow-run habitat. For these two methods, unit sampling distance was set at 20 m. Seining was undertaken in deeper-run and pool habitats, where this method is more suitable. A 10-m unit sampling distance was set for seining. This shorter distance was chosen because of the limited size of pool and deeper-run habitats in these streams and the typical extent of habitat sampled with a seine during stream fish inventories (Bonar *et al*. 2009). The mean area of stream sampled was: electrofishing 110 m^2 (range 31–228 m^2); hand capture 79 m^2 (range 22–211 m^2); and seining 65 m^2 (range 24–112 m^2). The dominant material in streambeds at sites where seining was carried out was typically finer (clay and sand) than at electrofishing and hand-capture sites (gravel, cobble, and bedrock). Sampling occurred between 29 July and 19 October 2010 and was completed before Rusty Crayfish initiated burrowing activity associated with winter hibernation (Hamr 2010).

At each unit, the sampling area was isolated with 3.2-mm mesh block nets. Electrofishing was undertaken with a Smith-Root Type 12A backpack electrofisher (pulsed DC settings: 300–400 V, 50–60 Hz, 4–6 ms; Smith-Root Inc., Vancouver, B.C.); one or two people, depending on channel width, used nets to pick up crayfish; and sampling rate was 10 s/m^2. Hand capture involved two or three people moving upstream, overturning rocks, and collecting crayfish by hand or using an aquarium net (Hamr 2007*). Seining was carried out by two or three people pulling a bag seine (3.2-mm mesh bag with 4.8-mm mesh wings) upstream. A minimum of three passes were made for each sampling unit. At each unit, effort was standardized among sampling passes. Additional sampling passes were made if a decline in crayfish catch (i.e., depletion) was not observed. Overall, the mean number of passes for each sampling method was similar (electrofishing 4.0; hand capture 4.1; and seine 4.3).

Crayfish captured at each pass were held in separate bins until processed. Individual crayfish were identified to species (Crocker and Barr 1968) and reproductive form: female, male form I (sexually active), and male form II (sexually inactive). For all crayfish, carapace length (CL) was measured to the nearest 0.1 mm and injuries (e.g., missing chelae) were noted. Batch weight of each species was measured to the nearest 0.01 g for each pass. Photographs were taken and voucher specimens were preserved in 70% ethanol for later confirmation of field identification. Voucher specimens were not retained after species identification was confirmed.

Data analysis

Population size (number of individuals) and capture probability were estimated for all crayfish species captured as well as for Rusty Crayfish using multiple-pass data and the maximum weighted likelihood method (Carle and Strub 1978). Catches of individual native species were low and inconsistent and, therefore, not suitable for analysis. The efficiency of sampling equipment is affected by crayfish size and sex (Alonzo 2001; Ogle and Kret 2008); therefore, estimates of capture probability were derived for each reproductive form of Rusty Crayfish and for four classes of CL (≤ 10 mm, 11–20 mm, 21–30 mm, and > 30 mm). Although small mature individuals have been reported, Rusty Crayfish with CL less than 20 mm are generally considered immature (Hamr and Berrill 1985; Hamr 2010). Multiple-pass data were analyzed with Removal Sampling (version 2) software (Seaby and Henderson 2007*). The constant probability of capture assumption was tested using a χ^2-based statistic (Seber 1982). Using the DeLury method (Ricker 1958*), capture probability was also estimated from the total biomass of all species of crayfish collected and the biomass of Rusty Crayfish.

Sampling events were assessed based on whether a decline in catch was observed with successive passes (i.e., depletion); capture probability; and e%, a measure of the precision of population estimates (calculated as 95% confidence interval*100/*N*) (Penczak and Romero 1990). Penczak and Romero (1990) proposed the following four-point scale to assess e%: 1, very good estimates (< 10%); 2, good estimates (11–25%); 3, adequate estimates (26–50%); and 4, poor estimates (> 51%).

As seining was used in a different habitat type, statistical comparisons among methods were limited to electrofishing and hand capture. Between-method differences in frequency of depletion were tested using Fisher's exact test (Sokal and Rohlf 1995). Differences in capture probability and the representation of each sex (proportion of Rusty Crayfish males in each sample; Alonzo 2001) were tested with the unpaired *t*-test. Percentage data were arc-transformed before analysis. Graphic inspection of length frequency distributions and the Kolmogorov-Smirnov (K-S) test (based on separate pooled datasets for all crayfish species and for Rusty Crayfish) were used to assess differences in the size of crayfish captured (Zar 1984; Dorn *et al*. 2005).

