

LENGTH-WEIGHT RELATIONSHIPS ASSOCIATED WITH GENDER AND SEXUAL STAGE IN THE NORTHERN CLEARWATER CRAYFISH, *ORCONECTES PROPINQUUS* GIRARD, 1852 (DECAPODA, CAMBARIDAE)

Thomas P. Simon¹, Kellene Quillen and Wendy E. Anderson: School of Public and Environmental Affairs, 1315 E. Tenth Street, Indiana University, Bloomington, IN 47403 USA

ABSTRACT. The northern clearwater crayfish, *Orconectes propinquus* Girard 1852, is a species native to eastern Canada and the northern United States. Growth patterns and relationships of body morphometrics were evaluated to understand its basic niche requirements. Growth and size relationships for gender, sexual phase for adults and juveniles, and chelae length and width relationships to interpret information on sexual dimorphism were determined. The log transformed carapace length-weight relationship for male form I ($y = 3.3201x - 4.0205$, $r^2 = 0.9645$, $F = 1302.9$, $p = <0.001$), and juveniles ($y = 3.2792x - 4.0171$, $r^2 = 0.8721$, $F = 709.2$, $p = <0.001$) showed positive allometric growth rates, whereas male form II ($y = 2.8831x - 3.4474$, $r^2 = 0.8268$, $F = 248.2$, $p = <0.001$) and female ($y = 2.9184x - 3.5313$, $r^2 = 0.9395$, $F = 1879.3$, $p = <0.001$) showed negative allometric rates of change with increasing length. Relationships between log transformed carapace length and carapace width ($y = 1.0425x - 0.3841$, $r^2 = 0.8964$, $F = 1945.8$, $p = <0.001$), carapace depth ($y = 0.9468x - 0.2816$, $r^2 = 0.9018$, $F = 2065.2$, $p = <0.001$), abdomen width ($y = 1.0413x - 0.4443$, $r^2 = 0.864$, $F = 1429.2$, $p = <0.001$), chelae width ($y = 1.2927x - 0.9998$, $r^2 = 0.6255$, $F = 375.9$, $p = <0.001$), and chelae length ($y = 1.3083x - 0.6281$, $r^2 = 0.7439$, $F = 653.6$, $p = <0.001$) for the total collected adult population grew at a negative allometric rate. According to the body condition index b , growth rates showed increasing weight with length but were within the expected allometric growth.

Keywords: morphometrics, allometry, growth, length-weight relationships, body condition Index

INTRODUCTION

Basic life history information is missing for the majority of the nearly 650 described species of crayfish (Moore et al. 2013), and there is limited understanding of growth and length-weight relationship information (Stein 1976; Romaine et al. 1977; Rhodes & Holdich 1984; Garvey & Stein 1993; Mazlum et al. 2007; Wang et al. 2011; Simon & Stewart 2014). Understanding patterns in growth can provide important community management information based on size, weight, and body condition between native and invasive species introductions.

The native distribution of northern clearwater crayfish, *Orconectes propinquus* Girard 1852 includes the northern USA and Canada (Page 1985; Hobbs 1989). It is native to Illinois, Indiana, Ohio, Pennsylvania, New York, southern Quebec and Ontario, with populations extending

west into Iowa and Minnesota (Page 1985). *Orconectes propinquus* is commonly found in streams across the state of Indiana and can be found in nearly every region of the state (Simon 2001).

The competitive advantage of *O. propinquus* is speculated to be based on its large size compared to other sympatric native species (Loughman & Simon 2011; Simon & Stewart 2014). This size advantage may be the result of size and weight differences caused by unequal growth of body parts (Lockwood et al. 2013). Change in growth of select structures is sexually dependent and rates may be characterized as either allometric or isometric based on the regression slope constant b (Mazlum et al. 2007). The b constant is considered a measure of body condition based on the cube law (Froese 2006). Growth is isometric when $b = 3$, and allometric when $b < 3$ or $b > 3$. This suggests that positive allometric growth occurs when organism weight increases more than length ($b > 3$) and negative allometric growth occurs when length increases more than weight ($b < 3$). Allometry may change

¹ Corresponding author: Thomas P. Simon, 812-332-4725 (phone), tsimon@indiana.edu.

during growth and sexual stage. These growth rate changes can result in differential expression based on gender or sexual maturation. This divergent expression may not provide a competitive advantage for females and juveniles.

The current study evaluates the relationships between growth, gender, and body morphology that would enhance competitive advantage among the northern clearwater crayfish. Length and weight, carapace, chelae, and abdomen relationships among male form I and II, female, and juvenile individuals of *O. propinquus* were investigated. This information will contribute to baseline information needs for evaluating introduced species life history attributes.

