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Cover Page Footnote

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Species and Life Stages of Odonata Nymphs Sampled with Large Drift Nets in Two Wisconsin rivers

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Abstract

Because relatively few nymphs of Odonata are caught in most drift studies, they have been inconsistently reported and little is known about the species and life stages that are predisposed to drift. We used large drift nets with relatively coarse mesh sizes (1500 μm) to sample late-instar odonate nymphs in two large rivers in Wisconsin. These nets were presumed to have advantages over smaller, conventional aquatic insect drift nets, including the capability to sample greater water volumes more quickly, sampling for longer periods of time before nets become clogged with debris, and a reduced likelihood of large, active insects escaping from the nets. Nymphs of 14 species of Odonata in five families were caught, but drift densities were low (0.042 m^{-3} overall; ≤0.007 m^{-3} for most species) and final instar nymphs (F-0) were collected less frequently than younger nymphs (F-1 through F≤4). Gomphidae comprised 83% of the nymphs collected, and three species of *Ophiogomphus* comprised 78% of the total in the St. Croix River. *Ophiogomphus howei* Bromley was the most commonly sampled species (drift density of 0.026 m^{-3}), with at least five instars collected.

Larval and nymphal forms of many aquatic insects drift downstream with the current in considerable numbers in an ecologically important phenomenon known as drift (Waters 1972, Muller 1974, Allan 1995). A number of categories of drift have been proposed, and some ecological reasons for drift behavior have been identified. Drift can have active or passive modes, and behavioral, diel, seasonal, and taxonomic attributes (reviewed by Brittain and Eikeland 1988). Odonates, however, have generally been thought to have a low predisposition to drift (e.g. Koetsier and Bryan 1995, Corbet 1999 [pp. 15, 394], Ward and Mill 2007) and the list of odonate species known to drift is likely only a small proportion of those that do so. It has been suggested that drift of odonates is largely passive and "accidental", occurring as a result of dislodgement by high flows (Leipelt 2005, Ward and Mill 2007), as opposed to the active drifting shown by some Ephemeroptera, Trichoptera, and Diptera. Benke et al. (1986) suggested that clinging animals such as odonates are more easily dislodged from substrates by the current than those with retreats or those that are attached to the substrate. Odonata nymphs have frequently been overlooked and inconsistently reported in drift studies, probably because the numbers collected have been small (e.g. Elliot 1967, Bishop and Hynes 1969, Cowell and Carew 1976, Winnell and Jude 1991, Brewin and Ormerod 1994, March et al. 2003, Bass 2004), and because part-grown nymphs of some species are difficult to identify.

Little is known about the life stages of Odonata that are predisposed to drift, but recently developed methodology (Tennessen 2016) has now facilitated the determination of the instar number of Anisoptera nymphs. Waters (1972) noted that the greatest drift of many aquatic insect species occurs in the younger life stages; this might be true for Odonata as well. For example, the small size of Zygoptera nymphs reported by Bishop and Hynes (1969) suggested that they were not full grown (< F-0).

The drift nets commonly used to sample aquatic insect drift tend to have net openings ≤0.09 m^{-2}, lengths of ca. 80–140 cm, and relatively fine mesh sizes of 250–500 μm (Anderwald et al. 1991). While nets of this size are appropriate for the great majority of aquatic insect taxa, they could be considered inappropriately small when larger aquatic

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insects such as nymphs of some odonate species are targeted. Sampling large volumes of water has been proposed as a means for providing information about downstream movement patterns of a variety of aquatic organisms, information that is difficult to obtain because of low drift densities (DuBois and Rackouski 1992, DuBois and Stoll 1995). Use of nets larger than those typically used to sample insect drift, including greater net lengths and larger mouth openings and mesh sizes, should have a number of advantages for sampling larger insects that occur infrequently in the drift. These advantages include the capability of sampling greater volumes of water per unit time, sampling for longer periods of time before nets become clogged with debris, sampling greater depth ranges of the water column, and a reduced likelihood that large, active insects could escape by crawling or swimming out of the net during the sampling period. We therefore used drift nets larger than those typically employed, to sample nymphs of Odonata in two large rivers in northern Wisconsin. One of the rivers, the St. Croix River in Burnett County, was sampled at a section where high densities of clubtail (Gomphidae) exuviae had previously been found (DuBois 2015). In so doing, we sought to gain increased insight into the species and life stages of nymphs of Odonata predisposed to drift in these rivers.