Results

Six species of crayfish were collected from stream sites: Appalachian Brook Crayfish, *Cambarus bartonii* (*n* = 131); Calico Crayfish, *O. immunis* (*n* = 2); Allegheny Crayfish (*n* = 1); Northern Clearwater Crayfish, *O. propinquus* (*n* = 32); Rusty Crayfish (*n* = 2082); and Virile Crayfish, *O. virilis* (*n* = 1). The Rusty Crayfish was collected from 65% of sampling units, comprised 90% of all crayfish captured and 85% of the crayfish biomass, and was the only species collected from four of the 11 streams sampled (Table 1). It was found in all Kawartha Lakes region streams except for Fleetwood Creek, where only a single Allegheny Crayfish was captured. Despite its presence in nearby lakes, the Rusty Crayfish was not collected from any Muskoka/Haliburton Lakes region streams. Only native crayfish species (Appalachian Brook Crayfish, Northern Clearwater Crayfish, and Virile Crayfish) were collected from these streams.

For all sampling methods, the Rusty Crayfish was typically collected during the first sampling pass (> 88% of sites where present). Overall, electrofishing resulted in the capture of all six species, whereas Calico Crayfish and Allegheny Crayfish were absent from both hand-capture and seining samples. The Appalachian Brook Crayfish was not collected during seining, and capture of the Northern Clearwater Crayfish and the Virile Crayfish required multiple hauls.

Seining was the least time-intensive method, requiring on average 1.2 min/m^2 for sampling and processing. Hand capture was the most time intensive at 3.9 min/m^2, while electrofishing required 2.6 min/m^2.

Population size

Electrofishing always resulted in crayfish capture. In contrast, no crayfish were collected at three hand-capture sites and five seining sites. For 53% of samples, we were able to estimate total crayfish population (all species) and capture probability. In most other cas-

TABLE 1. Comparison of catch data for Rusty Crayfish, *Orconectes rusticus*, using three sampling methods in 11 Ontario creeks.

Variable	Electrofishing	Hand capture	Seining
No. sites yielding Rusty Crayfish	10	9	7
Total number captured	918	1091	73
Females (%)	458 (49.9)	548 (50.2)	35 (48.0)
Form I males* (%)	174 (19.0)	158 (14.5)	22 (30.1)
Form II males* (%)	258 (28.1)	380 (34.8)	16 (21.9)
Total biomass (g)	2739	2555	332
Mean carapace length (mm [SE])			
Females	19.6 (0.30)	17.6 (0.24)	24.7 (1.16)
Form I males	23.0 (0.37)	24.3 (0.32)	25.8 (1.05)
Form II males	17.3 (0.27)	16.0 (0.19)	16.6 (1.15)

*Form I male = sexually active, form II male = sexually inactive.

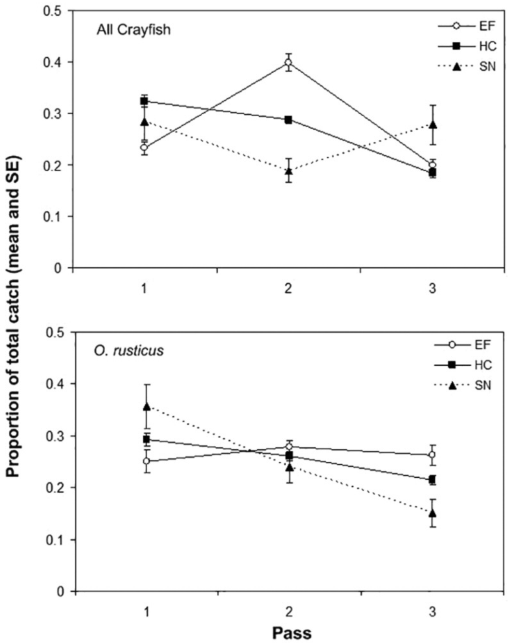

FIGURE 1. Proportion of total catch of crayfish of all species (above) and Rusty Crayfish, *Orconectes rusticus* (below) captured during the first three passes using three removal-based sampling methods in 11 Ontario creeks: electrofishing (EF), hand capture (HC), and seining (SN). Symbols indicate mean values and vertical lines represent standard errors of the mean.

es, either too few individuals were captured (42% of failed estimates), or there was no decline in catch with successive passes (46% of failed estimates) (Figure 1). There was no significant difference in the percentage of samples in which depletion occurred with successive passes between electrofishing (60%) and hand capture (66%) (exact test, P = 1.0). Depletion occurred less frequently (33%) in seined samples.

