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Evaluation of Four Larval Fish Sampling Methods in a Large Midwestern River

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ABSTRACT Understanding limitations of larval fish capture gears are critical for developing appropriate sampling protocols and interpreting catch data. We evaluated genera richness, genera diversity, assemblage similarities, abundance indices (i.e., density or catch per unit effort [CPUE]), and sample size requirements between a surface slednet and glow-stick light traps used in 2014 and 2015 and a benthic slednet and light-emitting diode light traps used in 2015 in the Minnesota River. The surface slednet captured the greatest number of larval fish genera (15) while the light-emitting diode light trap captured the fewest (1). Similarities of assemblages sampled was highest between surface and benthic slednets (58%) and lowest between the benthic slednet and LED light trap (0%). All evaluated gears had low and variable catch rates; the highest variability was observed for the LED light trap (CV = 800), and the lowest variability was observed for surface slednets (CV = 173). Slednets required less effort to detect a 25% change in total larval fish abundance compared to light traps. Low CPUEs or densities were possibly the result of suspended sediment loads (85.3 ± 8.5 Nephelometric Turbidity Units) that blocked light trap entrance slots and clogged net pores. Further, not targeting habitats critical to adult spawning and larval rearing (e.g., log jams or shallower or inside bends of meanders) may have influenced CPUEs and densities. We recommend modifications to evaluated sampling gears (e.g., nets with larger mesh sizes) or the evaluation of additional larval fish sampling methods (e.g., larval seines or pumps) coupled with a stratified random sampling protocol that incorporates complex habitats for sampling larval fish within the main channel of the Minnesota River or other river systems with similar high turbidity levels.

KEY WORDS larval fish relative abundance, larval fish densities, large rivers, sampling gear comparisons, standardized sampling

Assessing larval fish presence and abundance can help inform stocking decisions, index species restoration success, and identify environmental factors that regulate fish community dynamics (Avery 1996, Nemeth 2005, Kelso et al. 2012, Pulg et al. 2013). Interpretations of population dynamics and community structure of larval fishes vary depending on the habitat sampled and timing of sampling (Kelso et al. 2012) because of differential efficacy among fish species and habitats (Bonar et al. 2009). Thus, a variety of larval fish sampling methods have been used for collecting fish larvae (Kelso et al. 2012).

Riverine larval fish have typically been sampled with passive gears and active gears. The most commonly used passive gear for larval fish are light traps (Naus and Adams 2016) that attract and entrap positively phototaxic species (Kelso et al. 2012). Phototaxic responses vary among species (Mueller and Neuhauss 2010) and by individuals of the same species (Bulkowski and Meade 1983). Phototaxic larvae may be unable to visually detect light sources due to water clarity or unable to reach the light trap due to current velocity (Marchetti et al. 2004, Lindquist and Shaw 2005). Active larval fish sampling gears have included electrofishers (King and Crook 2002), centrifugal pumps (Gale and Mohr 1978), and a variety of seines (e.g., beach, purse, small mesh; Kelso et al. 2012). Most contemporary large-river sampling protocols use an actively towed 500–1,000-µm mesh ichthyoplankton net that is pushed or pulled through the water (e.g., Nannini et al. 2012, Cheshire et al. 2015, Mapes et al. 2015). Ichthyoplankton nets capture larval fish by filtering known volumes of water at specified depths within the water column (Kelso et al. 2012). Like passive gears, active gears also demonstrate bias depending on deployment

times and depths (Bosley et al. 1986), larval gear avoidance (Gartz et al. 1999), and habitat accessibility (Hayes et al. 2012). In flowing waters, ichthyoplankton nets can also be held stationary allowing them to passively fish (e.g., Killgore and Baker 1996). Due to known gear limitations, studies assessing larval fish assemblages often use multiple gear types (Niles and Hartman 2007, Pritt et al. 2015). However, deployment of multiple gears may not always be feasible due to needs for broad geographic sampling within time and fiscal restraints (Bonar et al. 2009).