Materials and Methods

Large drift nets were used to sample nymphs of Odonata in the Wisconsin waters of two rivers near the border of Wisconsin and Minnesota. The 288-km St. Louis River drains a watershed of 10,093 km^{-2} and is the largest U. S. tributary to Lake Superior. The study area was located immediately downstream of State Highway 23 bridge, on the south side of the river (N46.6579; W92.2837) in Douglas County. This area was in the upper estuary of the river where it approaches the western end of Lake Superior, and river width at the sampling site was ca. 200 m. The St. Croix River is a 272-km tributary of the Mississippi River that is listed as a National Scenic Riverway under the protection of the National Park Service. The study area (known as Riverside Landing) was located immediately downstream of State Highway 35 bridge, on the north side of the river (N46.0750; W92.2485) in Burnett County, and river width at the sampling site was ca. 65 m.

We used two large drift nets designed to sample the fry of lake sturgeon (*Acipenser fulvescens*) with D-shaped openings 75 cm wide x 54 cm high and opening areas of ca. 0.30 m^{-2} (Research Nets, Inc., P. O. Box 249, Bothell, Washington). The nets were made of 1500 μm mesh netting and were 3.5 m in length. Mesh of this size was anticipated to retain Anisoptera nymphs of at least the last four instars and Zygoptera nymphs of F-0 and F-1. The terminal collection cups were 10.5 cm x 30 cm. Nets were tied with nylon ropes to anchors placed 4 m upstream of each net. Anchors were dropped onto the substrate several minutes before the nets were set to ensure that odonates retained by the nets did not result from the upstream substrate being disturbed. In each river, one net was placed ca. 4 m from shore and the other ca. 7 m from shore. Water depths at the points of sampling in the St. Louis River ranged from 75–150 cm. In the St. Croix River, water depths were 60 cm for the nearshore net and 70 cm for the more distant net. Both nets were completely submersed for the duration of all samples. Because the usual time of maximum insect drift is just after complete darkness (Waters 1972, Wiley and Kohler 1984, Allan and Russek 1985, Benke et al. 1986, Brittain and Eikeland 1988), drift samples were taken post-dusk. On each date, the two nets were set at ca. 9 pm CST and three samples were taken per net, with 3-5 min breaks between samples to empty the collection cups. Current velocity (m s^{-1}) was measured once at the sampling site of each river, 4 m from shore, by averaging repeated measurements of the time taken for a float to travel 30.5 m, centered at the point of sampling.

In the St. Louis River, drift was sampled six times (three times each per net) on six evenings in spring 2007 (29 and 31 May; 4, 7, 12 and 14 June). Current velocity as measured on 29 May at the sampling site was 0.91 m s^{-1}. Each net sampled drift for an approximate average of 2.5 hr each evening (ca. 45 min per sample), but exact sampling times were not recorded for all samples. Numbers of drifting odonate nymphs were tallied for each sample. Samples were obtained in water temperatures ranging from 15.5 C to 22 C, and river discharges ranging from 73.6 m^3 s^{-1} to 116.0 m^3 s^{-1} (http://waterdata.usgs.gov/nwis/uv?04024000; accessed 7 October 2016). The median daily discharges for this site (109-yr dataset) were 110 m^3 s^{-1} for 29 May and 105 m^3 s^{-1} for 14 June (website above).

In the St. Croix River, three 30-min samples were taken with each net between 9 pm and 11:00 pm CST on 20 May 2008. Drift densities were estimated by dividing the number of odonate nymphs in a sample by the water volume sampled. Water volumes sampled (m^{-3}) were calculated by multiplying current velocity, the size of the submerged net opening area, and sampling time. Current velocity was 0.98 m s^{-1}; volumetric flow rate was 0.294 m^3 s^{-1} per net. Therefore, each

30-min sampling period strained a water volume of 529 m^{-3} (total water volume sampled during the evening = 3,175 m^{-3}). Samples were taken at a water temperature of 15 C, and river discharge was 39.6 $m^3 s^{-1}$ (http://waterdata.usgs.gov/usa/nwis/uv?05333500). The median daily discharge for this site (103-yr dataset) was 46 $m^3 s^{-1}$ for 28 May (website above). Weather was clear with no moon visible until after sampling had been completed.

Odonata nymphs were preserved in jars of 80% ethanol. Determinations were usually made to species using dichotomous keys and also by comparison with reared exuviae (F-0 to F-3 or F-4 for most species). Most nymphs of all stages collected possessed distinguishing characters in sufficient states of development to identify them to species, except that we did not attempt to separate nymphs of *Hylogomphus*, none of which were full grown. Instars were determined by relating the length of the hind wing sheaths (WL) to the maximum width of the head (HW) (Tennessen 2016). Ratio ranges for instars F-0 through F-4 are usually discretely grouped with little overlap for most North American families of Anisoptera (Tennessen 2016). Nymphs in instar F-4 and younger were grouped in one category. North American Zygoptera also exhibit gradually increasing WL to HW ratios as they molt into increasingly late instars, but the details of the ratios have not been worked out. Instars of older nymphs of Zygoptera (F-0 and F-1) were determined by supposition based on WL and total length of the nymph relative to those dimensions of reared exuviae. Voucher specimens are housed in the Odonata Collection of the Wisconsin Department of Natural Resources at the address of the senior author.