METHODS

All specimens ($n = 333$) used for measurement of *Orconectes propinquus* morphometry were collected from mostly ambient natural streams ($n = 51$), man-made ditches ($n = 2$), a mitigated wetland site ($n = 1$), and a few unknown locations across the state of Indiana ($n = 6$). Surveys were conducted in Indiana during May 2005 until May 2013. Sampling was restricted to daylight hours and all available habitats within a reach were sampled within a linear distance of 15 times the wetted stream width or 150 m minimum distance in lakes and wetlands along the shoreline margin using backpack electrofishing equipment. Data over this species native range was pooled in order to examine the relationships between growth, gender, sexual stage, and size.

A total of 123 females, 104 males ($n = 50$ form I and $n = 54$ form II), and 106 juveniles were measured using digital calipers to the nearest 0.1 mm. Individuals were segregated by gender and sexual stage groups. Two people measured the crayfish samples ($n = 76$ and $n = 257$) and measures were compared so that less than 5% variation in measurement error was achieved. All measured individuals had a full complement of chelae and walking legs. Regenerated chelae were not measured. Each individual crayfish was externally classified according to sex and reproductive state. All individuals were measured for morphometric variables and for wet weight (W_{WT}). (Technically, since our measurements are in grams, not newtons, this should be mass, not weight. However, since the literature going back almost 200 years has always referred to this as weight, we are

using weight instead of mass.) Weight (W_{WT}) was measured by placing the individual on paper towel to remove excess water, and then weighed with an accuracy of 0.001 g. Seven morphometric characteristics were measured for each specimen (see Simon & Stewart 2014) – carapace length (CL), postorbital carapace length (POCL) [from the anterior margin of postorbital spine to the posterior margin of the carapace], carapace width (CW), carapace depth (CD), chelae length (ChL), chelae width (ChW), and abdomen width (ABW). Based on similar studies of *Procambarus* (Mazlum et al. 2007; Wang et al. 2011) and *Orconectes* (Simon & Stewart 2014) crayfish species these morphological characters are related to sexual dimorphism and are influenced by environmental conditions and food resources.

Juvenile and adult specimens were distinguished by the presence or absence of reproductive organs (Hobbs 1989). Any possible relationship between smaller ($CL < 25$ mm) and larger ($CL > 25$ mm) specimens were determined by comparing the ratios between the means of above measurements and mean carapace length (CL/ABW, CL/POCL, CL/CW, CL/CD, CL/ChL, CL/ChW) of all individuals in each group.

The carapace length was considered as the independent variable for all relationships performed as it appears to be minimally affected by growth variations and sexual maturation among Decapoda (Lovett & Felder 1989). Regression analyses to determine the relationship between all measurements versus CL was investigated for each sex separately using the multiplicative model: $Y = aX^b$, where Y and X are the morphological dimensions and a and b the regression constants (StatSoft 2010). The relationships obtained were log transformed to the form $\log_{10} Y = \log_{10} a + b \log_{10} X$. The log transformation is preferred in order to better satisfy the assumptions of regression analysis (Sokal & Rohlf 1981). This allows the derivation of a single value from the analysis for the scaling relationship between the two-morphometric parameters. The allometry pattern for each parameter was established by testing the slope (b) of the regression equations against isometry ($H_0: b = 1$) applying the Student's t-test. Analysis of Covariance (ANCOVA) was used to compare the slopes b and carapace length between sexes, sizes, and sampling period (Zar 1984). The Kruskal–Wallis test (Zar 1984) was used

to identify possible differences in time, area, and size, at the 95.0% confidence level. The Mann-Whitney test compared independent samples, at the 95.0% confidence level (Sokal & Rohlf 1981), while a simple regression analysis was used to examine the relationship between *O. propinquus* morphological characters with the sex as a covariate.

RESULTS

The samples in the study area showed differences in mean carapace length between male and female, specifically the male carapace was larger than those of females (Mann-Whitney test, $p > 0.001$). The carapace length of sampled females ranged from 11.78 to 35.67 mm (average 23.96 ± 5.65 mm) and that of male form I from 12.16 to 34.16 mm (average 24.63 ± 5.11 mm), while male form II ranged from 12.22 to 33.49 mm (average $23.95 \text{ mm} \pm 5.14$ mm).