Riverine fisheries managers often establish main channel monitoring protocols to reliably track trends in larval fish abundance and species richness (Pritt et al. 2015), identify larval fish responses to changing conditions (e.g., climate change and invasive species establishment; Mapes et al. 2015), and verify reproduction for fishes of management interest (Braaten et al. 2008, Harvey et al. 2002). In the Minnesota River, Minnesota, USA, a goal of resource managers is to develop a sampling protocol that monitors trends in larval fish abundance and richness and reproductive activity for native species of concern such as lake sturgeon (*Acipenser fulvescens*), paddlefish (*Polyodon spathula*), and shovelnose sturgeon (*Scaphirhynchus platorynchus*), as well as for invasive species such as bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*H. molitrix*; Minnesota Department of Natural Resources 2013).

Magnan (1991) suggested that larval fish monitoring protocols should be based on efficiency, effectiveness, effort requirements, and time of year that will best meet management objectives. Such information is limited for the Minnesota River. Nickel (2014) provided initial information on larval fish sampling on the Minnesota River with a 1-year study using surface slednets and glow-stick light traps. Given temporal (1 year) and gear limitations (single light intensity for light traps and surface sampling with the slednet) of the Nickel (2014) study, annual variations in larval fish assemblages and gear effectiveness could not be sufficiently addressed. Our goal was to build upon the Nickel (2014) investigation by sampling similar time periods and locations but including gear modifications: adding slednet sampling at a different water depth and light trap sampling with increased light intensity using light-emitting diodes (LEDs). The objectives of the study were to 1) compare larval fish assemblages collected with slednets (benthic and surface) and light traps (glow-stick and LED); 2) quantify larval fish densities or relative abundance among gears; and 3) compare precision estimates and sample size estimates among the four gears. The collective results from these three objectives were then used to make recommendations for sampling larval fishes in the Minnesota River.

STUDY AREA

The Minnesota River originates at Big Stone Lake along the Minnesota-South Dakota border as a sixth-order river (Strahler 1957) and flows 530 km to its confluence with the Mississippi River near St. Paul, Minnesota (Musser et al. 2009). The Minnesota River is generally characterized as low gradient, productive, and turbid, with an annual hydrological regime driven by spring snowmelt and rainfall (Waters 1977). Approximately 79% of pre-settlement grasslands within the Minnesota River watershed have been converted to row-crop agriculture. Landscape conversion has reduced the time water spends on the landscape, increasing erosion potential (Thoma et al. 2005) and creating more intense hydrographs (Nelson 2015) that amplify sediment movement within the Minnesota River (Johnson et al. 2009).

Two reaches near the towns of Savage (river kilometer [RKM] 24–26) and Franklin (RKM 298–300), Minnesota, were sampled in 2014 and two reaches near Henderson (RKM 105–107) and New Ulm (RKM 234–236), Minnesota, were sampled in 2015 (Fig. 1). Reaches were selected to reflect habitat diversity present within the Minnesota River. Study reach channel widths varied from 55 to 140 m (mean [$\bar{X}$] ± 1 standard error [SE] = 70.3 ± 3.3). Median annual discharges from 2005 to 2015 recorded at United States Geological Survey gauging stations near study reaches at RKMs 64 and 313 were 98 and 40 m^3/s, respectively (Minnesota Pollution Control Agency 2016). Mean turbidity (± SE) during the same time frame (2005–2015) at the same gauging stations were 202.0 ± 43.1 and 55.5 ± 3.5 Nephelometric Turbidity Units (NTUs), respectively (Minnesota Pollution Control Agency 2016).

METHODS

Larval fishes were sampled approximately every other week from 15 May to 15 August 2014 at the Franklin and Savage study reaches (5 surveys at each reach) and from 23 April to 15 August 2015 at the Henderson and New Ulm study reaches (7 surveys at each reach). Within each study reach, 10 transects were established at 200-m intervals. Each transect was initiated on the left bank and extended at a 30–40° upstream angle to encompass all habitats across the river where gears could be deployed (>0.75 m in water depth). During 2014, one glow-stick light trap sample and a surface slednet sample was collected at each transect during each survey. A benthic slednet and a LED-light-source light trap were added as additional gears in 2015. We randomly selected the light source for each light trap so half of the transects (n = 5) during each survey were equipped with a glow-stick and half (n = 5) were equipped with a LED. The net method used at each transect was also randomly selected in 2015 so that half of the transects during each survey were sampled with the surface slednet (n = 5) and half with the benthic slednet (n = 5).