Estimates of total crayfish population were highest for hand-captured samples and lowest for seining (Table 2). In contrast, mean capture probability was highest for seining (Table 2). There were no significant differences in capture probability between electrofishing and hand capture (t = 0.23, P = 0.82). All estimates resulted in capture probability > 0.2, and, therefore, are considered valid (White *et al.* 1982*). However, capture probabilities for electrofishing and hand capture were below the level considered to provide consistently reliable results (≥ 0.4, Seber 1982). Based on e%, most electrofishing (83%) and hand-capture (86%) estimates were considered at least adequate; 60% of seining estimates were poor.

For 63% of sampling events at Rusty Crayfish sites, we were able to estimate population size and probability of capture. There was no significant difference in the frequency of depletion between electrofishing (60%) and hand-capture samples (70%) (P = 0.63), and the frequency of depletion (60%) was similar at seining sites. Differences among methods and estimates of population size and capture probability were similar for Rusty Crayfish and all crayfish species (Table 2). There were no significant differences in capture probability between electrofishing and hand capture (t = −0.57, P = 0.58). Based on e%, most estimates based on electrofishing (78%), hand capture (80%), and seining (60%) were considered at least adequate.

Biomass estimates were less suitable for comparing sampling methods than abundance data. For both Rusty Crayfish and all species, only 30% of sampling events permitted estimates of population size and capture probability based on biomass. However, capture probabilities for electrofishing and hand capture were similar to those derived from abundance estimates (Table 2).

Reproductive form

For Rusty Crayfish in all samples, the sex ratio was close to 1:1. Males captured by electrofishing and hand capture were more often form II, whereas seining resulted in the capture of more form I males (Table 1). There was little difference in the overall proportion of females captured by the three methods. No significant difference was detected in the proportion of males collected by electrofishing and hand capture (t = 0.06, P = 0.95).

For 40% of samples, we were able to estimate capture probability by reproductive form (Table 3). For

TABLE 2. Estimates of population size, in terms of abundance and biomass, and capture probabilities for all crayfish species and for Rusty Crayfish, *Orconectes rusticus*, based on three sampling methods used in 11 Ontario creeks: electrofishing (EF), hand capture (HC), and seining (SN).

	Population estimate (mean [SE])			Capture probability (mean [SE])		
	EF	HC	SN	EF	HC	SN
Abundance (no./m^2)						
All crayfish species	1.04 (0.31)	2.48 (1.12)	0.11 (0.05)	0.30 (0.04)	0.31 (0.04)	0.56 (0.13)
Rusty Crayfish	1.30 (0.41)	4.20 (1.80)	0.11 (0.04)	0.29 (0.03)	0.26 (0.04)	0.47 (0.05)
Biomass (g/m^2)						
All crayfish species	5.08 (1.65)	5.16 (2.02)	0.65 (0.48)	0.34 (0.08)	0.33 (0.07)	0.74 (0.26)
Rusty Crayfish	4.54 (1.51)	3.92 (2.03)	0.17 (n/a)*	0.33 (0.05)	0.40 (0.06)	1.0 (n/a)*

*Standard error of the mean (SE) not applicable as only one sample obtained.

TABLE 3. Probability of capture by reproductive form and carapace length of Rusty Crayfish, *Orconectes rusticus*, associated with three stream sampling methods in 11 Ontario creeks: electrofishing, hand capture, and seining.

	Capture probability (mean [range])		
	Electrofishing	Hand capture	Seining
Reproductive form			
Form I male*	0.34 (0.26–0.54)	0.36 (0.33–0.39)	0.26†
Form II male*	0.46 (0.25–0.66)	0.36 (0.10–0.38)	0.20 (0.02–0.39)
Female	0.16 (0.06–0.28)	0.43 (0.14–0.57)	0.34 (0.32–0.35)
Carapace length			
< 10.5 mm	0.42 (0.38–0.45)	0.53†	n/a
10.5–20.4 mm	0.10 (0.01–0.19)	0.45 (0.19–0.62)	0.02†
20.5–30 mm	0.45 (0.27–0.62)	0.32 (0.09–0.44)	0.42 (0.29–0.54)
> 30 mm	0.33 (0.10–0.49)	0.70 (0.68–0.71)	0.62†

*Form I male = sexually active, form II male = sexually inactive.
†Single sample.

electrofishing and hand capture, a decline in catch was most frequently associated with form I males (63% and 50% of cases). Alternatively, seining declines occurred least often for form I males (17% of cases). Although based on a small number of samples, capture probabilities for male Rusty Crayfish were lower during seining than the other two methods, and for females lower during electrofishing (Table 3). There were no obvious differences among reproductive forms in capture probability by hand capture or seine. For electrofishing, capture probabilities tended to be greater for males (Table 3).