Mean carapace length (CL \pm SD) and mean weight ($W_{WT} \pm$ SD) for the entire population of male and female were 24.12 ± 5.40 mm (range = 11.78–35.67 mm) and 3.93 ± 2.53 g (range = 0.34–12.09 g), respectively (Table 1). Mean carapace length (CL \pm SD), mean weight ($W_{WT} \pm$ SD), and their range were calculated for male and female as: $CL_{\text{male}} = 24.28 \pm 5.11$ mm (range = 12.16–34.16 mm), $W_{WT \text{ male}} = 4.24 \pm 2.54$ g (range = 0.34–11.06 g); $W_{WT \text{ female}} = 3.67 \pm 2.50$ g (range = 0.43–12.09 g), respectively. The normalized (\log_{10}) length-weight relationship for male form I was explained by the linear equation, where $y = 3.3201x - 4.0205$, $r^2 = 0.965$, $F = 1302.9$, $p \leq 0.001$, male form II was explained by the linear equation, $y = 2.8831x - 3.4474$, $r^2 = 0.863$, $F = 248.2$, $p \leq 0.001$, female length and weight ($y = 2.9184x - 3.5313$, $r^2 = 0.940$, $F = 1879.3$, $p \leq 0.001$), and juveniles ($y = 3.2792x - 4.0171$, $r^2 = 0.872$, $F = 709.2$, $p \leq 0.001$) (Fig. 1). Only male form I and juveniles showed positive allometric rates of weight change with increasing length (Table 1).

Mean carapace width (CW \pm SD), mean carapace depth (CD \pm SD), and their range for male and female were $CW_{\text{males}} = 11.69 \pm 2.78$ mm (range = 5.74–18.17 mm), $CD_{\text{male}} = 10.81 \pm 2.35$ mm (range = 5.06–15.47 mm), $CW_{\text{females}} = 11.25 \pm 2.91$ mm (range = 5.69–18.42 mm), and $CD_{\text{female}} = 10.52 \pm 2.48$ mm (range = 5.93–17.19 mm) (Table 1). The relationships between carapace length with carapace width ($y = 1.0425x - 0.3841$, $r^2 = 0.896$,

Table 1.—Descriptive statistics, estimated parameters (\log_{10}), and growth type of length-weight relationships for 333 individual *Orconectes propinquus*. SE = standard error of b ; CL = 95% confidence limits of b ; n = number of crayfish; +A = positive allometric growth; -A = negative allometric growth.

Sex and sexual form	n	Carapace length (mm)			Weight (g)			Parameters of the relationships					Type of growth
		Mean (SD)	Min	Max	Mean (SD)	Min	Max	a	b	SE (b)	CL (b)	r ²	
Male (I)	50	24.63 (5.11)	12.16	34.16	4.60 (2.76)	0.38	11.06	-4.021	3.320	0.092	3.135-3.505	0.964	+ A
Male (II)	54	23.95 (5.14)	12.22	33.49	3.91 (2.30)	0.34	9.91	-3.447	2.883	0.813	2.516-3.250	0.863	- A
Female	123	23.96 (5.65)	11.78	35.67	3.67 (2.50)	0.43	12.09	-3.531	2.918	0.067	2.785-3.052	0.940	- A
Juvenile	106	10.03 (1.94)	6.51	15.45	0.21 (0.15)	0.02	0.92	-4.017	3.279	0.123	3.035-3.523	0.872	+ A
Total	333		6.51	35.67		0.02	12.09						

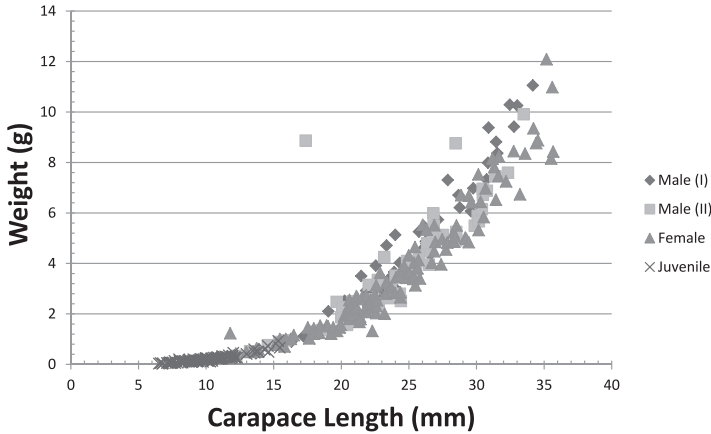


Figure 1.—Length-weight relationships for *Orconectes propinquus* Girard 1852 sexual phases. Diamonds = form I males; boxes = form II males; triangles = females; and x = juveniles.