Glow-stick light traps and LED light traps were the same quatrefoil design used by Nickel (2014) and included 2-mm

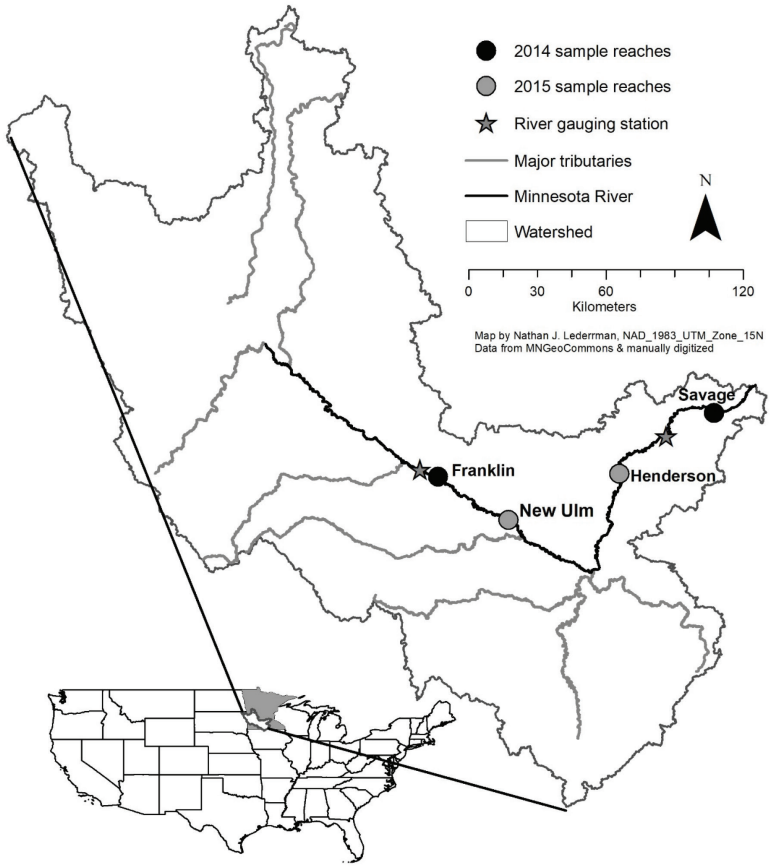

Figure 1. Study reaches where larval fish sampling gears were evaluated in the Minnesota River, Minnesota, USA, during 2014 and 2015. Included are U.S. Geological Survey river gauging stations (stars) near Morton and Jordan, Minnesota, USA.

slot openings and a light source suspended inside the trap from an eyebolt on the top plate (Fig. 2a). Glow-sticks (16 × 2 cm, FlashingBlinkyLights, California, USA) produced mean light intensity of 0.2 ± 0.1 lux directly outside of the light trap. Battery powered LEDs (two green LED lamps in a 12 × 4-cm polycarbonate resin body, KryptoLume, Utah, USA) increased light intensity to 1.2 ± 0.1 lux (379% increase) directly outside of the light trap compared to the glow-stick light source. Light traps were suspended directly beneath the water surface with a buoy tethered to a 9.1-kg cement block with 6 m of rope. Light traps were placed about 2 m from the bank in water deep enough to allow the light trap to be completely submerged but not deeper than the tether (i.e., 0.75–6.0 m deep). We set light traps between 0830 and 1200 and retrieved them 24 hr later.

The surface slednet was designed by Nickel (2014) and used a 500-µm mesh driftnet with a polyvinyl chloride pipe frame (Fig. 2b). Surface slednets were towed upstream along the sample transect parallel to the side of the boat at ground speeds ~1.6 km/h for about 5 min. In 2015, we altered the surface slednet to be able to sample near the benthic surface by attaching three detachable 13.5-kg sounding weights (Hoskin Scientific Ltd., British Columbia, Canada) that sank the sled to the river bed (Fig. 2b). We anchored the boat where the thalweg intersected the sample transect, lowered the weighted surface slednet to the river bottom, and allowed the slednet to soak for 5 min before lifting the net. A mechanical flow meter (General Oceanics, Miami, Florida, USA) suspended in the mouth of the net was used to estimate volume of water filtered for both surface and benthic samples. We conducted all slednet samples between 0830 and 1300.