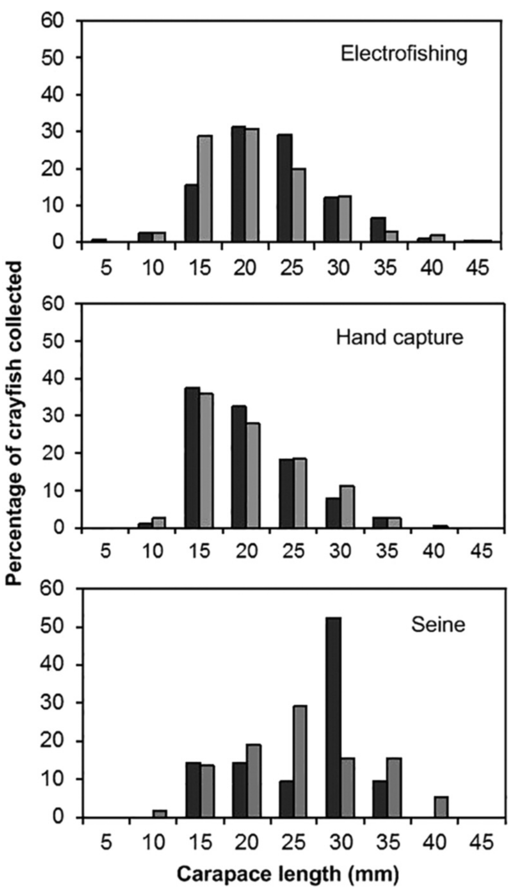

FIGURE 2. Distribution by carapace length of all crayfish species (black bars) and Rusty Crayfish, *Orconectes rusticus* (grey bars) captured by electrofishing, hand capture, and seining in 11 Ontario creeks.

Carapace length

A broad range of sizes of all crayfish species (CL range 5–43 mm) was collected during stream sampling (Figure 2). Electrofishing and hand capture resulted in a similar range of sizes, which included a greater proportion of small individuals (< 25 mm) than seining. However, CL distributions for samples collected by electrofishing and hand capture were significantly different (all species: $D = 0.15$; $P < 0.001$; Rusty Crayfish: $D = 0.13$; $P < 0.001$). Overall, hand-capture samples included a greater percentage of small individuals (CL 15–25 mm) than electrofishing.

For a third of samples, we were able to estimate capture probability for individual length classes. For hand capture and seining, a decline in catch was most frequently associated with larger Rusty Crayfish (CL 20–30 mm and > 30 mm). For electrofishing, declines occurred least often for the largest crayfish (CL > 30 mm), in only 18% of cases. Although based on a small number of samples, capture probabilities for most Rusty Crayfish length classes during hand capture were higher than other methods (Table 3). For electrofishing, capture probabilities were greatest for the smallest (< 10.5 mm) and the 20.5-30 mm length classes of Rusty Crayfish (Table 3). For the other two methods, differences in capture probability among length classes were also apparent. However, small sample sizes prevent meaningful comparisons.

Sampling injury

Cheliped loss was recorded for 8.8% of all crayfish collected by electrofishing, 6.3% by seine, and 5.4% by hand capture. Observed mortality was 1.4% for electrofishing and 2.2% for hand capture. No dead crayfish of any species was observed during seining. Most dead crayfish were found either crushed or with a cracked carapace.

Discussion

All three sampling methods assessed in this study were effective at detecting Rusty Crayfish. Although multiple-pass sampling may improve detection of native species, it was not a reliable approach for estimating capture probabilities or population sizes of Rusty Crayfish and other crayfish species. In most cases, failure of the removal model was observed as an increase in the number of crayfish captured after the first pass or too few individuals collected. The first sampling pass may disturb or draw out crayfish from areas of cover making them more vulnerable to capture in subsequent passes (Gladman *et al*. 2010). Removal sampling by both electrofishing and hand capture has been suitable for generating population estimates of other stream-dwelling crayfish (Rabeni *et al.* 1997; Alonzo 2001); however, in those studies, sampling was carried out in much smaller streams and channel width can have a strong influence on efficiency of a method (Zalewski and Cowx 1990). To improve catch efficiency, Alonzo (2001) also used low voltage and activated the electrode for only 1–2 seconds at a time. In our study, we used voltage output and sampling strategies typical of stream fish inventories in Ontario. Although affected by electrical current, crayfish do not display the same degree of galvanotaxis as many stream fish. Experimentation with different settings could improve our capture efficiency.