$F = 1945.8$, $p \leq 0.001$), carapace length with carapace depth ($y = 0.9468x - 0.2816$, $r^2 = 0.902$, $F = 2065.2$, $p \leq 0.001$), carapace length with abdomen width ($y = 1.0413x - 0.4443$, $r^2 = 0.864$, $F = 1429.2$, $p \leq 0.001$), carapace length with chelae length ($y = 1.3083x - 0.6281$, $r^2 = 0.744$, $F = 653.6$, $p \leq 0.001$), and carapace length with chelae width ($y = 1.2927x - 0.9998$, $r^2 = 0.626$, $F = 375.9$, $p \leq 0.001$) for the total adult specimens grew at a negative allometric rate. Mean carapace width ($CW \pm SD$), mean carapace depth ($CD \pm SD$), and their range were calculated for form I male, form II male, female, and juveniles respectively as: $CW_{MI} = 11.87 \pm 2.81$ mm (range = 5.74–18.17 mm), $CD_{MI} = 10.92 \pm 2.34$ mm (range = 5.55–15.47 mm), $CW_{MII} = 11.52 \pm 2.76$ mm (range = 5.86–16.96 mm), $CD_{MII} = 10.72 \pm 2.39$ mm (range = 5.06–15.33 mm), $CW_{female} = 11.25 \pm 2.91$ mm (range = 5.69–18.42 mm), $CD_{female} = 10.52 \pm 2.48$ mm (range = 5.93–17.19 mm), $CW_{juv} = 4.25 \pm 0.98$ mm (range = 2.32–7.38 mm), and $CD_{juv} = 4.29 \pm 0.93$ mm (range = 1.84–6.47 mm).

Sexual stage differences were observed between adult and juveniles. The carapace width (CW) growth rate increased at a negative allometric rate with weight for male form I, male form II, females, juveniles, and the entire population. ANCOVA tests showed that length-weight regression slopes and intercepts were not significantly different among sexes or sexual stage ($p > 0.05$). In addition, our results shows that form II male were 1.04 times heavier than form I male and 1.18 times heavier than females. Form I males were 1.95 mm larger than females,

while form II males were 1.67 mm larger than females. Mean total length and weight did not differ between males and females ($p > 0.060$); the only significant differences were detected among sexual stages ($p < 0.0001$).

Relationships among chelae length and width measurements for the population were evaluated for gender and sexual stage. Mean chelae length ($ChL \pm SD$), mean chelae width ($ChW \pm SD$), and their range were calculated for form I males, form II males, and females, respectively, as $ChL_{MI} = 18.55 \pm 6.08$ mm (6.56–32.58 mm), $ChW_{MI} = 7.69 \pm 2.48$ mm (3.05–12.63 mm), $ChL_{MII} = 17.00 \pm 5.76$ mm (7.19–27.57 mm), $ChW_{MII} = 6.82 \pm 2.99$ mm (2.72–19.17 mm), and $ChL_{female} = 13.73 \pm 4.33$ mm (5.53–25.70 mm), $ChW_{female} = 5.63 \pm 1.93$ mm (2.03–11.40 mm) (Table 2).

No significant difference was observed in mean ChL between form I and form II males ($p > 0.05$), but a significant difference was detected in mean ChL between males and females ($P < 0.05$). Form I and form II male had longer ChL than females. A similar trend was observed in mean ChW for form I and form II males, but a significant difference was observed between males and females ($p < 0.05$). Chela lengths and width increased with CL for both genders and sexual stages (Table 2). In addition, chelae length-weight relationships were positively correlated with gender and sexual states (Table 2). Although the slope and intercepts of regressions for ChL and ChW were similar for form I and form II males, the slope and intercepts of regression of females

Table 2.—Mean and standard deviation (SD) for chelae length (ChL) and chelae width (ChW) characteristics and parameters of the relationship between log ChL and log ChW of each sex and sexual form. SE = standard errors of *b*; CL = 95% confidence limits of *b*; N = number of crayfish; r^2 = coefficient of determination.

Sex and sexual form	n	Chelae length (mm)				Chelae width (mm)				Parameters of the relationships				
		Mean (SD)	Min	Max	Max	Mean (SD)	Min	Max	Max	<i>a</i>	<i>b</i>	SE (<i>b</i>)	CL (<i>b</i>)	r^2
Male (I)	50	18.55 (6.08)	6.56	32.58	12.63	7.69 (2.48)	3.05	12.63	12.63	-0.234	0.880	0.036	0.751–1.010	0.795
Male (II)	54	17.00 (5.76)	7.19	27.57	19.17	6.82 (2.99)	2.72	19.17	19.17	-0.340	0.945	0.085	0.774–1.117	0.702
Female	123	13.73 (4.33)	5.53	25.70	11.40	5.63 (1.93)	2.03	11.40	11.40	-0.904	1.191	0.073	1.046–1.336	0.685
Juvenile	106	5.41 (1.43)	2.14	10.09	4.13	2.12 (0.65)	0.78	4.13	4.13	-0.421	0.011	0.066	0.879–1.142	0.691

were significantly different (ANCOVA $p < 0.05$) from form I and form II males (Table 2). The relative growth rate of the abdomen in males did not present statistically significant results; however, females were significantly different from males (ANCOVA $p < 0.001$).