All larval fish samples taken from all gears were immediately fixed in a 10% buffered formalin solution. After 48 hr, each sample was filtered through a 53-µm sieve (Newark Wire Cloth Company, New Jersey, USA), and contents were preserved in 90% ethanol. Larval fish were sorted from sample contents, identified to genera under a dissecting microscope (Olympus, Massachusetts, USA) using larval fish keys by Auer (1982), Kay et al. (1994), and Simon and Wallus (2005), and counted.

Genera richness, diversity, and community assemblage similarities were calculated and compared among larval fish gears. A Shannon-Weiner diversity index was used to index diversity of the larval fish assemblage captured within each gear type and was calculated using the "vegan" package

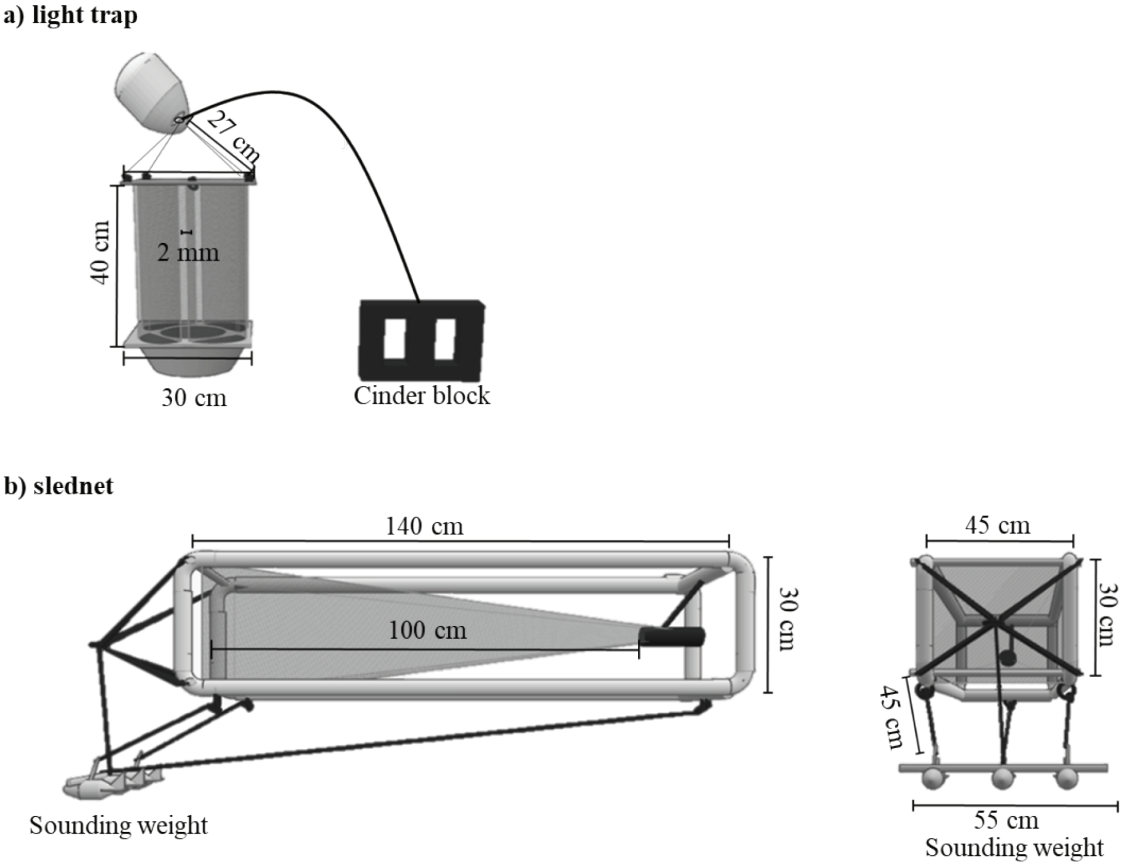

Figure 2. Schematic of the light trap (a) and slednet (b) used for sampling larval fish in the Minnesota River, Minnesota, USA, during 2014 and 2015. Sounding weights were attached to the surface net to adapt it to sampling at the benthic-water interface (i.e., benthic slednet).