Results

St. Louis River—Forty (40) nymphs representing six species of Odonata were collected during a total of ca. 21 hrs of sampling (Table 1). Thus, about two nymphs were retained per net-hour. *Argia moesta* (Hagen), *Macromia illinoiensis* Walsh, and *Gomphurus fraternus* (Say) were the species most commonly collected. Instars of Odonata from F-0 to F≤4 were all represented, with F≤4 being the largest category. No F-0 Anisoptera were collected and 40% of

Table 1. Numbers of drifting Odonata by instar in the St. Louis (left of slash) and St. Croix rivers (right of slash), and drift densities (all instars pooled) in the St. Croix River, Wisconsin (dash = no data).

	Instar				Instar	St. Croix River drift densities	
Species	F-0	F-1	F-2	F-3	F≤4	Totals	(# m^{-3})
Coenagrionidae							
Argia moesta (Hagen)	7/1	5/-	-/-	-/-	2/-	14/1	<0.001
Enallagma exsulans (Hagen)	2/1	-/1	-/-	-/-	1/-	3/2	<0.001
Ischnura verticalis (Say)	-/1	-/-	-/-	-/-	-/-	-/1	<0.001
Aeshnidae							
Basiaeschna janata (Say)	-/-	-/-	-/-	1/-	-/-	1/-	-
Gomphidae							
Dromogomphus spinosus Selys	-/-	-/-	1/-	-/1	3/-	4/1	<0.001
Gomphurus fraternus (Say)	-/-	-/-	1/-	1/-	6/-	8/-	-
Gomphurus ventricosus (Walsh)	-/1	-/1	-/-	-/1	-/2	-/5	0.002
Hylogomphus spp.*	-/-	-/1	-/3	-/3	-/6	-/13	0.004
Ophiogomphus anomalus Harvey	-/-	-/-	-/-	-/-	-/1	-/1	<0.001
O. howei Bromley	-/13	-/12	-/22	-/17	-/16	-/80	0.026
O. rupinsulensis (Walsh)	-/2	-/-	-/4	-/4	-/12	-/22	0.007
Progomphus obscurus (Rambur)	-/-	-/-	-/-	-/1	-/-	-/1	<0.001
Cordulegastridae							
Cordulegaster maculata Selys	-/-	-/-	-/-	-/-	-/1	-/1	<0.001
Macromiidae							
Macromia illinoiensis Walsh	-/1	2/-	1/1	3/-	4/2	10/4	0.001
Totals	9/20	7/15	3/30	5/27	16/40	40/132	0.042

**Hylogomphus adelphus* (Selys) and *H. viridifrons* (Hine) were both common in the reach sampled.

nymphs of that suborder were in the F≤4 category (Table 1). The two nets sampled a water volume of ca. 20,640 m^{-3} during six evenings, yielding a mean of one odonate nymph per 516 m^{-3} of water sampled and a mean drift density of 0.002 m^{-3}.

St. Croix River—Odonate nymphs totaling 132 individuals of 12 species were collected during six hours of sampling (Table 1). Each net caught 66 nymphs, yielding a mean of 22 nymphs per net-hour. Five species of Gomphidae comprised 93% of the total, and three species of *Ophiogomphus* made up 80%. *Ophiogomphus howei* Bromley was the most commonly collected species, comprising 61% of the Odonata drift total with a mean drift density of 0.026 m^{-3}. Final instar nymphs were collected less often than earlier instars, accounting for only 15% of the total (16% in the case of *O. howei*). The last five instars of *O. howei* were all found in the drift, but the instar with the greatest number of drifting nymphs was F-2 (Table 1). The total volume of water sampled by the two nets was 3,175 m^{-3}, with a mean of one Odonata nymph per 24 m^{-3} of water sampled (a mean drift density of 0.042 m^{-3}).