DISCUSSION

Studies focused on length-weight relationships reveal that sexual dimorphism is common in freshwater crayfish species (Lindquist & Lahti 1983; Holdich 2001; Mazlum et al. 2007; Wang et al. 2011; Simon & Stewart 2014). Difference in sexual dimorphism is a function of the rapid disproportionate growth of chelae in male compared to female genders. Differences in body size among sex and sexual stage was consistent with those reported in other studies (Stein 1976; Romaire et al. 1977; Rhodes & Holdich 1984; Garvey & Stein 1993; Mazlum et al. 2007; Wang et al. 2011; Simon & Stewart 2014). Juvenile crayfish grew at a positive allometric rate and rapidly attained adult sizes as in other members of the genus *Orconectes* (Simon & Stewart 2014).

The relative growth between the sexes differs only slightly as indicated by morphometric relationships, which is similar to *Orconectes virilis* (Simon & Stewart 2014). A positive allometry of all body relationships observed in male form I and juvenile, reflects the decreasing growth rate of these morphological characters in relation to CL, which is attributed to a sex-related variation (Wetzel 2002). Variation in abdomen width is commonly found in freshwater crayfish, but is always related to sex, sexual maturity, and size (Wetzel 2002). Widening of female abdomen width (ABW) reflects a sexually active female that is correlated with either swollen or white glair, dependent offspring, or remnants of egg stalks attached to pleopods. Wetzel (2002) found that only form I females mated with form I males and reinforced the view that wide abdomens are a reflection of the act of mating and rearing offspring. Reasons for female variation may include presence of ovigerous stages of ova development, instar development during the prolonged period of recruitment, and larval growth (Wetzel 2002). No reproductively active females were present in this study; none exhibited the widened ABW.

In this study, the length-weight relationships showed that males were heavier than females

Table 3.—Comparison of crayfish species body condition index factors (*b*) reflecting body condition for length-weight relationships.

Species and sexual form	Body condition factor	Study
	Length-weight	
<i>Orconectes propinquus</i>		Current study
Male	3.320	
Male form I	2.883	
Male form II	2.918	
Female	3.279	
<i>Orconectes virilis</i>		Simon & Stewart 2014
Male	3.85	
Male form I	3.66	
Male form II	3.95	
Female	3.73	
<i>Procambarus acutus</i>		Mazlum et al. 2007
Male	3.30	
Male form I	3.61	
Male form II	3.26	
Female	3.50	
<i>Procambarus clarkii</i>		Wang et al. 2011
Male	3.63	
Male form I	3.60	
Male form II	3.60	
Female	3.35	

of the same length. The largest male (34.16 mm CL) was shorter, but heavier (11.05 g) than the longest female (35.67 mm, weighing 8.429 g). No statistical difference in mean weight was observed; however, this is attributed to the accelerated development of the chelae in sexually mature form I males, whereas chelae of females grow slower throughout life. The relatively longer chelae of form I and form II males are due to sexually dimorphic change.

Body condition rates are used to evaluate competition and ecological relationships among a wide variety of species including crayfish (Lindquist & Lahti 1983). Body Condition Index factors comparisons among crayfish species show that *O. propinquus* exhibit the lowest body condition rates (Table 3). Condition factors (*b*) can be predictive to evaluate competition based on length-weight growth rates. Based on condition factors of *O. virilis* (Simon & Stewart 2014), *Procambarus acutus acutus* (Romaine et al. 1977), and *P. clarkii* (Wang et al. 2011),

O. propinquus would not be dominant in any introduced scenario between these four species (Table 3).

In summary, the length-weight relationship and condition factors observed in *O. propinquus* show the lowest body mass related to length among the crayfish species studied. We observed little morphometric or growth related differences between sex or sexual phase, including various male forms and female sexual phases, with the exception of female abdomen width. With increasing emphasis on the attainment of basic life history information for crayfish it will be necessary to consider differences among populations and possible intraspecific body shape differences associated with different habitats and water qualities.