(Oksanen et al. 2019) using the following equation:

$$H' = \sum -P_i * \log_e P_i,$$

where P_i is the proportion of species in a sample (Kwak and Peterson 2007). Values of H' closer to zero indicate a less-diverse fish assemblage and values further away from zero indicates a more-diverse fish assemblage. Schoener's percentage overlap index was calculated to quantify similarities in assemblages among each pairwise gear comparison (PSI; Schoener 1970). Schoener's percentage overlap index was calculated using the "spaa" package (Zhang 2016) using the following equation:

$$P_{jk} = \sum \text{minimum}\,(P_{ki}, P_{ji},),$$

where P_{ki} and P_{ji} are the count of species I in assemblage j and k, and minimum indicates the smallest of the two counts and is used in the summation (Kwak and Peterson 2007).

The total number of larval fish was used to calculate density (larvae/100 m^3 of water) for each slednet sample and total catch per unit effort (CPUE; larvae/trap night) for each light trap. Coefficient of variation (CV) was used as a measure of precision for larval fish density and CPUE for each gear type. A lower CV indicated higher precision (e.g., less variation) among samples (Zar 1996).

Sample size required to detect a 25% change in mean larval fish density or relative abundance was estimated for each gear with the power analysis formula described by Snedecor and Cochran (1989):

$$n = 2\left(z_\alpha + z_\beta\right)^2 (s^2/d^2)$$

where n is estimated sampling effort, z_α is the z-distribution deviate for the probability of a Type I error at a given level of significance, z_β is the z-distribution deviate for the probability of a Type II error at a given level of statistical power, *s* is the standard deviation of the abundance estimate (i.e., larvae per 100 m^3 of water for nets or larvae per trap for light traps) and d is the specified effect size. We used an alpha of 0.10, a beta of 0.20, and a power (1-" β") of 0.80 similar to Dembkowski et al. (2012). All analyses were performed with Program R version 3.3.3 (R Development Core Team 2014).

RESULTS

A total of 99 overnight glow-stick light trap samples and 100 surface slednet samples that filtered 22,515 m^3 of water ($\bar{X}$ ± SE = 225 ± 5 m^3/sample) were collected in 2014. In 2015, 64 overnight glow-stick light trap samples, 64 LED light trap samples, 70 surface slednet samples that filtered 19,564 m^3 of water (279 ± 8 m^3/sample), and 65 benthic slednet samples that filtered 8,655 m^3 of water (133 ± 15 m^3/sample) were collected.

A total of 213 larval fish representing 18 genera were captured collectively among all gears, reaches, and years. The LED light trap detected the fewest genera (1) and the surface slednet detected the most genera (15; Tables 1, 2). The surface slednet had the highest diversity index (H') during both years of sampling (1.8 in 2014, 2.1 in 2015; Table 1) and the LED light trap had the lowest (0 in 2015; Table 2). Schoener's PSI was highest between the 2015 surface slednet and 2015 benthic slednet (58%) and lowest between the 2015 benthic slednet and 2015 LED light trap (0%; Table 3).

Samples where zero larval fish were captured were common among all four gears, reducing CPUE or density estimates for each of those gears. The LED light trap had the highest percentage of samples where zero larvae were caught and the surface slednet had the lowest (Tables 1 and 2). Density and relative abundance tended to be higher during July and August compared to April, May, and June (Fig. 3). The highest mean CPUE (0.2 ± 0.1 larvae/trap-night) was observed in the 2014 glow-stick light traps, and the highest observed mean larval density was in the 2015 benthic slednet (0.5 ± 0.1 larvae/100 m3; Table 1). Coefficients of variation of mean CPUE or density estimates were high among all gears, but higher CVs were observed among light trap methods compared to slednet methods (Tables 1, 2). Effort required to detect a 25% change in mean CPUE or density was highest for light traps (>100 samples) compared to slednets (<64 samples; Tables 1, 2).