Electrofishing and visual sampling methods are often biased toward capture of large individuals and against cryptic taxa or life stages (Zalewski and Cowx 1990). Activity levels and use of cover and deeper habitats vary among crayfish species, sizes, and reproductive forms (Berrill and Arsenault 1982; Guiasu 1997; Davis and Huber 2007) and, therefore, can also be expected to affect capture probabilities. For electrofishing, Alonzo (2001) reported a difference in the probability of capturing small and large crayfish in small streams, but none between sexes. In our study, the low number of removal estimates prevented robust comparisons of capture probability among reproductive forms and length classes. However, capture probabilities were generally higher for males during electrofishing and for the smallest and largest crayfish during hand capture. All three methods collected crayfish of different sizes, with seining providing a greater representation of large individuals. The large size of Rusty Crayfish associated with seine hauls from pools is consistent with Davis and Huber (2007) who observed that large Rusty Crayfish prefer deeper water than smaller individuals.

Although changes in the size of native crayfish populations after Rusty Crayfish introduction into lakes have been thoroughly documented, research on such declines in streams and rivers has been less intensive (Jezerinac 1982, 1991; Daniels 1998). Recent stream surveys in the mid-west and eastern regions of the United States indicate the continuing spread of Rusty Crayfish and concurrent declines in native *Orconectes* species (Kuhlmann and Hazelton 2007; Kilian *et al*. 2010; Lieb *et al*. 2011b; Olden *et al*. 2011). Based on a broad-scale survey of the Kawartha Lakes region, Berrill (1978) found the Rusty Crayfish to be widespread and common and indicated that it was likely replacing Northern Clearwater Crayfish. At our Kawartha Lakes stream sites, the Rusty Crayfish was the dominant (and often only) crayfish species present, indicating that the shift in species composition in this region has persisted for several decades. Jezerinac (1982) reported that location within the watershed influenced the effect of the Rusty Crayfish, with Northern Clearwater Crayfish abundant in headwaters and small tributaries (where few Rusty Crayfish were present) and absent or in low numbers in the main stream channel (where Rusty Crayfish were abundant). Although the expanding distribution of the Rusty Crayfish in southern Ontario has begun to be tracked, factors influencing its effect on native crayfishes in flowing waters are not well understood and require greater attention.

As the likelihood of species detection is improved, the use of multiple sampling methods has been recommended for crayfish surveys (Holdich *et al*. 2002). Backpack electrofishing and seining provide the best combination of methods to detect the Rusty Crayfish, native species, and a broad range of sizes of crayfish in Ontario streams. Although electrofishing was most effective at detecting both native and non-native species, seining is more suitable for sites that are turbid, deep, or have substrates too soft for effective electrofishing. Hand capture (or hand searching) does not require expensive equipment and is associated with fewer safety concerns than electrofishing. However, it was the most labour-intensive method, resulted in the highest rate of mortality, and is less readily adaptable to current stream fish monitoring programs. Gladman *et al*. (2010) and United States National Park Service (2007*) both reported hand capture to be less efficient than electrofishing for sampling stream crayfish.

As applied in this study, multiple-pass sampling was not a reliable strategy to estimate crayfish population size. However, multiple passes are still preferable to single-pass sampling as this improves native species detection (Gladman *et al.* 2010), and a large percentage of individuals will be vulnerable to capture only after being disturbed by initial sampling efforts. If estimates of population size are required for stream reaches, the likelihood of failure could be reduced by pooling data from randomly distributed sampling sites and applying unbiased removal-type estimators (Heimbuch *et al*. 1997). In this study, we did not estimate detection probabilities for native crayfish. The design of stream monitoring efforts would benefit from additional studies that apply repeat survey designs (MacKenzie *et al*. 2002) to estimate method-specific detection probabilities.

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SUPPLEMENTARY MATERIAL:

TABLE S1. Crayfish catch data from each south-central Ontario stream sampling unit. For each method, electrofishing (EF), hand capture (HC), and seining (SN), the number of sampling passes is provided in parentheses. Note: Cb = *C. bartonii* (Appalachian Brook Crayfish), Oi = *O. immunis* (Calico Crayfish), Ob = *O. obscurus* (Allegheny Crayfish), Op = *O. propinquus* (Northern Clearwater Crayfish), Or = *O. rusticus* (Rusty Crayfish), and Ov = *O. virilis* (Virile Crayfish).

TABLE S2. Summary of catch data for Appalachian Brook Crayfish (*Cambarus bartonii*) and Northern Clearwater Crayfish (*Orconectes propinquus*) captured from Ontario streams using three methods: electrofishing, hand capture, and seining.