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- SUPPLEMENTAL MATERIALS
- Indiana: Brown County, Jackson Twp., Gravel Creek, Gravel Creek Rd, Story, 39.10718 N, –86.22673 W, 2 July 2008; Brown County, Middlefork Salk Creek, Poplar Rd, Story, 39.12683 N, –86.13673 W, 2 July 2008, Field No. MFS06US, INBS 3831; Brown County, Middlefork Salk Creek, Poplar Rd, Story, 39.12683 N, –86.13673 W, 2 July 2008, Field No. MFSC06DS, INBS 3832; Brown County, Gravel Creek, Gravel Creek Rd, Story, 39.10718 N, –86.22673 W, 2 July 2008, Field No. MFSC14US, INBS 3833; Brown County, Gravel Creek, Elkinsville Rd, Story, 39.09169 N, –86.28108 W, 2 July 2008, Field No. MFSC15US, INBS 3835; Brown County, Hamilton Creek, Mt. Neb. Rd, 39.08792 N, –86.17157 W, 2 July 2008, Field No. MFSC21US, INBS 3839; Brown County, Hamilton Creek, Christianburg Rd, Christianburg, 39.08693 N, –86.15695 W, 2 July 2008, Field No. MFSC23US, INBS 3841; Brown County, Hamilton Creek, Christianburg Rd, Christianburg, 39.08693 N, –86.15695 W, 2 July 2008, Field No. MFSC23DS, INBS 3842; Brown County, unnamed creek, Blue Creek Rd, Elkinsville, 39.05613 N, –86.27207 W, 2 July 2008, Field No. MFSC25US, INBS 3843; Brown County, Happy Hollow Lake Creek, Bellsville Pike, New Bellsville, 39.14452 N, –86.10815 W, 2 Jul 2008, Field No. MSC48US, INBS 3849; Brown County, Washington Twp., unnamed trib. of Salt Creek, D/S bridge at Crooked Creek Rd., 0.1 mi from SR 46, 2 mi. E Belmont, (Simon), 39.154756 N, –86.305369 W, 6 July 2010, Field No. TPS 92-75 Cob; Brown County, Washington Twp., Middle Fork Salt Creek, SR 46 Bridge, Nashville, (TP Simon), 39.20079 N, –86.248267 W, 1 June 2005, Field No. TPS05-50; Brown County, Hamble Twp., unnamed trib. of Salt Creek, D/S bridge at intersection of N. Peoga Ridge Rd. and Gatesville Rd .2 mi N, TRS: 9N 4E 25/36/ 30 SE ¼, (Simon), 39.261817 N, –86.143267 W, 6 July 2011, Field No. TPS-92-6814JUL2010; Clay County, Jackson Twp., 300 E Shady Lane, N W, 2 June 2006; Clay County, Jackson Twp., TPS 92-91 COB, N W, 3 June 2006, Clay County, Jackson Twp., TPS 92-68, N W, 4 June 2006; Clay County, Jackson Twp., TPS 92-103, N W, 5 June 2006; Clay County, Jackson Twp., TPS 92-94, N W, 6 June 2006; Clay County, Jackson Twp., unnamed trib., Boon, Hoosierville, (J Burskey, M. Herbert, TPS), 39.47431 N, –87.09138 W, 8 June 2006, Field No. JLB06062, INBS 2292; Clay County, Dick Johnson Twp., unnamed trib., 950 N Bee Ridge, (J Burskey, M. Herbert, T), 39.52639 N, –87.16421 W, 2 June 2006, Field No. JLB06065, INBS 2299; Clay County, Dick Johnson Twp., unnamed trib., 200 W Perth, (J Burskey, M. Herbert, TPS), 39.58467 N, –87.14317 W, 2 Jun 2006, Field No. JLB06066, INBS 2301; Clay County, Van Buren Twp., Croys Creek, 1300N Shady Lane, (J Burskey, M Herbert, TPS), 39.57536