DISCUSSION

The light trap and slednet represent some of the more commonly used large river larval fish sampling gears (Niles and Hartman 2007, Kelso et al. 2012, Mapes et al. 2015, Pritt et al. 2015). Few studies have compared these gears to each other or have tested modifications of those to increase catches of larval fishes in riverine systems. Despite efforts to improve larval catches, gears evaluated here may still be limiting abilities to draw inferences about larval fish production. Only 40% of the known genera in the Minnesota River were captured across all gears used in this study. Low catches of larval fish were also found among all gears leading to highly skewed data, creating challenges in identifying gears to monitor abundance, and quantifying specific sampling periods for larval fish within the Minnesota River. Despite the restrictiveness of results, we were still able to make recommendations for future larval sampling efforts.

Previous research has shown the ability to capture genera varies widely among larval fish gears. In the Kanawha River, West Virginia, USA, Rider and Margraf (1997) used a modified Isaacs-Kidd ichthyoplankton net (n = 912) and sampled 21 of 40 genera (53%) during the larval stage that Messinger and Chambers (2001) noted as being present. Niles and Hartman (2007) sampled 12 of the Kanawha River genera (30%) using benthic sleds (n = 720), larval activity traps (n = 360), and light traps (n = 360). Passive light traps captured 45% more genera compared to the benthic slednet (Niles and Hartman 2007). Gale and Mohr (1978) captured more species with an active ichthyoplankton net compared to a passive net. In flow-through backwaters of the upper Missouri River, Fisher (1999) captured 18 genera of larval fish, including 15 genera in glow-stick light traps and 13 genera in surface trawls. In our study, the glow-stick light trap and LED light trap captured fewer genera than expected based off the fish community present, but the benthic slednet and surface slednet performed at a level comparable to other studies.

Abundance indices among capture gears also vary markedly from prior research. Holland-Bartels et al. (1995) reported capturing nearly 1,000 times more larvae/100 m in the main channel of the Mississippi River than we did in the Minnesota River with similar surface trawls. Similarly, mean benthic slednet and light trap catch rates from our study were more than 50 times lower than catch rates of similar gears reported by Niles and Hartman (2007) from the main channel of the Kanawha River. However, our results were more similar to those of Nickel (2014; e.g., 0.1 v. 3.2 larval fish/trap night and 0.4 v. 1.0 larval fish/100 m3) than other research. Results indicate that abundance may vary among systems and gear selection should be system specific.

Various larval fish capture gears have been recommended depending on study system characteristics (e.g., fish population, spatial/temporal sampling, habitat). Niles and Hartman (2007) and Neal et al. (2012) recommend the use of light traps to sample larval fish in rivers. Those studies sampled shallow, slower water velocity areas within the river. Increases in discharge may reduce the effectiveness of light traps (Lindquist and Shaw 2005). Systems or sample areas that are deeper and have higher steady flow (i.e., main channel, channel boarders, side channels) may be more conducive to net gears (e.g., benthic slednet or surface slednet) that exploit the limited swimming capabilities of larvae instead of those requiring active swimming to the gear (glow-stick light trap or LED light trap).

Recommended capture gear should also take other system-specific factors, such as sediment levels, into consideration. Deployed light traps slow water velocities, which appeared to cause sediment to accumulate in the entrance slots. This sedimentation potentially prevented larval fish from entering

Table 1. Genera richness, Shannon-Weiner Diversity Index (H'), percentage samples capturing no larvae, mean CPUE (number/trap night ±1 standard error), coefficient of variation of mean CPUE (CV), and the number of samples (*n*) required to detect a 25% change in larval fish abundance for light trap methods from the Minnesota River, Minnesota, USA, during 2014–2015.