Discussion

Compared with standard aquatic insect drift nets available for purchase from biological supply retailers, the large drift nets we used had mesh sizes several times larger, about 20 times greater net lengths, and more than 3 times the combined area of the openings. These larger dimensions should have conferred advantages for capturing later instar nymphs of Odonata, including much more water volume sampled per unit time, less back pressure, longer sampling times before the nets become clogged with plant debris, and less-frequent escape of nymphs once inside the net. Although we did not make comparative measurements of these aspects of net functionality (larger vs. smaller nets), the large nets we used did perform as anticipated in straining large volumes of water without clogging with debris. A mesh size of 1500 μm appeared to be a reasonable compromise for sampling late-instar nymphs of Odonata. Small nymphs might have passed through mesh of this size, but early instar nymphs (F-5 or younger) are usually difficult to identify and are therefore less often useful. Conventional aquatic insect drift nets with mesh sizes ranging from 250–500 μm would retain earlier instar nymphs more reliably than the nets we used, but they would also clog with vegetative debris more quickly and would be subject to greater water resistance. If concerns arise about larger-mesh nets failing to retain early instar nymphs of Odonata, using multiple net sets incorporating different opening and mesh sizes could improve representation of large and small taxa groups.

Comparing our relatively low Odonata drift densities with those reported in other studies is inconclusive in all but a most general sense because relatively few studies have reported drift densities of gomphids, and those few have provided disparate results. Obi and Conner (1986) reported a low mean drift density of 0.07 nymphs per 100 m^{-3} of *Gomphus* sp. in the lower Mississippi River in Louisiana. Pendergrass (2006) found odonates, including five genera of Gomphidae (not including *Ophiogomphus* however), to be common in the benthos of the Blanco River, Texas, but odonates were absent from the drift. Conversely, Cloud (1973) reported a relatively high mean drift density of 5.5 nymphs per 100 m^{-3} of *Ophiogomphus* spp. for six semi-monthly samples from the Brazos River, Texas. DuBois and Smith (2016) used a marking study in a small river in Wisconsin to assess the downstream movement of F-0 nymphs of a robust population of *Ophiogomphus rupinsulensis* (Walsh) from fall through spring of the following year. They found that nearly all marked nymphs moved some distance downstream, in a few cases 75 m or more, but most nymphs (79%) moved less than 30 m. Kennedy and Benfield (1979) did not provide drift densities for the New River, Virginia, but noted that nine species of Odonata were collected in the drift (of 22 species of Odonata known to occur in the river), among them *Ophiogomphus aspersus* Morse and *O. howei*. In a particularly intriguing study, Arai (1993) noted that nymphs of *Stylogomphus suzukii* (Matsumura in Oguma) inhabited upper reaches of mountain streams in Japan, but that exuviae were found along lower reaches; these findings led him to surmise that the nymphs somehow moved downstream. In synthesis, these results raise the possibility that species of *Ophiogomphus*, and perhaps *Stylogomphus* as well, might have relatively high propensities to drift. However, despite the presumed advantages of the large nets we used, the odonate drift densities we measured, even in a gomphid-rich section of the St. Croix River, were not especially high, and were indeed much lower than those Cloud (1973) reported for *Ophiogomphus*.

Although drift densities were only approximate in the St. Louis River because sampling times on that river were approximated, it is clear that numbers of drifting odonates there were only about one tenth of those in the St. Croix River, despite samples being taken at the same time of season and day. This difference could be due to differences in the productivity of the two rivers, reach-scale differences in habitat, or species-specific differences in propensity to drift. The highest densities of gomphid exuviae found along 10 rivers in northern Wisconsin by DuBois (2015) were in the same section of the St. Croix River sampled in this study. Further, the St. Croix River is rich in species of *Ophiogomphus*, a genus which may have a relatively high propensity to drift, whereas *Ophiogomphus* nymphs were not collected in the St. Louis River. It was not unexpected that *O. howei* was the dominant species in the St. Croix River drift (F-0 nymphs of that species comprised 84% of the drifting F-0 gomphids collected): this species also made up ca. 2/3 of the total number of gomphid exuviae collected on shore in June 2008 within just a few meters of our drift sampling point (DuBois 2015, Table 3, Trial #5).

Drift-netting is not commonly practiced specifically for the collection of odonates, and our results concord with many other studies in showing that large volumes of water must be strained to capture a relatively small number of specimens. The fact that most of the nymphs we collected drifting were not in their final instar further compromises the usefulness of drift sampling for odonates because partly-grown nymphs can be difficult to identify, even at the genus level. However, in circumstances in which sampling for odonates using other methods is impractical, drift-net collections can supply useful information about species presence. Pringle and Ramirez (1998) noted that drift sampling in biodiversity assessment is potentially useful because it provides integrated samples of various habitats (advantageous if the habitats of some species are unknown or difficult to sample), it is relatively 'clean' in that target organisms are not mixed with substrate materials, and it is non-destructive of benthic habitats. Thus, drift sampling can be seen as a complementary tool in the assessment of aquatic invertebrate assemblages (Calisto and Goulart 2005).

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