N, -87.03456 W, 2 June 2006, Field No. JLB06068, INBS 2308; Clay County, Brazil Twp., Otterco, SR 59, Cardonia, (J Burskey, M Herbert, TPS), 39.12525 N, -87.12524 W, 2 June 2006, Field No. JLB06070, INBS 2315; Clay County, Dick Johnson Twp., N BR Otter Creek, Rockruch Church, Perth, (J Burskey, M Herbert, TPS), 39.59304 N, -87.17594 W, 2 June 2006, Field No. JLB06071, INBS 2316; Clay County, Cass Twp., unnamed creek, 700 E Poland, (J Burskey, M, Herbert, T), 39.4429 N, -87.9706 W, 3 June 2006, Field No. JLB06074, INBS 2323; Clay County, Washington Twp., McIntyre Creek, 200 N LAP corner, (J Burskey, M Herbert, T), 39.41501 N, -87.02196 W, 8 June 2006, Field No. JLB06076, INBS 2328; Clay County, Perry Twp., unnamed trib., 300 S Hickory Island, (J Burskey, M Herbert, TPS), 39.34651 N, -87.237943 W, 8 June 2006, Field No. JLB06081, INBS 2339; Clay County, Harrison Twp., unnamed creek, 1100 S Barrick Corner, (J Burskey, M Herbert, TPS), 39.22697 N, -87.09078 W, 9 June 2006, Field No. JLB06084, INBS 2345; Clay County, Harrison Twp., unnamed creek, 900 S Barrick Corner, (J Burskey, M Herbert, TPS), 39.25607 N, -87.0766 W, 3 June 2006, Field No. JLB06083, INBS 2349; Clay County, Harrison Twp., Connelly Ditch, Clay City, (J Burskey, M Herbert, TPS), 39.30834 N, -87.12373 W, 9 June 2006, Field No. JLB06090, INBS 2358; Gibson County, Montgomery Twp., Coffee Bayou, CR 50 S Bridge 3 mi E Skelton, (TP Simon), 38.35591 N, -87.715187 W, 23 May 2005, Field No. TPS05-79; Greene County, Stockton Twp., Buck Creek, 1100W Hoosier, (J Burskey, M Herbert, TP Simon), 39.06857 N, -87.14802 W, 26 May 2006, Field No. JLB06141, INBS 2467; Greene County, Highland Twp., unnamed creek, 650 N Calverville, (J Burskey, M Herbert, TP Simon), 39.12044 N, -86.901183 W, 26 May 2006, Field No. JLB061358, INBS 2456; Greene County, Stockton Twp., Black Creek, White Rose, (J Burskey, M Herbert, TP Simon), 39.01565 N, -87.21257 W, 3 May 2006, Field No. JLB06146, INBS 2484; Knox County, Widner Twp., Maria Creek, Kerns Rd, Freelandville, (J Burskey, M Herbert, TP Simon), 39.84276 N, -87.36537 W, 11 May 2005, Field No. JLB06032, INBS 2229; Knox County, Widner Twp., Unnamed, BBRD Freelandville, (J Burskey, M Herbert, TP Simon), 38.87047 N, -87.33417 W, 5 June 2006, Field No. JLB06033, INBS 2232; Knox County, Busserson Twp., Marsh Creek, 875 N Oaktown, (J Burskey, M, Herbert, TP Simon), 38.85416 N, -87.40137 W, 7 June 2006, Field No. JLB06038, INBS 2245; Knox County, Ralmyra Twp., Kessinger ditch, SR 50/150, Fritchton, (J Burskey, M Herbert, TP Simon), 38.67584 N, -87.37235 W, 6 Jun 2006, Field No. JLB06040, INBS 2250; Lawrence County, Pleasant Run Twp., Guthrie Creek, D/S SR 50 Bridge, 0.2 from Back Creek Rd. 2.5 mi N Leesville, (TP Simon), 38.874025 N, -86.306256 W, 18 July 2010, Field