Year	Gear	Genera richness	H'	Zero catch (%)	CPUE (#/trap night)	CV	*n*
2014	Glow-stick	6	1.2	89	0.17 (0.09)	525	109
2015	Glow-stick	0	NA	100	0.00 (0.00)	NA	NA
2016	LED	1	0.0	98	0.02 (0.02)	800	NA

Table 2. Genera richness, Shannon-Weiner Diversity Index (H'), percentage samples capturing no larvae, mean density (no./100m³ of water ±1 standard error), coefficient of variation of mean densities (CV), and the number of samples (*n*) required to detect a 25% change in larval fish abundance for slednet methods from the Minnesota River, Minnesota, USA, during 2014–2015.

Year	Gear	Genera richness	H'	Zero catch (%)	CPUE (#/trap night)	CV	*n*
2014	Surface	10	1.8	61	0.33 (0.06)	171	15
2015	Surface	14	2.1	56	0.37 (0.08)	174	21
2015	Benthic	8	1.7	74	0.50 (0.14)	226	63

Table 3. Percentage similarity index (PSI; %; Schoener 1970) of larval fish assemblages between each pairwise comparison of evaluated gear by year. Comparisons to 2015 glow-stick light traps were omitted as no larval fish were captured with that gear during that year.

	2014 Glowstick light trap	2014 Surface slednet	2015 Benthic slednet	2015 LED light trap
2014 Surface slednet	21			
2015 Benthic slednet	36	58		
2015 LED light trap	11	8	0	
2015 Surface slednet	16	52	58	3

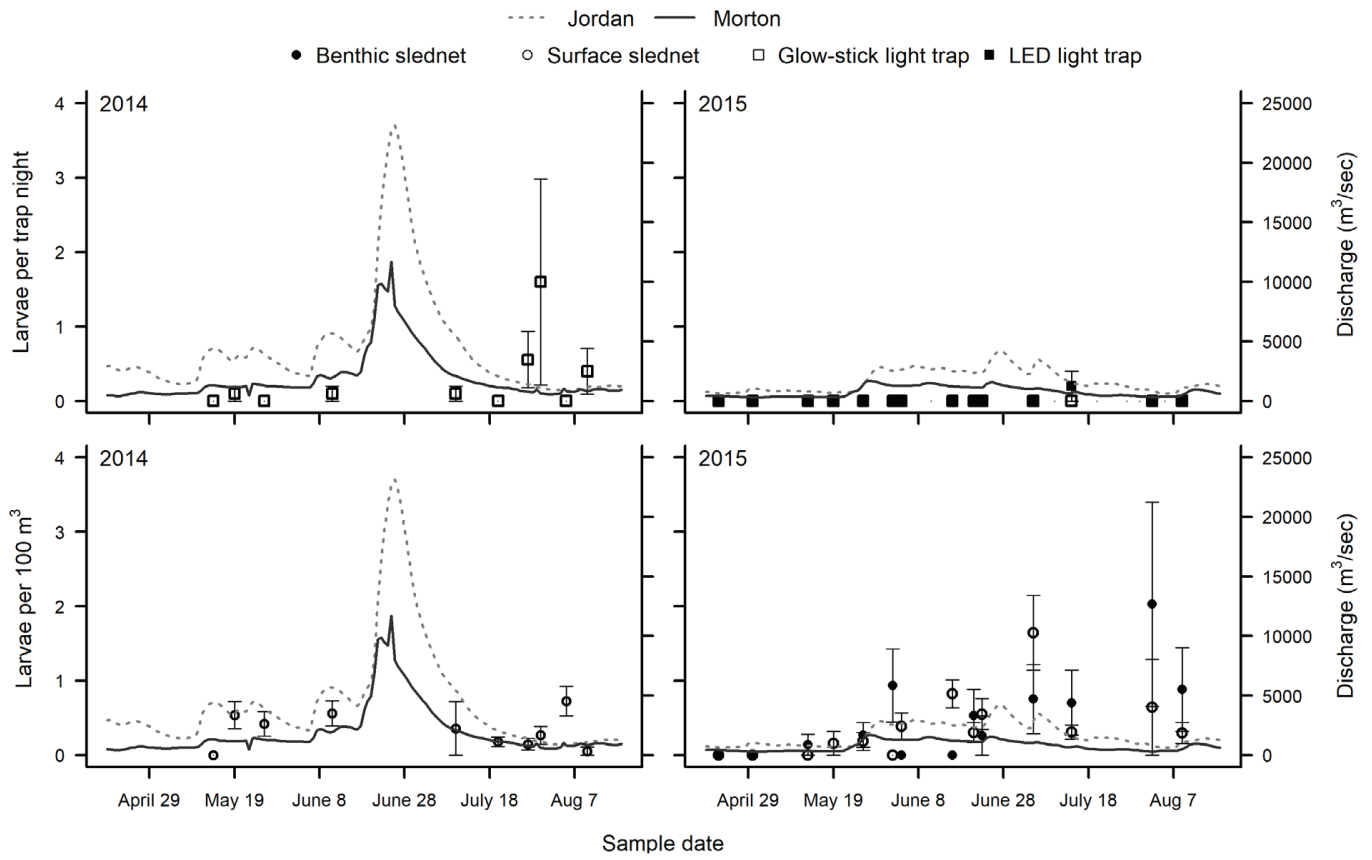

Figure 3. Larval fish abundance indices from the Minnesota River, Minnesota, USA, during 2014 (left panels) and 2015 (right panels) from light traps (top panels; larvae per net night) and slednets (bottom panels; larvae per 100 m³ of water). Bars indicate ±1 standard error. Discharge measures of the Minnesota River taken by the U.S. Geological Survey near Jordan, Minnesota (grey dotted line), and Morton, Minnesota (black line), are overlaid on each plot.

the trap, lowering CPUEs. Suspended sediment may have also clogged net pores, which could reduce filtration rates and increase net avoidance by larval fishes (Isermann et al. 2002), ultimately reducing abundance estimates. Reducing sample or soak time for any of these gears may result in lower catch rates among samples, but these shorter times may allow for more locations within specific time periods to be sampled, potentially increasing precision.

Another consideration to improve catch rates of larval fishes would be to test other sampling gears not included in this study that are less likely to be impacted by sedimentation. For example, larger mesh ichthyoplankton nets (i.e., 1,000-µm or larger) could increase filtration capabilities and filter water for longer time intervals (i.e., >-5 minutes) providing more representative density estimates and reduce the number of zero catches and variability. Durable pumps can intake water and have the ability to limit the effects of suspended sediment on filtering efficiency compared to towed nets (Mohlenberg 1987). Another possible sampling gear would be a larval seine with a greater mesh size that reduces the impacts of sediment and could potentially detect more individuals (Post et al. 1995). An understanding of how additional capture gears function within the Minnesota River and the sample sizes required should be determined prior to including them in a monitoring program.

Location and timing of sampling may have also impacted observed catch rates and genera detected across gears evaluated in our study. Sample transects of this study were placed every 200 m, no matter the habitat present. Complex habitats (e.g., log jams) and shallower, slower water velocity areas (e.g., inside bends of meanders) within the main channel serve as critical adult spawning habitat and larval fish refuge (Slipke et al. 2005) but were not targeted in this study. Incorporating complex habitats using a stratified random sampling protocol may provide a more representative picture of the larval fish assemblage in the main channel. In addition, bi-weekly sampling may have missed important pulses of drifting larval fishes. Spawning periods of many fishes can be as short as a few days (Neal et al. 2012), and pulses of drifting larvae may last only three days (Tan et al. 2010). Increasing sampling frequency (e.g., weekly or continuous) and number of samples collected may improve

catch rates and potentially reduce variability in catches.

MANAGEMENT IMPLICATIONS

Larval fish sampling protocols should be based on the goals of sampling. If the goal is to determine genera richness, we recommend a multiple gear approach that includes the combination of the slednet and light trap. If only a single gear is to be used, slednets had lower variability in abundance, detected a greater number of species, produced higher diversity indices, and required fewer samples to assess larval fish assemblages compared to light trap methods and is recommended. However, because of low and variable catch rates of evaluated gears, monitoring abundance may be difficult with any of these gears and underscores the challenges associated with sampling larval fish in large river systems. Thus, continued evaluation of additional larval fish capture gears, such as larger mesh nets, pumps, or larval seines, in a high-frequency, habitat-stratified sampling design that encompasses complex habitats (e.g., log jams, inside bend of meanders) in a river system should be considered.

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