No. TPS05-70; Monroe County, Monroe Creek, Monroe Creek Rd, 39.1146 N, -86.46969 W, 2 July 2008, Field No. LM63US, INBS 3850; Monroe County, Monroe Creek Tributary, Monroe Creek Rd, 39.1145 N, -86.46818 W, Field No. LM63US, INBS 3860; Monroe County, Monroe Creek Tributary, Huges Rd, Handy, 39.11539 N, -86.47111 W, 2 July 2008, Field No. LM37DS, INBS 3866; Monroe County, Monroe Creek Tributary, Huges Rd, Handy, 39.11612 N, -86.47137 W, 2 July 2008, Field No. LM38DS, INBS 3869; Monroe County, Indian Creek Twp., unnamed trib. of Clear Creek, U/S W. Tom Phillips Rd., between S. Burch Rd. and S. Breeden Rd., 1 mi. S. Stanford, (TP Simon), 39.076031 N, -86.668358 W, 18 July 2010, Field No. TPS92-97; Monroe County, Perry Twp., Clear Creek, d/s Country Club Road bridge 6 mi S Bloomington, (TP Simon), 39.13557 N, -86.5335 W, 24 May 2012, Field No. TPS12-011; Monroe County, Bloomington Twp., Jordan River wetland, u/s Jordan River garage 5 mi E Bloomington, (TP Simon), 39.16779 N, -86.51395 W, 10 May 2012, Field No. TPS12-005; Monroe County, Bloomington Twp., West Br Jackson Creek, u/s Rhorer Road, 6 mi SE Bloomington (TP Simon), 39.13581 N, -86.50811 W, 22 May 2013, Field No. TPS12-010; Owen County, Washington Twp., Rattlesnake Creek, 75 S Southport, (J Burskey, M Herbert, TP Simon), 39.27888 N, -86.80404 W, 12 June 2006, Field No. JLB06151, INBS 2495; Owen County, Franklin Twp., unnamed creek, Hoot Rd, Arney, (J Burskey, M Herbert, TP Simon), 39.24731 N, -86.88777 W, 12 June 2006, Field No. JLB06152, INBS 2496; Owen County, Franklin Twp., unnamed creek, Hoot Rd, Arney, (J Burskey, M Herbert, TP Simon), 39.24731 N, -86.88777 W, 12 June 2006, Field No. JLB06153, INBS 2499; Owen County, Morgan Twp., unnamed creek, 500 N Beamer, (J Burskey, M Herbert, TP Simon), 39.36384 N, -86.91877 W, 12 June 2006, Field No. JLB06155, INBS 2506; Owen County, Harrison Twp., unnamed creek, Quincy Rd, Quincy, (J Burskey, M Herbert, TP Simon), 39.45584 N, -86.70132 W, 13 Jun 2006, Field No. JLB06161, INBS 2523; Owen County, Wayne Twp., unnamed creek, Moore Rd, Carp, (J Burskey, M Herbert, TP Simon), 39.39566 N, -86.71476 W, 13 Jun 2006, Field No. JLB06166, INBS 2536; Ripley County, Washington Twp., South Hogan Creek, CR625E 5 mi. E of Versailles, (TP Simon), 39.076031 N, -86.668358 W, 17 May 2005, Field No. TPS05-60 Burrows Clear; Sullivan County, Jefferson Twp., Maria Creek, 1050 S Carlisle, (J Burskey, M Herbert, TP Simon), 38.92437 N, -87.33298 W, 22 May 2006, Field No. Field No. JLB06099, INBS 2373; Sullivan County, Curry Twp., Turman Creek, 100 N Farharsburg, (J Burskey, M Herbert, TP Simon), 39.23004 N, -87.4095 W, 23 May 2006, Field No. JLB06107, INBS 2392; Sullivan County, Fairbanks Twp., W Fk Turman Creek, 850 N Scott City, (J Burskey,

M Herbert, TP Simon), 39.2082 N, -87.47838 W, 24 May 2006, Field No. JLB06120, INBS 2422; Vigo County, Fayette Twp., Coal Creek, Libertyville Pl, Libertyville, (J Burskey, M Herbert, TP Simon), 39.58861 N, -87.51873 W, 8 May 2006, Field No. JLB6001, INBS 2140; Vigo County, Fayette Twp., Coal Creek, Shepardsville Rd, Shepardsville, (J Burskey, M Herbert, TP Simon), 39.6064 N, -87.41516 W, 8 May 2006, Field No. JLB6002, INBS 2141; Vigo County, Lost Creek Twp., Snake Creek, Mainstreet, Seelyville, (J Burskey, M Herbert, TP Simon), 39.50836 N, -87.26758 W, 9 May 2006, Field No. JLB06005, INBS 2149; Vigo County, Lost Creek Twp., Miami Garden Rd, Ehrmandale, (J Burskey, M Herbert, TP Simon), 39.54218 N, -87.19978 W, 9 May 2006, Field No. JLB06007, INBS 2155; Vigo County, Prairie Creek Twp., Prairie Creek, French Drive, (J Burskey, M Herbert, TP Simon), 39.28839 N, -87.50438 W, 10 May 2006, Field No. JLB06014, INBS 2157; Vigo County, Sugar Creek Twp., E Little Sugar Creek, Concannon Rd, Terre Haute, (J Burskey, M Herbert, TP Simon), 39.50947 N, -87.47329 W, 8 May 2006, Field No. JLB06015, INBS 2179; Vigo County, Riley Twp., Little Honey Creek, Old Riley Rd, Riley, (J Burskey, M Herbert, TP Simon), 39.41353 N, -87.34412 W, 10 May 2006, Field No. JLB06021, INBS 2196; Vigo County, Honey Creek Twp., unnamed creek, Eaton Rd, Youngstown, (J Burskey, M Herbert, TP Simon), 39.36852 N, -87.36021 W, 10 May 2006, Field No. JLB06022, INBS 2200.